BIO-SUSTENTABILIDADE E BIO-SEGURANÇA ALIMENTAR, INOVAÇÃO E QUALIDADE ALIMENTAR

23-26 de outubro de 2022

Castelo Branco

Sociedade Portuguesa de Química

Instituto Politécnico de Castelo Branco

Instituto Politécnico de Castelo Branco
Escola Superior Agrária

Câmara Municipal
Castelo Branco

EuChemS
European Chemical Society
Division of Food Chemistry

Histórico do "Encontro de Química dos Alimentos (EQA)"

I	1993	Santarém 19-22 de dezembro	Encontro de Química dos Alimentos	Jorge Justino – Instituto Politécnico de Santarém
II	1995	Aveiro 19 a 21 de julho	Encontro de Química dos Alimentos	Ivonne Delgadillo – Universidade de Aveiro
III	1997	Algarve 24-26 de março	Alimentação Mediterrânica	Nídia Braz - Escola Superior Tecnologia do Algarve
IV	1999	Coimbra 1-4 de junho	Qualidade e Inocuidade dos alimentos, segurança alimentar	Maria Irene Silveira - Universidade de Coimbra
V	2001	Porto 8-11 de maio	Qualidade, Segurança e Inovação	Alcina M. M. B. Morais – Universidade Católica
VI	2003	Lisboa 21 a 24 de junho	Novas perspetivas sobre Conservação, Processamento e qualidade de alimentos	Maria Leonor Nunes e Narcisa Maria Bandarra – IPIMAR
VII	2005	Viseu 12 a 15 de abril	Alimentos: tradição e inovação, saúde e segurança	Dulcineia Ferreira – Instituto Politécnico de Viseu
VIII	2007	Beja 4 a7 de março	Alimentos tradicionais, alimentos saudáveis e rastreabilidade	Silvina Ferro Palma – instituto Politécnico de Beja
IX	2009	Angra do Heroísmo 29 abril a 2 maio	Qualidade e a segurança alimentar	Célia C. G. Silva – Universidade dos Açores
X	2011	Braga 3 a 6 de julho	Cem Anos de Química em Portugal	João Paulo André – Universidade do Minho
XI	2012	Bragança 16 a 19 setembro	Qualidade dos alimentos: novos desafios	Joana Amaral – Instituto Politécnico de Bragança
XII	2014	Lisboa 10 a 12 de setembro	Composição Química, Estrutura e Funcionalidade: a ponte entre alimentos novos e tradicionais.	Isabel Sousa e Anabela Raymundo - ISA/ULisboa
XIII	2016	Porto 14 a 16 de setembro	Disponibilidade, valorização e inovação: uma abordagem multidimensional dos alimentos	Beatriz Oliveira, Victor Freitas e Ada Rocha – FFUP e FCNAUP
XIV	2018	Viana do Castelo 6 a 9 de novembro	Indústria, Ciência, Formação e Inovação	M. Rui Alves e Manuela Vaz Velho – Instituto Politécnico de Viana do Castelo
XV	2021	Madeira, Funchal 5 a 8 de setembro	Estratégias para a Excelência, Autenticidade, Segurança e Sustentabilidade Alimentar	José Câmara – Universidade da Madeira
XVI	2022	Castelo Branco 23 a 26 de outubro	Bio-sustentabilidade e Bio-segurança alimentar, Inovação e qualidade alimentar	Ofélia Anjos – Instituto Politécnico de Castelo Branco

Livro de Resumos

XVI Encontro de Química dos Alimentos

Ficha Técnica

Título

Livro de Resumos do XVI Encontro de Química dos Alimentos - Bio-Sustentabilidade e Bio-Segurança Alimentar, Inovação e Qualidade Alimentar

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Sociedade Portuguesa de Química

Esta publicação reúne os trabalhos apresentados no XVI Encontro de Química dos Alimentos: Bio-sustentabilidade e Bio-segurança alimentar, Inovação e qualidade alimentar, Castelo Branco 2022, e inclui ainda o programa científico do encontro.

As doutrinas expressas em cada um dos resumos são da inteira responsabilidade dos autores.

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Nota de Abertura

É com o maior prazer que dou as boas-vindas a todos os participantes no XVI EQA estando convicta que serão 4 dias de trabalhos intensos com excelentes comunicações, mas também, associado de um programa social para que se possa continuar a debater ideias e parcerias de trabalhos futuros ou para que apenas possamos descontrair um pouco.
Agradeço a todos o enorme interesse e participação neste evento que se traduz em cerca 320 inscrições e 330 resumos submetidos o que demonstra, não apenas o grande interesse nacional na temática, como também a expressiva comunidade científica que todos os dias trabalha arduamente em prol do desenvolvimento do sector agroalimentar nacional.
A todos os membros da Comissão Científica agradeço a Vossa disponibilidade e a preciosa colaboração principalmente nas sugestões dadas durante as nossas "Flash meeting", seleção dos resumos submetidos para as comunicações orais e amizade, pois quando trabalhamos entre profissionais e amigos o trabalho flui mais facilmente.
A tarefe que me incumbiram em setembro de 2021, XV EQA na Madeira, não foi fácil, por termos apenas um ano para organizar o evento, e por ser um período Pós-Pandemia em que com a retirada de grande parte das restrições anteriormente impostas, houve um grande número de eventos e outros trabalhos que se atrasaram devido às referidas restrições, e, consequentemente, todos tivemos de nos distribuir por inúmeras tarefas. Espero não vos desiludir, pois a responsabilidade da organização deste evento é grande devido à elevada qualidade e nível científico a que já nos habituaram ao longo das anteriores 15 edições do EQA.
Um Muito Obrigado ao Instituto Politécnico de Castelo Branco e Escola Superior Agrária do mesmo Instituto que me apoiaram desde o início neste desafio.
Um abraço forte e sentido a todos os colegas da Comissão Organizadora pela colaboração, suporte e simpatia. Tenho, no entanto, de fazer um agradecimento especial ao Carlos Antunes e à Soraia Inês Pedro, incansáveis desde o primeiro momento e sempre com uma palavra amiga e de ânimo quando eu começava a acusar o cansaço. A vossa entrega, trabalho, dedicação, espírito de equipa, colocando as vossas teses de Mestrado e Doutoramento em standby, foram insuperáveis e da minha parte nunca serão esquecidas.
Os eventos só são possíveis com a colaboração de todos e por isso um Bem-Haja enorme e todos os nossos patrocinadores que com o seu apoio garantiram que nos conseguíssemos juntar a partilhar e discutir ciência, para um bem nacional maior, que é o sector alimentar.
Neste contexto os nossos agradecimentos vão também para as entidades listadas de seguida.

1) Municípios da Região:
   - Camara Municipal de Castelo Branco, que desde o primeiro dia se envolveu em facultar tudo o necessário para o sucesso do Evento;
   - Comunidade Intermunicipal da Beira Baixa (constituída pelos municípios de Castelo Branco, Idanha-a-Nova, Oleiros, Penamacor, Proença-a-Nova e Vila Velha de Ródão);
   - Câmara da Sertã;
   - Câmara do Fundão.

2) Patrocinadores do XVI EQA:
   - Bruker Portugal, sponsor Diamante;
   - ILC, sponsors Ouro;
   - Isaza Scientific, Soquímica, Specanalítica, Waters, Avantor e Normax, Sponsor Prata;
   - Dias de Sousa e Schreiber Food, sponsor Bronze;

3) Outros apoios:
- Labor Spirit;
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- Meltagus;
- Focar Momentos;
- Farinha e Amaro;
- Herdade Tapada da Tojeira;
- Fio da Beira;
- Geocakes.

Tenho ainda de agradecer o excelente trabalho e apoio dado pelo Secretariado da SPQ, Dr. Leonardo Mendes e Dra. Cristina Campos dada a sua disponibilidade, eficiência e paciência com toda a gestão do site, registos e inscrições. Muito obrigado!
Tendo como temática central a Bio-sustentabilidade e Bio-segurança alimentar, Inovação e qualidade alimentar, onde serão apresentados e discutidos os seguintes temas: Química Analítica; Inovação e caracterização de novos produtos; Compostos bioactivos; Ómica na análise de alimentos; Autenticidade de alimentos; Segurança alimentar; Embalagens alimentares; Micotoxinas, microplásticos, alergénios; Resíduos de pesticidas e medicamentos e Quimiometria, o congresso constitui uma oportunidade única e privilegiada para que todos os intervenientes possam continuar a estreitar relações, estabelecerem novos contactos e parcerias, agregarem novos colaboradores nas suas equipas, catalisar a criação de redes entre os diferentes intervenientes com vista ao desenvolvimento de sinergias conducentes à excelência e sustentabilidade dos alimentos e do sector agroalimentar.

Tratando-se de um encontro da área alimentar é importante realçar que, infelizmente e historicamente, sempre aconteceu: Peste, Guerra e Fome, pelo que todos temos pela frente um grande desafio humanitário em que os alimentos desde a sua produção à tecnologia e desenvolvimento tecnológico no processamento alimentar tomarão um lugar fulcral.
Neste contexto toda a comunidade científica está empenhada em encontrar soluções para produzir mais alimentos de qualidade nutricional elevada, reduzir o desperdício com base no reaproveitamento e economia circular, otimização de processos de modo a garantira qualidade dos alimentos e aumentar o seu tempo de vida útil. Temos um logo caminho pela frente, mas conhecendo a excelência dos investigadores portugueses nesta temática, sei que temos esperança nas soluções vindouras.
Contamos ainda que esta estadia permita aos participantes a consciencialização da diversidade de experiências que a região tem para oferecer e que com esse conhecimento serão certamente atraídos a voltar mais tarde para uma estadia mais longa para um período de cooperação científica ou apenas lazer pessoal, fundamental nesta vida extremamente agitada que todos temos atualmente. É muitas vezes nestas pausas que nos surgem ideias fantásticas para a melhoria continua da nossa prestação e apoio à comunidade em Geral.

Um Bem-Haja a todos

Ofélia Anjos
(Chairperson do XVI EQA)

Índice

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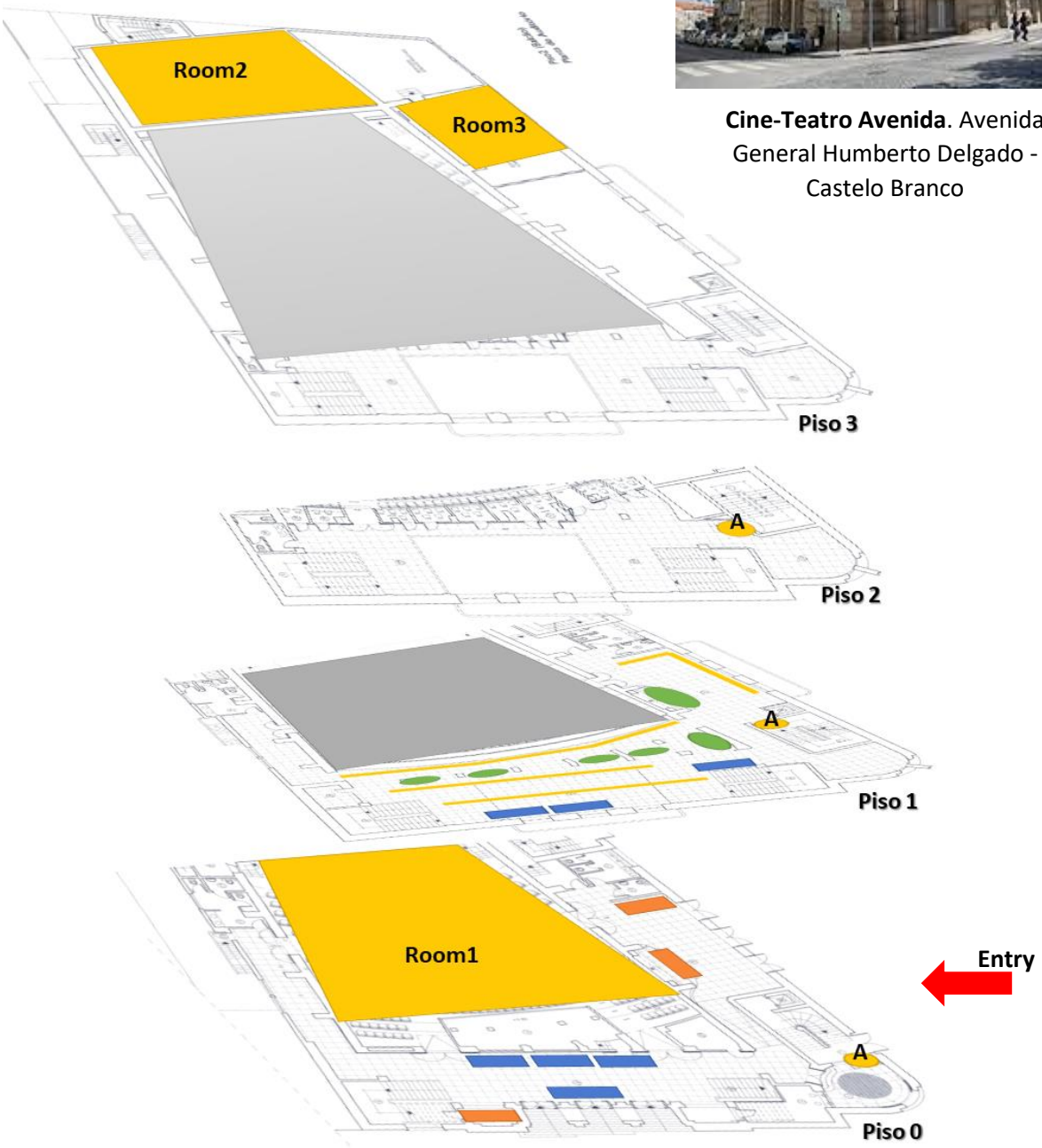

PROGRAM

Sunday – 23rd of October 2022

16:00 - 16:45	Registration
17:00 - 17:45	Open Session
17:45 - 18:30	**Plenary Lecture** The future of foods: foods for the future *Antonio Vicente*
18:30 - 18:50	**Keynote Lecture** Applications of non-destructive strategies for agri-food quality and safety monitoring: trends and challenges *Ana Soldado*
18:50 - 19:15	**Sponsor Lecture** One Step Ahead of Routine Analysis - True Mass Analytical Solutions for Wines *Rui Rocha, Bruker*
19:30 - 21:00	***Welcome drink***

Monday – 24th of October 2022

ROOM 1	**Chairperson -** *Manuel Coimbra*
09:00 - 09:45	**Plenary Lecture** How urgent is sustainability in food production – rethinking old practices *Isabel Sousa*
09:45 - 10:00	**OC** Monitoring Contaminants in Food: from Food Production to "One Health" approach *Andreia Freitas, Marta Leite, Sara Leston, Jorge Barbosa, Fernando Ramos*
10:00 - 10:15	**OC** Characterization of coagulase-negative *Staphylococci* as potential starter cultures to substitute the addition of nitrate in the production of meat products *Maria Pedro Teixeira, Maria José Fernandes, Maria Helena Fernandes, Patricia Bernardo, Mariana Camoez, Ons Bouchami, Maria Miragaia, Maria João Fraqueza*
10:15 - 10:30	**OC** *Byssochlamys nivea* ascospore germination and inactivation by hyperbaric storage – dependence of thermal and nonthermal pre-activation steps *Carlos A. Pinto, Alireza M. Ganjeh, Maria Holovicova, Miroslav Habán, Marta Habanova, Jorge A. Saraiva*
10:30 - 10:45	**OC** Determination and human health risk assessment of mercury in fish samples *Caio Silva Assis Félix, João Batista Pereira Júnior, Jucelino Balbino Da Silva Júnior, Allan S. Cruz, Kelly Graças Fernandes Dantas, Sérgio Luís Costa Ferreira*
10:45 - 11:35	**Coffee Break / Poster Session**

ROOM 1	**Chairperson -** *Lilian Barros*
11:35 - 12:00	**Sponsor Lecture** Perform food analysis with revolutionary 2400 GC - fast, sensitive, sustainable, connected *Luca Piatti, ILC*
12:00 - 12:15	**OC** Regulated, non-regulated and emerging multi-mycotoxins in raw milk: UHPLC-QTrap-MS/MS method validation for control of biosafety measures *Marta Leite, Andreia Freitas, Jorge Barbosa, Fernando Ramos*
12:15 - 12:30	**OC** Vibrational spectroscopy applied to *Arbutus unedo* fruit spirit characterization *Carlos Alberto Lopes Antunes, Ilda Caldeira, Soraia Inês Pedro, Sara Canas, Ofélia Anjos*
12:30 - 12:45	**OC** FT-Raman methodology applied to study the effect of seasoning time of Fino Sherry Casks® in Brandy de Jerez elaboration

	Guerrero-Chanivet, María, Anjos, Ofélia, García-Moreno; M. Valme, Valcárcel-Muñoz, Manuel J., Guillén-Sánchez, Dominico A.
12:45 - 14:15	**Lunch**
ROOM 1	**Chairperson -** *Vitor Freitas*
14:15 - 14:35	**Keynote Lecture** Food/Herb-Drug Interactions: What we should know *Maria Graça Campos*
14:35 - 14:55	**Keynote Lecture** DNA-based approaches for the authentication of complex foods *Joana Amaral*
14:55 - 15:20	**Sponsor Lecture** Food safety, quality and authenticity - Agilent solutions for food and beverage characterization and quality control assays *Rodrigo Tomaz, SOQUIMICA*
15:20 - 15:35	**OC** Effect of ultrasonic treatment on the physicochemical, bio-functional properties and digestibility of chayote *(Sechium edule (Jacq.) Swartz*) seed protein isolates *Elsa F. Vieira, Simão Silva, Suene Souza, Cristina Delerue-Matos*
15:35 - 15:50	**OC** Integrated Bioprocess for Structured Lipids, Functional Sugars, and Glucose Production using Olive Pomaces *Suzana Ferreira-Dias, Fátima Peres, Natália M. Osório, Isabel Miranda*
15:50 - 15:57	**FC** Multiple correspondence analysis of microbiological profiles as markers of authenticity of PDO cheeses *Rocha, Rui, Vaz Velho, Manuela, Pinto, Ricardo, Fernandes, Paulo, Santos, Joana*
15:57 - 16:04	**FC** Acylated and polyacylated anthocyanins as a pallet of natural colours *Ana Rita Pereira, Alexandra Borges, Victor de Freitas, Nuno Mateus, Fernando Pina, Joana Oliveira*
16:04 - 16:11	**FC** Preparation of a nutraceutical from prickly pear flower extracts: Determination of biological properties *Jacinta Ribeiro, Dulcineia F. Wessel, Susana M. Cardoso, Ricardo M. Ferreira*
16:11 - 16:55	**Coffee Break / Poster Session**
ROOM 1	**Chairperson -** *Isabel Coelhoso*
16:55 - 17:10	**OC** Brown macroalgae-rich extracts as potential food ingredients: a holistic extraction approach *Ana R. Circuncisão, Manuel A. Coimbra, Susana M. Cardoso*

17:10 - 17:25	**OC** Fruit pomace macromolecular antioxidants: from wastes towards innovative food applications *Ana Fernandes, Nobre C, Pinheiro A C, Iva Fernandes, Nuno Mateus, Victor de Freitas*
17:25 - 17:55	**EQA – Group meeting**
20:00 - 23:00	**Gala dinner**

Monday – 24th of October 2022

ROOM 2	**Chairperson -** *Manuela Pintado*
09:45 - 10:00	**OC** Red tomato vs. yellow tomato: which is healthier? A comparative study of nutritional and antioxidant traits of tomato farmer's varieties *Mikel Añibarro-Ortega, José Pinela, Lillian Barros, Ana Maria Carvalho, Isabel C. F. R. Ferreira*
10:00 - 10:15	**OC** Olive oils from the Douro valley: influence of the different sub-region on the quality and composition *Nuno Rodrigues, Kevin Silva, Susana Casal, José Alberto Pereira*
10:15 - 10:30	**OC** Nutritional characterization of gilthead seabream *(Sparus aurata)* fed with *Pelvetia canaliculata* supplemented diet: a biorefinery approach for seaweed biomass valorization *Carla Tecelão, Damiana Pires, Ricardo Passos, Beatriz do Carmo, Carolina F. Tchobanov, Sara Forte, Mariana Vaz, Madalena Antunes, Marta Neves, Teresa Baptista*
10:30 - 10:45	**OC** Effect of linseed (*Linum usitatissimum*) as an alternative to xanthan gum, over the physicochemical and sensory properties of pasta fortified with macro and microalgae *Daniela Almeida, Filipa R. Pinto, Maria Manuel Gil*
10:45 - 11:35	**Coffee Break / Poster Session**
ROOM 2	**Chairperson** - *Célia da Silva*
12:00 - 12:15	**OC** DNA-based techniques for the entomological authentication of honey: comparison of high-resolution melting (HRM) analysis and next generation sequencing (NGS) approaches *Mónica Honrado, Andreia Quaresma, Ana Rita Lopes, Dora Henriques, M. Alice Pinto, Joana Amaral*

12:15 - 12:30	**OC** Multielement analysis to trace authenticity using potential markers of PDO pears and PGI apples cake fillings *Ana M.S. Costa, Elisabete Coelho, Lina Carvalho, Eugénio Soares, Eduarda Pereira, Manuel A. Coimbra*
12:30 - 12:45	**OC** Detection and quantitation of added water in octopus using a rapid and non-destructive method based in Time Domain Reflectometry (TDR) *Bárbara Teixeira, Helena Vieira, Sandra Martins, Rogério Mendes*
12:45 - 14:15	**Lunch**
ROOM 2	**Chairperson -** Anabela Raimundo
15:20 - 15:35	**OC** Impact of origin on the nutritional evaluation of dark chocolates from Africa and America *António Panda, Nuno Alvarenga, Ana Partidário, Manuela Lageiro, Cristina Roseiro, João Lita da Silva, João Dias*
15:35 - 15:50	**OC** Nutritional and physicochemical analysis of quince from Cova da Beira region: similarities, differences and particularities *Guido R. Lopes, Ana Martins, Alexandra Camelo, Ana Rodrigues, Helena Beato, Luisa Paulo, Mafalda Resende, Mário Cristovão, Marlene Mota, Christophe Espírito Santo*
15:50 - 15:57	**FC** Study of the amino acids profile of *Coffea canephora* silverskin from different geographical origins *Susana Machado, M. Beatriz P. P. Oliveira, Jesus Simal-Gandara, Rita C. Alves*
15:57 - 16:06	**FC** Effect of heat treatment on the quality and composition of canned tuna coating liquid *Nuno Ferreiro, Karina Duarte, Conceição Fernandes, José Alberto Pereira, Nuno Rodrigues*
16:04 - 16:11	**FC** Effect of moisture on the characteristics of hazelnut kernel during storage *Paula M. R Correia, Ana Filipe, Ana Cristina Ferrão, Elsa Ramalhosa, Raquel Guiné*
16:11 - 16:55	**Coffee Break / Poster Session**
ROOM 2	**Chairperson -** *Ilda Caldeira*
16:55 - 17:10	**OC** Chia *(Salvia hispanica* L.*)* whole flour: phenolic profile and authenticity *Walter Nei Lopes dos Santos, Bárbara Elizabeth Alves de Magalhães*

17:10 - 17:25	**OC** Antineurodegenerative and antioxidant properties of bioactive compounds extracted from olive seeds of three cultivars by ultrasound-assisted extraction *Irene Gouvinhas, Juliana Garcia, Daniel Granato, Ana Barros*

Monday – 24th of October 2022

ROOM 3	**Chairperson -** *M. Manuela Vaz Velho*
09:45 - 10:00	**OC** Is it possible to prepare coffee infusions resembling espresso coffee brews? The role of carbohydrates *Guido R. Lopes, Cláudia P. Passos, Manuel A. Coimbra*
10:00 - 10:15	**OC** Study of odorant compounds and sensory changes associated with wine spirit ageing using chestnut wood and Limousin oak under different technologies *Ilda Caldeira, Ofélia Anjos, Cláudia Vitória, Tiago A. Fernandes, Sofia Catarino, Sara Canas*
10:15 - 10:30	**OC** Colorimetric labels based on pyranoflavylium-cellulose acetate films for food intelligent packaging *Vânia Gomes, Ana Sofia Pires, Nuno Mateus, Victor de Freitas, Luís Cruz*
10:30 - 10:45	**OC** Inactivation of *Escherichia coli* and *Listeria monocytogenes* in raw goat milk by pulsed electric fields and mild heating *Alexandre Romão, M. Rui Alves, Alberta Araújo, Carla Barbosa, Paulo Fernandes*
10:45 - 11:35	**Coffee Break / Poster Session**
ROOM 3	**Chairperson -** *Silvina Palma*
12:00 - 12:15	**OC** Cowpea immature pod purée: an innovative functional food product for elderly *Catarina Passão, Alfredo Aires, Miguel Rodrigues, Luís Ferreira, Irene Gouvinhas, Ana Barros*
12:15 - 12:30	**OC** *Chritmum maritimum* L. as natural preservative: in vitro antioxidant activity assessment, phytochemical characterization and nutritional profile *Sónia Pedreiro, Maria Lopes, Artur Figueirinha, Olga Cardoso, Carlos Cavaleiro, Fernando Ramos*
12:30 - 12:45	**OC** New insights into phenolic compounds-proteins complexes as natural emulsifiers in mayonnaise models *Ana Catarina Ribeiro, Rosa Perez-Gregorio, Susana Soares, Nuno Mateus, Victor Freitas*

12:45 - 14:15	**Lunch**
ROOM 3	**Chairperson -** *Beatriz Oliveira*
15:20 - 15:35	**OC** Pumpkin by-products as a source of preservative compounds for food application: valorization of industrial bioresidues towards a sustainable system *Maria Gabriela Leichtweis, Adriana K. Molina, Carla Pereira, Tânia C.S.P. Pires, Ricardo Calhelha, Maria Beatriz Oliveira, Isabel C.F.R. Ferreira, Lillian Barros*
15:35 - 15:50	**OC** Exploring the effects of *Cynara cardunculus* L. besides milk clotting: antioxidant properties *Cássia H. Barbosa, Mariana A. Andrade, Fernanda Vilarinho, Ana Luísa Fernando, Ana Sanches Silva*
15:50 - 15:57	**FC** Fruit and vegetable pomaces from Juice Industry as a source of bioactive compounds *Saeed Salari, Joana Ferreira, Ana Lima, Isabel Sousa*
15:57 - 16:04	**FC** Evaluation of antioxidant capacity and phenolic composition of muffins fortified with grape pomace from the Douro region *Rui Dias Costa, Irene Gouvinhas, Ana Barros*
16:04 - 16:11	**FC** Virtual screening of medicinal compounds present in mushrooms as potential tyrosinase inhibitors *Carlos S. H. Shiraishi, Miguel A. Prieto, Lillian Barros, Rui M.V. Abreu*
16:11 - 16:55	**Coffee Break / Poster Session**
ROOM 3	**Chairperson -** *Fernando Ramos*
16:55 - 17:10	**OC** On the quest for low calorie carbohydrate-based sweeteners *Pedro A. R. Fernandes, Bruna L. Antunes, Sónia S. Ferreira, Cláudia Nunes, Elisabete Coelho, Manuel A. Coimbra*
17:10 - 17:25	**OC** Valorisation of halophyte plants produced in Portugal: from nutritional value to bioactivity *Elsa Mecha, Juliana Oliveira, Sheila Oliveira-Alves, Fábio Andrade, João Sousa, Andreia B. Silva, Maria R. Bronze, Ana Teresa Serra*

Tuesday – 25th of October 2022

ROOM 1	**Chairperson -** *Suzana F Dias*
09:00 - 09:45	**Plenary Lecture** Unveiling the chemical nature of food aromas: in the path of the multi-sensoriality *Silvia Rocha*
09:45 - 10:00	**OC** Effect of pasteurisation on bioactive compounds of human milk *María Luisa Fernández-Sánchez, Sara Escudero Cernuda, Karelmar López Benítez, Ana Belen Soldado Cabezuelo, María Teresa Fernández Fernández-Arguelles, Belen Fernández Colomer*
10:00 - 10:15	**OC** Chemical composition and biological activity of different residues obtained from the wine industry *Cristina N. Duarte, Maria Inês Dias, Sandrina A. Heleno, Lillian Barros, Celestino Santos-Buelga, Rolando C. S. Dias, Joana S. Amaral*
10:15 - 10:30	**OC** Olive phenols stability and selective extraction steps value in olive leaves *Andreia F.R. Silva, Manuel A. Coimbra, Artur M.S. Silva, Susana M. Cardoso*
10:30 - 10:45	**OC** Subcritical Water Extraction of chestnut shells: A promising source of bioactive compounds *Ana Sofia Ferreira, Diana Pinto, Ana Margarida Silva, Jaroslava Švarc-Gajić, Paulo Costa, Cristina Delerue-Matos, Francisca Rodrigues*
10:45 - 11:35	**Coffee Break / Poster Session**
ROOM 1	**Chairperson -** *Raquel Guiné*
11:35 - 12:00	**Sponsor Lecture** PFAS Analysis – Overcoming challenges to meet regulatory limits with a total solution *Alberto Méndez, Waters*
12:00 - 12:15	**OC** Obtaining nutritionally enriched emulsified alternative - mayonnaise with *Tenebrio molitor flour* *Maribel Aybar, Sara Simões, Joana Sales, Joel Santos, Diogo Castelo-Branco, Ana Tasso, Diogo Figueira, Anabela Raymundo*
12:15 - 12:30	**OC** Yeast polysaccharides as food ingredients for clean label dressings and sauces *Sofia F. Reis, Pedro Fernandes, Vítor J. Martins, Sara Gonçalves, Luís Ferreira, Vítor M. Gaspar, Manuel A. Coimbra, Elisabete Coelho*
12:30 - 12:45	**OC** Microbiological evaluation of vacuum-packed low sodium sliced cold-smoked rainbow trout *(Oncorhynchus mykiss)* stored under refrigeration

	Fraqueza MJ, Teixeira MP, Bernardo P, Fernandes MH, Fernandes MJ, Lira C, Alfaia C, Gonçalves A, Camacho C, Nunes ML
12:45 - 14:15	**Lunch**
ROOM 1	**Chairperson -** *Ana Barros*
14:15 - 14:35	**Keynote Lecture** Behind the scenes of agri-food waste - from the health benefits to potential applications *José Câmara*
14:35 - 15:00	**Sponsor Lecture** IR & FT-NIR Quality Control of Milk *Eva Monteiro, Bruker*
15:00 - 15:15	**OC** Optimized microwave-assisted extraction of fish oil from fish industry by-products *Matilde Rodrigues, Beatriz de la Fuente, Cristina Caleja, André Almeida, Maria Inês Dias, José Pinela, Lillian Barros*
15:15 - 15:22	**FC** Post-harvest conservation of chestnut *(*cv. Martaínha*)*, comparison of two controlled atmospheres during 60 days *Mário Cristóvão, Alexandra Camelo, Ana Martins, Ana Resende, Ana Riscado, Ana Rodrigues, Ana Silveira, Cátia Batista, Guido Lopes, Helena Beato, Luísa Paulo, Okta Pringga, Rita Ramos, Christophe Espírito Santo*
15:22 - 15:29	**FC** Bioactivity, rheology, texture and chemical characterization of Halloumi cheese fortified with *Chlorella vulgaris* biomass *Hector Hernandez, Catarina Prista, Maria Cristiana Nunes, Anabela Raymundo*
15:29 - 15:36	**FC** Biobased polymers of chitosan incorporated with *Aloysia citrodora* and *Cymbopogon citratus* essential oils for packaging fresh poultry meat *Sofia J. Silva, João R.A. Pires, Carolina Rodrigues, Ana Luisa Fernando, Arlindo Gomes, Lúcia Silva*
15:36 - 15:43	**FC** Microalgae as natural colorants in pastry products *Tatiana Pereira, Sónia Barroso, Maria M. Gil*
15:43 – 15:50	**FC** Influence of phytochemical composition and biological activity of Portuguese honeys from different botanical sources and geographical origins *Soraia Santos, Miguel Maia, Irene Gouvinhas, Ana Barros*
15:50 - 16:15	**Coffee Break / Poster Session** **Social program**

Tuesday – 25th of October 2022

ROOM 2	**Chairperson -** *Rui Alves*
09:45 - 10:00	**OC** Bioplastics based on chitosan and micro/nanocellulose loaded with sage essential oil for extending shelf-life of fresh poultry meat *João R.A. Pires, Raquel Pereira, Victor G.L. Souza, Helena Godinho, Ana Luísa Fernando*
10:00 - 10:15	**OC** 3D Priting of snacks containing *Tenebrio molitor* flour *Francisco Herdeiro, Maria Otilia Carvalho, Maria Cristiana Nunes, Anabela Raymundo*
10:15 - 10:30	**OC** Development of a clean label meat free alternative to deli ham *Beatriz Caetano, Capucine Godinot, Norton Komora, Adriana Ferreira, Anabela Raymundo, Isabel de Sousa*
10:30 - 10:45	**OC** Grass pea sweet miso as a clean label ingredient for innovative vegan emulsions *Sara Simões, Diogo Castelo-Branco, Diogo Figueira, Ana Tasso, Catarina Prista, Anabela Raymundo*
10:45 - 11:35	**Coffee Break / Poster Session**
ROOM 2	**Chairperson -** *António Mendonça*
12:00 - 12:15	**OC** Influence of the particle size and extraction process of pear pomace in their health-promoting properties *Ferreira J, Tkacz K, Turkiewicz I, Sousa I*
12:15 - 12:30	**OC** *Prunus lusitanica* L. fruits as a source of bioactive compounds *Ana Abrão, Nelson Fernandes, Amélia Silva, Raúl Domínguez Perles, Ana Barros*
12:30 - 12:45	**OC** WPI active edible coatings to prevent cheese color defects *A. R. Ferraz, O. Anjos, M. L. Serralheiro, C.M.B.S. Pintado*
12:45 - 14:15	**Lunch**
ROOM 2	**Chairperson -** Dulcineia Wessel
15:00 - 15:15	**OC** The mycoestrogen zearalenone and rice: occurrence and risk assessment *LJG Silva, I. Encarnação, A.M.P.TPereira, S.C.Duarte, A. Pena*

15:15 - 15:22	**FC** Luminescence Sensors based on nano-MOFs to detect biogenic amines *Candela Melendreras García, Pablo Álvarez García, Enrique Álvarez Rubiera, Elena Lastra Bengochea, Francisco Javier García Alonso, Francisco Ferrero, Adrián Vizcaíno, Juan Carlos Campo, Marta Valledor, Ana Soldado, José M. Costa Fernández*
15:22 - 15:29	**FC** Heavy metals and metalloids in shrimps from northwest Portuguese coast *Maria Luz Maia, Agostinho Almeida, Cristina Soares, Luís M. S. Silva, Cristina Delerue-Matos, Conceição Calhau, Valentina Fernandes Domingues*
15:29 - 15:36	**FC** Arsenic in Portuguese rice. Is there any risk? *A. Pereira, A. Silva, L. Silva, S. Duarte, A. Pena*
15:36 - 15:43	**FC** The effect of the drying process on the composition of two varieties of prickly pear (*Opuntia ficus indica*) *Gaudêncio Semedo, Carolina Rodrigues, Victor G.L. Souza, Ana Luísa Fernando*
15:40 - 16:15	**Coffee Break / Poster Session**
16:15 - 19:00	**Social Program**

Tuesday – 25th of October 2022

ROOM 3	**Chairperson -** *Carla Tecelão*
09:45 - 10:00	**OC** Nutritional and bioactive traits of Kweli® red raspberry cultivated in Portugal Matilde Rodrigues, Ana Luísa Vara, Jonava Petrovic, Maria Inês Dias, António Nogueira, Marina Sokovic, Isabel C.F.R. Ferreira, José Pinela, Lillian Barros
10:00 - 10:15	**OC** Development of low-fat vegan emulsions with the incorporation of citrus fiber Cláudia Maia, Sara Simões, Diogo Castelo-Branco, Diogo Figueira, Ana Tasso, Anabela Raymundo
10:15 - 10:30	**OC** In vitro and in vivo antioxidant activity of 3D snacks enriched with different microalgae species Sónia Oliveira, Alberto Niccolai, Liliana Rodolfi, Isabel Sousa, Anabela Raymundo
10:30 - 10:45	**OC** Chemical characterization and bioactive potential of coffee pulp, a by-product of coffee industry Marlene Machado, Liliana Espírito Santo, Susana Machado, Anabela Costa, Helena Ferreira, M. Beatriz P. P. Oliveira, Rita C. Alves

10:45 - 11:35	**Coffee Break / Poster Session**
ROOM 3	**Chairperson -** *Irene Gouvinhas*
12:00 - 12:15	**OC** Wheat-based canned products nutritional properties *Cláudia Novais, Carla Pereira, Leticia Álvarez Rodríguez, Margarita Barrero Antón, Isabel C.F.R. Ferreira, Lillian Barros*
12:15 - 12:30	**OC** Interactions between beer phenolic compounds and human salivary proteins: insights toward astringency and bitterness perception *Leonor Gonçalves, Mónica Jesus, Carlos Guerreiro, Elsa Brandão, Paulo Magalhães, Susana Soares, Nuno Mateus, Victor de Freitas*
12:30 - 12:45	**OC** Taste properties of Royal Gala apple fruit: a combination of molecular and sensory analysis *Mónica Jesus, Elsa Brandão, Nuno Mateus, Victor de Freitas, Susana Soares*
12:45 - 14:15	**Lunch**
ROOM 3	**Chairperson -** *Cristina Roseiro*
15:00 - 15:15	**OC** Melon byproducts as source of sustainable multifunctional foods ingredients *Ricardo Gómez-García, Cristobal N. Aguilar, Ana R. Madureira, Manuela Pintado*
15:15 - 15:22	**FC** Effects of gastrointestinal digestion on the anti-inflammatory properties of phlorotannins from *Himanthalia elongata* *Marcelo D. Catarino, Ana Rita Circuncisão, Catarina Marçal, Bruno Neves, Maria Teresa Cruz, Artur M. S. Silva, Susana M. Cardoso*
15:22 - 15:29	**FC** First identification and characterization of sugars in Borututu roots *"Cochlospermum angolense"* from Kwanza Norte (Angola) *Nsevolo Samba, Radhia Aitfella Lahlou, Mpanzu Nelo, Lúcia Silva, Jesus Miguel Lopez Rodilla*
15:29 - 15:36	**FC** Chemical characterization and antioxidant activity of wild mushroom extracts *Marco Nunes, Vanessa Silva, Gilberto Igrejas, Alfredo Aires, Rosa Carvalho, Lillian Barros, Patrícia Poeta*
15:36 - 15:43	**FC** Exploring the technological potential of *Salicornia ramosissima* as a mineral accumulator *Maria Lopes, Mário Roque, Carlos Cavaleiro, Fernando Ramos*
15:45 - 16:15	**Coffee Break / Poster Session**
16:15 - 19:00	**Social Program**

Wednesday – 26th of October 2022

ROOM 1	**Chairperson -** *Aida Moreira da Silva*
08:45 - 09:05	**Keynote Lecture** Collaborative ecosystems addressing priority themes for the Agrifood sector *Deolinda Silva*
09:05 - 09:30	**Sponsor Lecture** ICP-MS and GC-MS: an invaluable tool in food analysis *Carlos Sousa, João Cappelle, Luísa Moura, Specanalitica*
09:30 - 09:45	**OC** *Opuntia spp.* cladodes: Can this be a source of pectin for the food industry? *Carolina Rodrigues, Bilge Sayın Börekçi, Victor G. L. Souza, Isabel Coelhoso, Ana Luísa Fernando*
09:45 - 10:00	**OC** Moderate Pressure Pasteurisation at Room Temperature as a new quasi-energetically costless nonthermal preservation methodology – a case study on milk *Álvaro Tomaz de Lemos, Ivonne Delgadillo, Jorge Alexandre Saraiva*
10:00 - 10:15	**OC** Liquefaction Optimization of Peel of Potato *Solanum tuberosum L*. var Monalisa *Yuliya Dulyanska, Luísa Cruz-Lopes, Bruno Esteves, Raquel P.F. Guiné, José Vicente Ferreira, Idalina Domingos*
10:15 - 10:30	**OC** Study on the incorporation of seaweed in fresh cheese *Daniela Melo Borges, Susana Isabel Chaves Ribeiro, Célia Costa Gomes Silva*
10:30 - 10:45	**OC** Extending Shelf Life of Fresh Red Raspberry *(Rubus idaeus L.* cv. 'Kweli'*)* Using an Eco-friendly Antifungal Active Package *Tiago M. Vieira, Luísa Brito, Margarida Moldão-Martins, Vítor D. Alves*
10:45 - 10:52	**FC** Mayonnaise with table olive flours: development and characterization of an innovative product *Elsa Ramalhosa, Catarina Oliveira, José Alberto Pereira, Susana Casal, Nuno Rodrigues*
10:52 - 10:59	**FC** Ultrasound extraction to improve the sensory profile of microalgal biomass *Rachelle Riviere, Filipa Vinagre, Joana Ferreira, António Pagarete, Maria Cristiana Nunes, Anabela Raymundo*
10:59 - 11:06	**FC** Production of a sustainable, healthy and plant-based food under controlled conditions: Innovative miso *Rafaela Santos, Mariana Mota, Anabela Raymundo, Catarina Prista*

11:06 - 11:13	**FC** Raspberry fruit stabilization for its valuation in the development of muffins *Sílvia Petronilho, Diana Pimenta, Manuel A. Coimbra, Cláudia P. Passos*
11:13 - 11:45	**Coffee Break / Poster Session**
ROOM 1	**Chairperson -** *Christophe Espírito Santo*
11:45 - 12:00	**OC** Design of "Pera Rocha do Oeste" structured fruit with agar and locust bean gum: nutritional, antioxidant, textural and sensorial properties *Ana Luísa Leitão Correia, Elsa F. Vieira, Maria João Ramalhosa, Rui M. Alves, Carla Barbosa, Cristina Delerue-Matos*
12:00 - 12:15	**OC** Non-commonly used Edible Vegetable Substrates for Fermentation: An Alternative and Sustainable Source for Innovative and Healthy Food Products *Sofia Carapinha, Maria Ramos, Daniela Correia, Mayumi Delgado, Diogo Castelo Branco, Diogo Fernandes, Diogo Figueira, Anabela Raymundo, Catarina Prista*
12:15 - 12:30	**OC** Vegetable extracts as alternatives to nitrite in cured meat sausages *Patrícia Bernardo, Maria José Fernandes, Maria Helena Fernandes, Maria Pedro Teixeira, Luís Patarata, Maria João Fraqueza*
12:30 - 12:45	**OC** Evaluation of corncob as carbon source in the production of xanthan gum *Meirielly Jesus, Fernando Mata, Rejane Batista, Denise Santos Ruzene, Ricardo Albuquerque, Manuela Vaz-Velho, Francine Padilha, Daniel Pereira Silva*
12:45 - 13:00	**End Session**
13:00 - 14:15	**Lunch**
	Social Program

Wednesday – 26th of October 2022

ROOM 2	**Chairperson –** *Angelina Pena*
09:30 - 09:45	**OC** The phenolic profile for the discrimination of honeydew honey with origin in *Quercus pyrenaica oak* *Soraia I. Falcão, Rania Slama, Kheira Moufida Mouffok, Olga Escuredo, M. Shantal Rodriguez, M. Carmen Seijo, Miguel Vilas-Boas*
09:45 - 10:00	**OC** Chemical and bioactive characterization of *Euterpe oleracea Mart.* *Izamara de Oliveira, Márcio Carocho, Tiane Finimundy, Tânia Pires, Josiana Vaz, Celestino S. Buelga, Isabel C.F.R. Ferreira, Sandrina Alves Heleno, Lillian Barros*

10:00 - 10:15	**OC** A modified high-performance liquid chromatographic method for simultaneous quantification of skatole and androstenone in pig's backfat *Ricardo Pereira-Pinto, Mário Barros, Manuela Vaz-Velho, Fernando Mata, Preciosa Pires*
10:15 - 10:30	**OC** Chemical properties and microbiological quality of three hazelnut varieties cultivated in Portugal *Ana Cristina Ferrão, Raquel Guiné, Marco Silva, Paula Correia*
10:30 - 10:45	**OC** Study of the interaction between lysozyme and chlorogenic acid by fluorescence spectroscopy *Daiana Leithardt, Cândida Tomaz, António G. Mendonça*
10:45 - 10:52	**FC** Chemical characterization of almond varieties natives from Algarve region *Luís Carreira, Alcinda Neves, António Marreiros, Soukaïna El-Guendouz, Ângelo Luís, Fátima Peres, Soraia I. Pedro, Ofélia Anjos, Graça Miguel*
10:52 - 10:59	**FC** Application of edible coatings, supplemented with extracts of macroalgae and halophyte plants, in fillets of mackerel *(Scomber scombrus)* to reduce fat content in frying processes *Neves M, Freire C, Antunes M, Silva Susana, Tecelão C.*
10:59 - 11:06	**FC** Extraction of chlorophylls from the aerial parts of carrots *(Daucus Carota* L.*)* for the development of alternative natural colorants *Adriana Katherine Molina Vargas, Leonardo Correia Gomez, Carla Pereira, Miguel Ángel Prieto, Isabel C.F.R. Ferreira, Lillian Barros*
11:13 - 11:45	**Coffee Break / Poster Session**
ROOM 2	**Chairperson –** *Vitor Alves*
11:45 - 12:00	**OC** Valorization of strawberry, blueberry, and raspberry bioresidues for application in food industry *Leonardo Gomes, Carla Pereira, Maria Dias, Miguel Prieto, Isabel Ferreira, Lillian Barros*
12:00 - 12:15	**OC** Hydrolysable tannins in aged wine spirits: a fresh perspective using alternative ageing technology and high-resolution mass spectrometry *Tiago A. Fernandes, Alexandra M.M. Antunes, Oliveira Alves, S.C., Caldeira, I., Ofélia Anjos, Sofia Catarino, Sara Canas*
12:15 - 12:30	**OC** The unsung ingredients of salt pan waters: sulfated polysaccharides *Sónia S. Ferreira, Cláudia Nunes, Manuel A. Coimbra*

12:30 - 12:45	**OC** The dynamics of sustainability claims and certifications in new food products *Luís Rodrigues, João Ferreira, Bruno Henriques, Dalila Vieira*
12:45 - 13:00	**End Session**
13:00 - 14:15	**Lunch**
	Social Program

Wednesday – 26th of October 2022

ROOM 3	**Chairperson –** Elisabete Coelho
09:30 - 09:45	**OC** Novel type of *Camellia sinensis* green tea rich in polyphenols and L-theanine, a promotor of cognitive functions *Lisete Sousa Paiva, Elisabete Lima, Madalena Motta, José António Bettencourt Baptista*
09:45 - 10:00	**OC** Evaluation of microalgae enriched gluten-free bread as functional food *Marco António da Costa Freitas, Ferreira, J.P., Nunes M.C., Raymundo, A.*
10:00 - 10:15	**OC** Development of a Clean Label mayonnaise using fruit flour *Maria Vieira, Simões S., Castelo-Branco D., Figueira D., Tasso A., Raymundo A.*
10:15 - 10:30	**OC** Microalgae biomass as a relevant source of vitamin B12, in vegetarian and vegan diets *Albano Joel Moreira Santos, Isabel Sousa, Anabela Raymundo*
10:30 - 10:45	**OC** Crop rotation and irrigation regime affects the nutritional and chemical profile of *Cichorium spinosum* *Beatriz H. Paschoalinotto, M.A. Prieto, Compocholi M, N. Polyzos, S.A. Petropoulos, Isabel C.F.R. Ferreira, Maria Inês Dias, Lillian Barros*
10:45 - 10:52	**FC** Revalorization of *Prunus avium L.*: Determination of bioactive compounds *Erika N. Vega, Maria Inês Dias, Virginia Fernández Ruiz, Lillian Barros, Patricia Morales*
10:52 - 10:59	**FC** Study on the effect of the concentration and drying of microalgae on *Chlorella vulgaris* and *Arthrospira platensis* enriched pasta *Rafael Quinta, Helena Cardoso, Joana Silva, Patrícia Fradinho, Cristiana Nunes, Anabela Raymundo*

10:59 - 11:06	**FC** Hypocholesterolemic functional food based in polysaccharides: from structure to function *Filipe Coreta-Gomes, Maria João Moreno, Fernanda Machado, Cláudia Nunes, Manuel A. Coimbra*
11:13 - 11:45	**Coffee Break / Poster Session**
ROOM 3	**Chairperson -** *Elsa Ramalhosa*
11:45 - 11:52	**FC** Cheese prototypes enriched with microalgae: impact on structure, chemical composition, and sensory acceptance *Rita Falcão, Valentina Pinheiro, Cátia Ribeiro, Anabela Raymundo, Isabel Sousa, Maria Cristiana Nunes*
11:52 - 11:59	**FC** Obtention of lipid enriched extracts from microalgae for food applications *Jéssica Costa, Sónia Barroso, Tatiana Pereira, Clélia Afonso, Maria M. Gil*
11:59 - 12:06	**OC** The effects of dried halophyte as a salt substitute: a preliminary randomized study *Pereira T, Barroca MJ, Moreira da Silva A, Caseiro*
12:06 - 12:20	**FC** Chemical profile and nutraceutical features of cape gooseberry fruit and fruiting calyx *Mikel Añibarro-Ortega, Maria Inês Dias, Jovana Petrović, Marina Soković, Jesús Simal-Gándara, José Pinela, Lillian Barros*
12:45 - 13:00	**End Session**
13:00 - 14:15	**Lunch**
	Social Program

POSTERS PRESENTATIONS

First Group – 23rd/24th October

T2	Tema 2 - Inovação de Produtos e Tecnologias
PC01	**Salt content control of tuna loins during processing: coccion operation** Maria Cunha, M. Rui Alves, Carla Barbosa
PC02	**Structure and performance of polysaccharides extracted from brown seaweeds of occidental Portuguese coast. A pilot study** Meirielly Santos de Jesus, Fernando Mata, Mário Barros, Manuela Vaz-Velho, Preciosa Pires
PC03	**Integrated approaches for socio-economic boosting of the sustainable production and consumption of Montesinho mushrooms** Ana Saldanha, Maria Inês Dias, Leonardo Corrêa Gomes, José Pinela, Ângela Fernandes, Anabela Martins, Ana Maria Carvalho, Sílvia Nobre, Manuel A. Coimbra, Lillian Barros, Carla Pereira
PC04	**Bio-based hybrid molecules for coloring and preservative purposes** Cláudia Novais, A. K. Molina, Rui Abreu, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Carla Pereira, Lillian Barros
PC05	***Attalea speciosa* mesocarp flour in-depth characterization and its application for the development of new bakery products** Souza MVS, Saldanha A, Pereira C, Ivanov M, Sokovic M, Steinmacher NC, Dias MI, Barros L
PC06	**Microbiological evaluation of vacuum-packed low sodium sliced cold-smoked rainbow trout (*Oncorhynchus mykiss*) stored under refrigeration** Fraqueza MJ, Teixeira MP, Bernardo P, Fernandes MH, Fernandes MJ, Lira C, Alfaia C, Gonçalves A, Camacho C, Nunes ML
PC07	**Advantages and disadvantages of flavouring olive oils** Sandra Lamas, Nuno Rodrigues, António M. Peres, José Alberto Pereira
PC08	**Influence of winemaking on the quality of Vinhão wines** Iveta Rodrigues, Marta Macedo, Fernando Gonçalves
PC09	**Production of Isoamyl Butyrate by bioimprinting lipase-catalyzed esterification of isoamyl alcohol and butyric acid.** Cleide Mara Faria Soares, Amanda Beatriz Caversan Pereira, Jéssica Jéssi Carvalho de Melo, Josu Lopez Fernandez, Eliana Setsuko Kamimura, Suzana Ferreira Dias, Francisco Valero
PC10	**Molecular mechanism of lipase-mediated synthesis of flavoring compounds: The impact of enzyme active site hydrophobicity** Cleide Mara Faria Soares, Amanda Beatriz Caversan Pereira, Josu Lopez Fernandez, Eliana Setsuko Kamimura, Suzana Ferreira-Dias, Francisco Valero, Matheus Mendonça Pereira
PC11	**Bread Waste into Beer: Optimizing bread incorporation in beer production** Pedro Coelho, Catarina Prista, Isabel Sousa,

PC12 **Salt and sugar-reduced strategies: An approach to mustard formulation.**
Beatriz Ouro, Nelson Pereira, Catarina Luís, Ana Cristina Ramos, Diogo Figueira, Diogo Castelo-Branco, Ana Tasso, Margarida Moldão Martins, Marta Abreu, Elsa M Gonçalves

PC13 **Development of high-quality sauces with probiotic potential based on fermented green tomatoes**
Mahsa Farrokhi, Nelson Pereira, Manuela Vida, Paula Martins, Cláudia Correia, Ana Cristina Ramos, Elsa M Gonçalves, Margarida Vieira, Marta Abreu

PC14 **Assessment of alternatives to salt and sugar in healthy Ketchup formulations**
Catarina Luís, Nelson Pereira, Beatriz Ouro, Ana Cristina Ramos, Margarida Moldão Martins, Diogo Figueira, Diogo Castelo-Branco, Ana Tasso, Elsa M Gonçalves, Marta Abreu

PC15 **Development of Cobrançosa "functional olive oils" by co-processing techniques**
Madalena Marques, Suzana Ferreira-Dias, Cecília Gouveia, Conceição Vitorino, Miguel Mourato, Luisa L. Martins, Fátima Peres

PC16 **Bread development with partial replacement of wheat flour by sorghum flour (*Sorghum bicolor* (L.) Moench) germinated and *in natura***
Nadia Cristiane Steinmacher, Diogo Salvati, Eduardo Henriques Ledur, Beatriz H. Paschoalinotto, Maria Inês Dias, Carla Pereira, Lilian Barros

PC17 **Influence of Germination Process in Sorghum Grains (*Sorghum Bicolor* L. Moench) on the Starch Gel Technological Characteristics**
Nadia Cristiane Steinmacher, Eduardo Henrique Ledur, Diogo Salvati, Carolina Castilho Garcia, Glaucia Cristina Moreira, Maria Dias, Carla Pereira, Lillian Barros

PC18 **Effect of protein fortification and hydrocolloids coatings and on shelf life Sarrajão fillets (*Sarda sarda*)**
Joana Solinho, Rita Pinheiro

PC19 **How *Cynara cardunculus* ecotypes affect the production of Castelo Branco PDO cheese - a case study**
Mário Cristovão, Alexandra Camelo, Ana Martins, Ana Resende, Ana Riscado, Ana Silveira, Cátia Baptista, Guido Lopes, Helena Beato, Luísa Paulo, Luís Pinto de Andrade, Cristina Pinheiro, Paulo Barracosa, Christophe Espírito Santo

PC20 **Technological characteristics of pineapple jellies produced with psyllium**
Francielle Fernandes Felippe, Ígor Henrique de Mello Rodrigues Ciolin, Nádia Cristiane Steinmacher, Daiane Cristina Lenhard, Gláucia Cristina Moreira, Carolina Castilho Garcia

PC21 **Pressurized solvent extraction for the production of fish protein hydrolysates from industry by-products**
Bianca Marques, Tânia Ribeiro, Manuela Pintado, José A. Teixeira, Cristina M. R. Rocha

PC22 **Antioxidant activity of alginate edible films containing plant extracts**
Ana Isabel Lopes, Sara Silva, Lillian Barros, Cristina Caleja, Eliana Pereira, Freni Tavaria, Manuela Pintado

PC23 **VEGarum: Innovation of an ancestral gastronomic delicacy through the fermentation of Portuguese macroalgae**
Ana Catarina S. Costa, Gonçalo Soeiro, Rafaela Santos, Anabela Raymundo, Catarina Prista, Marisa Santos

PC24 **Biodegradable films produced with arabinoxylan extracted from corn fiber**
Verónica Weng, Vitor Alves, Isabel Coelhoso, Carla Brazinha

PC25 **Sequential extractions of red seaweed biomass: a cascading biorefinery approach to the recovery of multiple value-added products from *Gracilaria vermiculophylla***
Joana Gomes-Dias, José A Teixeira, Cristina Rocha

PC26 **Valorization of Acorn Starch and Polyphenols by Pulsed Electric Fields**
Luís M. G. Castro, Carlos A. Pinto, Sérgio C. Sousa, Elisabete M. C. Alexandre, Jorge A. Saraiva, Manuela Pintado

PC27 **Combination of hydrothermal treatments and enzymes for the valorization of chestnut shell residues**
Beatriz Gonçalves-Lima, Joana Gomes-Dias, Catarina Teixeira-Guedes, José A Teixeira, Cristina Rocha

PC28 **Linking abiotic stress treatments to shelf-life extension: fresh-cut carrot as a case study**
Carla Alegria, Elsa M. Gonçalves, Cristina Ramos, Marta Abreu

PC29 **Acorn Starch and Polyphenol Extraction by High Hydrostatic Pressure**
Luís M. G. Castro, Carlos A. Pinto, Sérgio C. Sousa, Elisabete M. C. Alexandre, Jorge A. Saraiva, Manuela Pintado

PC30 **Influence of the storage in bottle on the antioxidant activity of wine spirit aged by sustainable technology of micro-oxygenation with Limousin oak staves**
Sílvia Lourenço, Sheila Oliveira-Alves, Tiago A. Fernandes, Ofélia Anjos, Ilda Caldeira, Sofia Catarino, Sara Canas

PC31 **Ketchup processing under ohmic heating: effects on physical-chemical properties**
Gabriela Alves Moreira, Ricardo N. Pereira, Ana Tasso, Diogo Castelo-Branco, Diogo Figueira, José A. Teixeira, Cristina M. R. Rocha

PC32 **Ohmic heating as an innovative green technology for the boiling stage of the brewing process**
Gonçalo Carvalho, Ricardo Pereira

PC33 **Production of fibrillar protein aggregates under the effects of electric fields**
Rita Leal, Zita Avelar, Rui M. Rodrigues, Ricardo N Pereira

PC34 **Effects of ohmic heating on cellular morphology of *Chlorella vulgaris* – effects on proteins extraction**
Luís Machado, Maria Silva, Pedro Geada, José A. Teixeira, Ricardo N. Pereira

PC35 **Extraction bioactive molecules from *Coelastrella* sp. LFR1 biomass using ohmic heating**
Vitor Sousa, Luís Loureiro, Ricardo Pereira

PC36 **Whey-gelatine film combined with lactic acid bacteria to prevent cheese fungal contamination**
Sofia P.M. Silva, José António Teixeira, Célia C.C.G. Silva

PC37 **Macroalgae-based nanoparticles: current status and potentially emerging applications in the food industry**
P. Barciela-Alvarez, M. Carpena, A. Perez-Vazquez, L. Cassani, Jianbo Xiao, J. Simal-Gandara, M.A. Prieto

PC38 **Influence of pulp preparation in the sensorial, nutritional and antioxidant properties of a mixed "Pera Rocha do Oeste" and strawberry structured product**
Ana Luísa Leitão Correia, Elsa Vieira, Maria João Ramalhosa, Cristina Delerue-Matos

PC39 **Pressurized liquid extraction for the recovery of bioactive compounds from seaweeds for the food industry application**
A. Perez-Vazquez, M. Carpena, P. Barciela-Alvarez, L. Cassani, Hui Cao, J. Simal-Gandara, M.A. Prieto

PC40 **Dynamic sensory characterization of functional chocolate ice creams using Temporal Check-All-That-Apply (TCATA) methodologies**
Ricardo Isaías, Rui Costa Lima, Célia Rocha, Sandra Guimarães, Mahnoor Ayub, Miguel Cerqueira, António Vicente, Luís Miguel Cunha

PC41 **Effect of pulsed electric fields and mild heating combination on physicochemical properties of goat milk**
Alexandre Romão, Alberta Araújo, Verónica Solheiro, Paulo Fernandes, M. Rui Alves

PC42 **Sunflower oil enriched with bioactive compounds from Sea Fennel (*Crithmum maritimum* L.) flowers by ultrasound-assisted extraction**
Gabriela Sousa, Célian Pasquet, Carla Tecelão, Suzana Ferreira-Dias

PC43 **Sub-lethal pressure pre-treatments for subsequent shorter and improved egg yolk thermal pasteurization**
Ana C. Ribeiro, Susana Casal, José Lopes-da-Silva, Jorge A. Saraiva

PC44 **Influence of electrical stimulation on the final pH of bovine carcasses and evolution of cooling temperature in washed and unwashed carcasses**
Silvina Ferro Palma, Henrique Palma Gonçalves, Maria João Carvalho, Inês Fernandes

PC45 **Optimization and characterization of cultivation substrates for edible mushroom species – the *MicoCoating* initiative.**
Carla Miranda, Catarina Nunes, João Nunes

PC46 **Characterization of waste biomass generated in mushroom production and their potential for the extraction of bioactive compounds for food coatings**
Carla Miranda, Catarina Nunes, Carolina Nunes, João Nunes

PC47 **Innovative edible coatings to increase the shelf life of smoked sausages**
Sónia Ribeiro, Catarina Nunes, Diana Farinha, João Nunes

PC48 **Development of bioactive food products and ingredients using endogenous Portuguese agricultural resources for healthy nutrition**
Sónia Ribeiro, Catarina Nunes, Diana Farinha, João Nunes

PC49 **Natural food ingredients from quince peel: Towards a "zero-waste" production system**
Alexis Pereira, Mikel Añibarro-Ortega, António Nogueira, José Pinela, Lillian Barros

PC50 **Nutritional quality of mealworm (*Tenebrio molitor*) oil obtained by extrusion**
Adriana K. Molina, Beatriz Helena Paschoalinotto, Mikel Añibarro-Ortega, Carla Pereira, José Pinela, Vasco Teixeira Esteves, Maria Inês Dias, Lillian Barros

PC51 **Application of autochthonous lactic acid bacteria as starter cultures for ewes' milk cheese production**
Sandra Gomes, Nuno Alvarenga, Maria Paula Esteves, António Pedro Louro Martins, Helena Araújo-Rodrigues, José Soares, Manuela Pintado, Paulo Serol, Célia Lampreia, Maria João Carvalho, João Dias, Olga Amaral, Antónia Macedo, Miguel Floro, Manuela Costa, Maria Teresa Santos

PC52 **Microencapsulation of a phenolic-enriched fraction of *Gunnera tinctoria* with natural polymers: starch, pectin, and a starch/pectin complex**
Faezeh Fathi, Samad N. Ebrahimi, Alireza Fathi, Rita C. Alves, M. Beatriz P. P. Oliveira

PC53 **Evaluation of coagulation kinetics using cardoon flower extracts and rennet in sheep milk from different origins in the Alentejo region**
Gomes S, Pina I, Dias J, Caeiro J, Martins J, Alvarenga N, Martins APL

PC54 **Physical characterization and preservation studies of a clean label mayonnaise containing carrot powder**
Luisa Castro, Sara Simões, Joana Sales, Diogo Castelo Branco, Diogo Figueira, Ana Tasso, Vítor D. Alves, Margarida Moldão-Martins

PC56 **Brewer's spent yeast as an emulsifier for vegan and clean label sauces**
Sara Gonçalves, Pedro Fernandes, Sofia F. Reis, Vítor J. Martins, Manuel A. Coimbra, Elisabete Coelho

PC56 **Effect of different edible coatings on the preservation of whole apples**
Diana Melo Ferreira, Liliana Espírito Santo, Maria Beatriz Oliveira, Carla Barbosa

T3 Tema 3 - Compostos Bioativos

PC01 **Flavonoids profile by UPLC-MS/MS of taperebá (*Spondias mombin* L) fruit peel from Cerrado biome - Brazil**
Eliana Fortes Gris, Eduardo A Ferreira, Nilton T. V. Junqueira, Giovanna Oliveira de Brito1, Fulvio Mattivi, Urska Vrovsek

PC02 ***Anisophyllea boehmii* food potential: Chemical composition and antioxidant activity**
Alcides M.S. Lofa, Maria E. Romero, Maria D. Lopez, Ricardo Ferreira, Isabel Sousa

PC03 **Antioxidant, Anti-hypertensive and Anti- Alzheimer activities of *Porphyra* sp.: the effect of extraction time**
Maria Sapatinha, Ana Oliveira, Rogério Mendes, Narcisa Maria Bandarra, Carla Maria Feio Pires

PC04 **Phenolic compounds from sea buckthorn leaves modulate ROS generation and inflammation markers in human cells**
Daniel Granato, Amanda dos Santos Lima, Liciana Azevedo

PC05 **Evaluating phenolic compounds in ethanolic extracts of cherry pit**
Yuliya Dulyanska, Maria João Lima, Paula Correia, Manuela Ferreira, Anabela Fragata, Ana Paula Cardoso, Maria João Barroca, Aida Moreira da Silva, Luísa Cruz-Lopes, Bruno Esteves, José Vicente Ferreira, Idalina Domingos, Raquel Guiné

PC06 **Effect of harvesting time on the composition and biological activities of *Alaria esculenta***
Silvia Blanco, Maria Sapatinha, Ana Oliveira, Mick Mackey, Julie Maguire, Simona Paolaci, Rogerio Mendes, Narcisa Bandarra, Carla Pires

PC07 **The impact of extraction temperature and solution concentration on the antioxidant activity of sweet cherry seeds' extracts**
Yuliya Dulyanska, Margarida Cunha, Maria João Lima, Paula M. R. Correia, Manuela Ferreira, Anabela Fragata, Ana Paula Cardoso, Maria João Barroca, Aida Moreira da Silva, Luísa Cruz-Lopes, Bruno Esteves, José Vicente Ferreira, Idalina Domingos, Raquel P. F. Guiné

PC08 **Nutritional and chemical study of the fruits of *Rubus fruticosus* L. var. 'Triple Crown' as a food source with high antioxidant capacity**
A. K. Molina, Leonardo Corrêa Gomes, Carla Pereira, Maria Inês Dias, Miguel Ángel Prieto, Isabel C.F.R. Ferreira, Lillian Barros

PC09 **Bioaccessibility of phenolic compounds from white quinoa flour**
Walter Nei Lopes dos Santos, Barbara Elisabeth Alves de Magalhaes, Thais Luz de Souza, Aníbal de Freitas Santos Júnior

PC10 **Evaluation of the potential preservative capacity of pumpkin (*Cucurbita maxima Duchesne*) by-products**
Maria Gabriela Leichtweis, Adriana K. Molina, Carla Pereira, Tania C. S. P. Pires, Ricardo Calhelha, Neji Tarchoun, Maria Beatriz Oliveira, Isabel CFR Ferreira, Lillian Barros

PC11 **Dietary polyglycosylated anthocyanins, the smart option? Towards their stability and bioavailability**
Helder Oliveira, Catarina Roma-Rodrigues, Iva Fernandes, Alexandra Fernandes, Pedro Baptista, Victor de Freitas, Nuno Mateus

PC12 **Microwave Assisted Extraction of Pinus pinaster bark under different process conditions**
Diana Barros, Ana Cristina Duarte, Manuela Vaz-Velho

PC13 **Screening Methodologies to Extract Polyphenols from Olive Oil Pomace**
V. Martins, T. B. Ribeiro, M. Pintado, R.M.S.C. Morais, A.M.M.B. Morais

PC14 **Comparison of theaflavin-3,3'di-O-gallate content, as a 3CLPro SARS-CoV-2 inhibitor in different *Camellia sinensis* tea plantation zones**
José Baptista, Lisete Paiva

PC15 **The surplus value of bromelain as a potential therapeutical agent for COVID-19 and SARS-CoV-2 infectivity: Bromelain content in different parts of pineapple plant**
José Baptista, Sabrina Alves, Lisete Paiva

PC16 **Optimization extraction of natural antioxidants from Galega kale by-products using response surface methodology**
Solange Fernandes, Nelson Pereira, Manuela Lageiro, Ana Cristina Ramos, Vítor Alves, Marta Abreu, Elsa M. Gonçalves

PC17 **Influence of olive anthracnose and olive fruit fly on bioactive compounds of Cobrançosa olive oils**
Cecília Gouveia, Suzana Ferreira-Dias, Conceição Vitorino, Helena Oliveira, Fátima Peres

PC18 **Extraction of bioactive compounds from *Mastocarpus stellatus* using ultrasound and microwave-assisted extraction: a comparative study**
Maria Luz Maia, Elsa F. Vieira, Clara Grosso, Loic Hilliou, Cristina Delerue-Matos

PC19 **Chitosan/Alginate coating functionalized with essential oils: A bio-based proposal for meat preservation**
Jorge Miguel Magalhães Viera, António Augusto Martins de Oliveira Soares Vicente, Joana Teresa Rodrigues Martins, Cíntia Gomes Mendes, Ana de Mira Geraldo, Alfredo Manuel Franco Pereira, Mariana Monteiro Araújo de Lemos Gil

PC20 **Application of soaking and cooking waters as prebiotics**
Angela Daniela Carboni, Gonçalo Nuno Martins, Ayelén Amelia Hugo, Andrea Gómez-Zavaglia, Paula Cristina Castilho

PC21 **Assessing green tea catechins' effect on gluten-driven activation of intestinal CD4+ T cells from celiac disease patients**
Ricardo Dias, Serena Vitale, Ilaria Mottola, Nuno Mateus, Carmen Gianfrani, Victor Freitas

PC22 **Phenolic composition and antioxidant properties of Cowpea Portuguese Landraces**
Catia Nunes, Juliana Oliveira, Elsa Mecha, Ana T. Serra, Maria Manuela Veloso, Maria R. Bronze

PC23 **Solid lipid nanoparticles produced with beeswax as oral carriers of quercetin**
Andreia Mendes, Raquel F. S. Gonçalves, Luís Abrunhosa, António A. Vicente, Joana Teresa Rodrigues Martins

PC24 **LC-MS profiling and biological evaluation of methanolic extracts from local varieties of *Brassica species***
Carmo Serrano, M. Conceição Oliveira, Andreia Soares, Carla Pereira, Maria Inês Dias, Maria José Alves, Lillian Barros, Ana Dias, V. Rolim Lopes, Ana M. Barata

PC25 **Valorization of citrus by-products through the evaluation of their antioxidant capacity**
Ana Rita Soares Mateus, Angelina Pena, Silvia Barros, Raquel Sendón, Ana Sanches Silva

PC26 **Valorization of by-products: comparison of the antioxidant capacity of pistachio (*Pistacia vera* L.) and peanuts shells (*Arachis hypogaea* L.)**
Ana Rita Soares Mateus, Angelina Pena, Silvia Barros, Raquel Sendón, Ana Sanches Silva

PC27 **Implementation of an innovative methodology, FPSE, for extraction of free polyphenols from food matrices**
Patricia A. Nóbrega, Jorge A.M. Pereira, José S. Câmara

PC28 **Production of biodegradable edible coatings from algae polysaccharides**
Gabriela Sousa, Margarida Moldão-Martins, Carla Tecelão, Vítor Alves

PC29 **The effect of transition metals on coniferaldehyde oxidation in wine spirits model solutions**
Oliveira-Alves Sheila, Lourenço S, Fernandes TA, Anjos O, Caldeira I, Sofia Catarino, Sara Canas

PC30 **Valorization of a food industry orange waste as biostimulant plant growth: use of vibrational spectroscopy to early access their chemical composition**
Carmo Horta, Berta Riaño, Cláudia Vitória, María Cruz García-González, Ofélia Anjos

PC31 **Comparison of two HPLC methods with derivatization to assess γ-aminobutyric acid (GABA) contents in brown rice flours and rice bran**
Cristiana Pereira, Manuela Lageiro, Ana Castanho, Cristina Roseiro, Carla Brites

PC32 **Phenolic compounds as mycorrhization signaling molecules in micropropagated chestnut (*Castanea sativa* Mill.) seedlings**
José Pinela, Maria Inês Dias, Patrícia Ferreira, Maria de Fátima Oliveira, Anabela Martins, Andreia Afonso, Lillian Barros

PC33 **Evaluation of bioactive coatings in post-harvest physical and mechanical properties of cherries**
Joana Domingues, Henrique Soares, Fernanda Delgado

PC34 **Influence of the extracting solvent on the antioxidant activity and bioactive compounds of grape stems**
Ana F. Vinha, Liliana Espírito Santo, Carla Sousa, Anabela. S. G. Costa, M. Beatriz P. P. Oliveira

PC35 **Coffee by-products as sources of bioactive compounds: a comparative study**
Marlene Machado, Helena Ferreira, M. Beatriz P. P. Oliveira, Rita C. Alves

PC36 **By-products from seaweed production: protein content and amino acids profile**
Filipa B. Pimentel, Marlene Machado, Rita C. Alves, M. Beatriz P.P. Oliveira

PC37 **Development of highly effective growth strategies aiming at improving *Dunaliella salina* biomass production for food applications**
Vitor Sousa, António Vicente, Óscar Dias, Pedro Geada

PC38 **Comparison of roasted coffee and coffee silverskin extracts: effects on a malignant pancreatic cell line (AsPC-1)**
Nelson Andrade, Juliana A. Barreto Peixoto, Cláudia Silva, M. Beatriz P. P. Oliveira, Fátima Martel, Rita C. Alves

PC39 **Bacterial biofertilization improves production and modifies organic chemical composition of blueberry**
José David Flores-Félix, Ana C. Gonçalves, Sara Meirinho, Filipa Amaro, Cristina G. Viguera, Diego A. Moreno, Gilberto Alves, Paula Guedes de Pinho, Luís R. Silva

PC40 **Development of bold naturally colored ice creams using organic Spirulina**
Monize Bürck, Camilly Fratelli, Anna Rafaela Cavalcante Braga

T4 Tema 4 - Autenticidade e rastreabilidade dos alimentos

PC01 **Ethical Food Entrepreneurship (EFE) - Erasmus+ Project: Food for People – Planet – Profit**
Elsa Ramalhosa, Paula Whyte, Anna-Maria Saarela

PC02 **Botanical identification of honey from Natural Park of Montesinho: improving honey DNA extraction methodology**
Sónia Soares, Justina Mikučiūnaitė, Rui Sérgio Barbosa, Cristina Delerue-Matos

PC03 **Optimization of molecular methods for the identification of commercial strains of *Saccharomyces cerevisiae***
Nikol Parra, Mariangie Castillo, Manhaz Khadem, José S. Câmara

PC04 **Differentiation of Arabica coffee from distinct geographical origin based on integration of volatilomic profiles and chemometrics**
Carolina Andrade, José S. Câmara, Rosa Perestrelo

PC05 **Assessment of volatilomic fingerprint of apple ciders as a powerful tool to find putative geographical biomarkers**
Antonio Sousa, José Vereda, Sónia Medina, Regina Pereira, José S. Câmara, Rosa Perestrelo

PC06 **Discrimination of Italian saffron samples using µQUEChERS-dSPE/UHPLC-PDA and chemometric analysis of their bioactive composition**
Pedro Afonso Teles, Rosaria Cozzolino, José S. Câmara, Jorge A.M. Pereira

T7 Tema 7 - Quimiometria na ciência dos alimentos

PC01 **Critical study of moisture determination in fish samples using microwave radiation**
Sérgio Luis Costa Ferreira, Icaro Sacramento Almeida Porto, Saulo Vitor Araujo Dantas, Jucelino Balbino da Silva Junior, João Batista Pereira Junior

Second Group - 25/ 26 October

T1 Tema 1 - Química Alimentar: Estrutura, Composição e Qualidade Alimentar

PC01 **Changes of the physicochemical characteristics of aged wine spirits during the storage in bottle**
Sílvia Lourenço, Amélia Soares, Deolinda Mota, Ofélia Anjos, Ilda Caldeira, Sheila Oliveira-Alves, Sara Canas

PC02 **Fatty Acid composition of *Brassica rapa* landraces from Portugal**
Ana Carvalho Partidário, Cristina Serrano, Violeta Rolim Lopes, Ana Maria Barata

PC03 **Nutritional and chemical profile of grape pomace generated in red wine production**
Gomes, L.C., Dias, M.I., Pereira, C., Prieto, M. A., Ferreira, I.C.F.R, Barros, L.

PC04 **Quantification of mineral elements in leafy vegetables from Portuguese markets**
Laura Abreu, Luísa Louro, Miguel Mourato

PC05 **Variation of the chemical composition of red wine (Alicante Bouschet and Syrah) during the first eight months of maturation in new and reused oak wood barrels**
Carlos Antunes, Luísa Potêncio, Cristina Canavarro, Fátima Peres, Cecília Gouveia, António Ramos, Ofélia Anjos, Manuela Carmona, José Hipólito

PC06 **Grape composition during ripening, in two cultivars and different sites of "Beira Interior" region**
António Ramos, Fátima Valério, Cecília Gouveia, Carlos AL Antunes, Cristina Canavarro, Fátima Peres, Ofélia Anjos

PC07 ***Cynara cardunculus* L. flowers enzymatic profiles and ewe's cheese yield**
Ana Rita Fonseca, Cristina Conceição, Paulo Barracosa, Maria F. Duarte

PC08 **Interaction of Sodium Caseinate with Caffeic Acid by Fluorescence Spectroscopy Analysis**
Ana Martins, Daiana Leithardt, Cândida Tomaz, António G. Mendonça

PC09 **Quantification and characterization of polyphenol content in apple products**
José Carlos Teixeira, Susana Soares, Rosa Perez-Gregorio, Nuno Mateus, Victor Freitas

PC10 **Method validation for determination of amino acids in feedstuffs by HPLC**
Ana Teresa Gramacho, Maria Teresa Dentinho, Ana Teresa Belo

PC11 **Pulsed electric field technology as pretreatment to enhance strawberries (*Fragaria ananassa*) drying efficiency and physicochemical quality**
Enrique Pino Hernández, Patrícia Antunes, Marco Alves, Marta B. Evangelista

PC12 **Comparative evaluation of regional olive cultivars for potential transformation into table olives**
Ana Cristina Ramos, Elsa M Gonçalves, Marta Abreu, António Cordeiro

PC13 **Application of an electronic nose to differentiate extra virgin olive oils according to the geographical origin: Côa versus Douro regions**
Nuno Ferreiro, António M. Peres, Ana C. A. Veloso, José Alberto Pereira, Nuno Rodrigues

PC14 **Blend olive oils: mixing two monocultivar olive oils (Cobrançosa and Arbequina) for improving sensory and chemical characteristics of olive oils**
Ana Rodrigues, José Alberto Pereira, Nuno Rodrigues

PC15 **Endogenous Algarve pomegranate to improve nutritional benefits in the development of a pie**

Manuel Serra, Vera Margarida de Deus Nunes Gonçalves, Ezequiel António Marques Pinto, Natacha Alentejano, Ludovina Rodrigues Galego

PC16 **Sensory and chemical characteristic of white monovarietal wines produced from varieties more adapted to abiotic stress**
Rita Roque, Ilda Caldeira, Ofélia Anjos, Sílvia Lourenço, João Amaral, Miguel Damásio, Ricardo Egipto, José Silvestre

PC17 **Influence of pH on lipid oxidation, lipolysis and proteolysis susceptibility of pork dry cured sausage**
Luis Canhoto, Helena Gonçalves, Cristina Serrano, Carlos Santos, Cristina Roseiro

PC18 **Vitamin A and vitamin D contents of cow's milk and plant-based milk alternatives: preliminary comparison**
I. C. Antunes, R. Bexiga, C. Pinto, D. Gregório, H. Gonçalves, C. Santos, L. C. Roseiro, M. A. G. Quaresma

PC19 **Olfactometric approach to screen the odorant compounds profile in red monovarietal wines produced from varieties more adapted to abiotic stress**
Francesco Agostinelli, Ilda Caldeira, Patrícia Martins, Sun Baoshan, Miguel Damásio, Ricardo Egipto, José Silvestre

PC20 **Rheological properties of cranberry suspensions as affected by temperature and solid concentration**
Ígor Henrique de Mello Rodrigues Ciolin, Daiane Cristina Lenhard, Nádia Cristiane Steinmacher, Gláucia Cristina Moreira, Carolina Castilho Garcia

PC21 **Olive oils quality from the Trás-os-Montes region: comparative analysis of the last three years**
Sandra Lamas, Nuno Rodrigues

PC22 **Quality of tomatoes produced in a hydroponic system with nutritive solution of pre-treated agro-industrial wastewater**
Alexandra Afonso, Pedro Correia, Tânia Correia, Mariana Regato, Maria João Carvalho, Idália Costa, Fátima Carvalho, Ana Lopes, Adelaide Almeida, Carlos Ribeiro

PC23 **A look upon the adsorption of different families of polyphenols to salivary oral models: understanding the secondary mechanisms in astringency**
Carlos Guerreiro, Elsa Brandão, Mónica de Jesus, Leonor Gonçalves, Nuno Mateus, Victor de Freitas, Susana Soares

PC24 **PDO Serpa cheese volatiles and descriptive analysis**
Antónia Macedo, Maria João Carvalho, Paulo Henrique Silva, António Ferreira, Maria do Rosário Bronze

PC25 **Contribution to the chemical characterization of Algarve grapes and wines native's varieties "Negra Mole" and "Crato Branco"**
Joana Marcos, Vera Francisco, Manuel Serra, Ludovina Galego, Carlos Afonso

PC26 **Atlantic Yellowfin tuna loin protein content and quality**
Beatriz Resende, Leonor Nunes, Breixo Ventoso, Carlos Santos, Cristina Roseiro, Mário Quaresma

PC27 **The Atlantic yellowfin tuna (*Thunnus albacares*) fatty acid signature**
Valentina Domingues, Sara Sousa, Monica Rosas, Breixo Ventoso, Maria Leonor Nunes, Cristina Delerue-Matos, Mário Alexandre Gonçalves Quaresma

PC28 **Valorisation of endogenous wild fruits in Alto Minho region, Northern of Portugal: Centesimal composition and fatty acids profile of *Myrtus communis* L.**
Jéssica Domingues, Susana Casal, Ana Patrícia Guedes, Márcio Meira, Nuno Vieira e Brito, Ana Paula Vale, Isabel Afonso

PC29 **Characterization and Valorisation of endogenous wild berries in Northern Portugal: Centesimal composition and fatty acids profile of Tramazeira (*Sorbus aucuparia* L.)**
Márcio Meira, Ana Guedes, Susana Casal, Jéssica Domingues, Ana Paula Vale, Nuno Vieira e Brito, Isabel Afonso

PC30 **Physicochemical composition of eggs from Portuguese Autochthonous Poultry Breeds**
Márcio Meira, Isabel Afonso, Júlio Cesar Lopes, Ana Paula Vale, Jéssica Domingues, Virgínia Ribeiro, Rui Dantas, José Leite, Nuno Vieira e Brito

PC31 **Analysis of biometric parameters of the pine cone and pine nuts of *Pinus pinea* L. from Viseu Dão Lafões region**
Neves C.M.B, Macena M.W., Costa D.V.T.A., Pereira C.A.C., Wessel D.F

PC32 **Hydroponic systems for lettuce cultivation using treated olive mill wastewater**
Margarida Oliveira, Alexandra Afonso, Adelaide Almeida, Maria João Carvalho, Ana Lopes, Fátima Carvalho

PC33 **Valorization of waste and by-products from wine industry: chemical composition of grape skin, seed and stalk of *loureiro* and *espadeiro* varieties from the vinho verde region**Jéssica Domingues, Ana Cristina Rodrigues, Ana Isabel Ferraz, Maria Isabel Valin Sanjiao, Susana Mendes, Ana Paula Vale, Isabel Afonso

PC34 **Influence of the type of cocoa beans on phase transition, rheology, and fat bloom formation of dark chocolates**
José Cartas, Nuno Alvarenga, Ana Partidário, Manuela Lageiro, Cristina Roseiro, Maria João Carvalho, António Melgão, Catarina Melgão, Carlos Ribeiro, Pedro Prazeres, João Dias

PC35 **Organic acids profile of water kefir beverages produced from pineapple waste extract**
Jéssica Menezes, Natália Hata, Jéssica Ressutte, Ofélia Anjos, Wilma Spinosa

PC36 **Nutritional composition of omnivorous and carnivorous wild freshwater fish from Tagus River basin**
Paulo, L., Carmo, V., Beato, H., Pitacas, I., Rodrigues, A.M.

PC37 **Influence of the preservation method on the nutritional profile, antioxidant activity and free sugar content of coffee pulp**
Anabela S. G. Costa, Clàudia S. Pujol, M. Beatriz P. P. Oliveira, Jesus Simal-Gandara, Rita C. Alves

PC38 **Study of the amino acids profile of *Coffea canephora* silverskin from different geographical origins**
Susana Machado, M. Beatriz P. P. Oliveira, Jesus Simal-Gandara, Rita C. Alves

PC39 **Influence of organic vs. conventional production on the nutritional profile and antioxidant activity of roasted coffee**
Anabela S. G. Costa, Nina Barczyk, M. Beatriz P. P. Oliveira, Jesus Simal-Gandara, Rita C. Alves

PC40 **Comparison of the amino acid profile in young and adult of wild boar male (*Sus scrofa*) meat**
Susana Machado, Thiago F. Soares, Josman D. Palmeira, Rita T. Torres, M. Beatriz P. P. Oliveira, Jesus Simal-Gandara, Rita C. Alves

PC41 **Nutritional profile and mineral content of *Sonchus asper*: a Wild Edible Plant from the Mediterranean area**
Beatriz Helena Paschoalinotto, Miguel Angel Prieto Lage, Maria Compocholi, Nikolas Polyzos, Spyridon A. Petropoulos, Isabel C.F.R. Ferreira, Maria Inês Dias, Lillian Barros

PC42 **Effects of the fertilization system on the chemical profile of *Ribes rubrum* L.**
Luís Palmeira, Adriana K. Molina, Carla Pereira, Maria Inês Dias, Isabel C.F.R. Ferreira, Lillian Barros

PC43 **"Pingo de Mel" fig as a rich source of phytochemicals with antioxidant and antimicrobial properties**
Luís Palmeira, Adriana K. Molina, Carla Pereira, Tânia C.S.P. Pires, Maria Inês Dias, Isabel C.F.R. Ferreira, Lillian Barros

PC44 **Nutritional assessment of fresh, salted and soaked European catfish**
Galego, P., Paulo, L., Cristóvão, M., Beato, H., Vasconcelos, V., Barata, L., Rodrigues, A. M.

PC45 **Additive, synergistic, and antagonistic effects of a mixture of fruit and vegetables**
Suzylena Levy, Mafalda Alexandra Silva, Helena S. Costa, Maria Eduardo Figueira, Tânia Gonçalves Albuquerque

PC46 **Vitamin E profile of agri-food by-products: biological relevance and potential applications**
Diana Melo Ferreira, Rita Carneiro Alves, Maria Antónia Nunes, Joana Correia Lobo, Maria Beatriz Oliveira

PC47 **Green coffee from organic and conventional production: chlorogenic acids profile and safety assessment regarding pesticides and mycotoxins**
Rita C. Alves, Susana Machado, Marlos E. Z. Fontana, Ingrid D. dos Santos, Anabela S. G. Costa, Joana C. Lobo, Ionara R. Pizzutti, M. Beatriz P. P. Oliveira

PC48 **Chemistry characterization of Scottish grain and malt spirits aged in Sherry Casks® and Brandy casks**
Butrón-Benítez, Daniel, Valcárcel-Muñoz, Manuel J., Guerrero-Chanivet, María, García-Moreno, M. Valme, Guillén-Sánchez, Dominico, A.

PC49 **Diaion™ HP20LX resin to improve antitumoral activity of *Gunnera tinctoria* extracts in a pancreatic cell line**
Faezeh Fathi, Anabela S. G. Costa, Nelson Andrade, Cláudia Silva, Fátima Martel, Rita C. Alves, M. Beatriz P. P. Oliveira

PC50 **Determination of arsenic, cadmium, and lead in shellfish samples collected in Todos os Santos Bay - São Francisco do Conde City**
Jucelino B. Silva Junior, Ícaro S.A. Porto, Rui J.L. Garcia, Jorge M.P. Gomes, Saulo V.A. Dantas, Antônio F.S. Queiroz, Sérgio L. C. Ferreira

PC51 ***Talinum paniculatum* (Jacq.) Gaertn: evaluation of antioxidant and antimicrobial potential**
Caroline Toigo Marcon, Daniela Cristina Elsenbach, Daneysa Lahis Kalchne, Marines Paula Corso, Lillian Barros, Joana S. Amaral, Cristiane Canan

PC52 **Thermal stability of organic Spirulina (*Arthrospira platensis*) biomass**
Camilly Fratelli, Monize Bürck, Anna Rafaela Cavalcante Braga

PC53 **Nutritional and chemical characterization of ivy gourd (*Coccinia grandis* (L.) Voigt)**
Daniela Cristina Elsenbach, Rossana Cardoso, Ângela Fernandes, Cristiane Canan, Lillian Barros, Joana S. Amaral

PC54 **Techno-functional and physicochemical properties of lentil flour with different particle sizes**
Angela Daniela Carboni, Gonçalo Nuno Martins, Cristina Ferrero, María Cecilia Puppo, Paula Cristina Castilho

PC55 **Use of edible flowers as new potential antioxidant additives for food stability**
Cristiana Breda, Irene Gouvinhas, Berta Gonçalves, Ana Paula Silva, Ana Barros

PC56 **Soil fertilization practices as a tool for enhancing wines characteristics – assessment of the amino acid and volatile content of Aragonez wines.**
Pereira C, Mendes D, Martins N, Garcia R, Gomes da Silva M, Cabrita MJ

T5 Tema 5 - Segurança Alimentar

PC01 **Food and nutritional security in Brazil and the covid-19 pandemic: a literature review**
Vanessa Alves Ferreira, Thales Henrique de Brito

PC02 **The application of the one health concept in meat and meat products supply chain**
Joana Santos, Manuela Vaz Velho, Carla Ramos, Marta Vasconcelos

PC03 **Risk assessment of toxic metal contamination in the reuse of treated wastewater for urban vegetable irrigation**
Hugo Félix Silva, Ana Maria Barreiros, André Lima, José Coelho, Cristina Oliveira, Nelson Silva, Manuel Matos

PC04 **Host-guest system based on sulfonatocalixarene and pyranoflavylium dye for biogenic amine sensing during food spoilage**
Ana Sofia Pires, Nuno Basílio, Vânia Gomes, Nuno Mateus, Victor de Freitas, Luis Cruz

PC05 **Microbiological quality assessment of two portuguese PDO cheeses - Serra da Estrela and Azeitão**
Rui Rocha, Manuela Vaz Velho, Paulo Fernandes, Joana Santos

PC06 **Parasites and its Zoonotic Potential in Fishery Products from the Portuguese Markets**
Paula Ramos

PC07 **Impact of pH on food preservation by hyperbaric storage and potential of a novel food pasteurization process**
Vasco Lima, Carlos A. Pinto, Jorge A. Saraiva

PC08 **Validation of critical control points for non-thermally processed egg-based desserts**
Juliana Pinheiro, José M. Pestana, Manuela Guerra, Cátia Morgado, Eduardo Tondo, Carlos Brandão

PC09 **Identification of Critical Control Points of Ceviche production**
Luís Pequeneza, José M. Pestana, Manuela Guerra, Cátia Morgado, Eduardo Tondo, Carlos Brandão

PC10 **Multi-mycotoxins assessment: occurrence in infant and children food**
Soraia Sá, Sara Cunha, Miguel A. Faria, José de Oliveira Fernandes

PC11 **Assessment of six phthalates and one adipate in commercial beer samples**
Cheila Pereira, Sara Cunha, José de Oliveira Fernandes

PC12 **Levels of contaminant metals in three species of holothurians from Portugal: Seasonal, sex and tissues variations**
Sabrina S. Sales, Mercedes González-Wangüemertd, Maria F. Pessoa, Narcisa M. Bandarra, Helena M. Lourenço

PC13 **Bioaccumulation of heavy metals in the liver of marine fish species from different trophic levels caught off Portuguese Exclusive Economic Zone (EEZ)**
Miguel Mourato, Luisa Louro, Helena M. Lourenço, Susana Gonçalves, Paula Mota, M. Leonor Nunes, Mário Quaresma

PC14 **Contaminant metals in bivalve molluscs from Portugal**
Susana Gonçalves, Maria Fernanda Martins, Narcisa M. Bandarra, Helena M. Lourenço

PC15 **Uncertainty of chemical and microbiological quantitative methods: scenarios for conformity assessment of live bivalves**
Sónia Pedro, Susana M. Rodrigues, Helena M. Lourenço

PC16 **Water quality on small ruminants' dairy farms in Castelo Branco region**
Maria Conceição Mesquita, Maria Helena Martins, Maria Manuela Goulão, Renata Pedro, Ana Rita Martins, Inês João, Francisco Rodrigues, Cristina Santos Pintado

PC17 **Pilot survey of Nitrates, Nitrites, and histamine in raw Tuna in Sashimi**
Maria Monte, José Fraga, Sofia Duarte, Liliana Silva, André Pereira, Angelina Pena

PC18 **Determination of Aflatoxin M1 in milk and cheese from the Azores**
Nuno Rego, Sofia Duarte, Eduarda Silveira, Anabela Almeida, Liliana Silva, André Pereira, Angelina Pena

PC19 **Influence of wood type on polycyclic aromatic hydrocarbons profile in a Portuguese traditional fermented sausage**
Cristina Roseiro, Helena Gonçalves, Carlos Santos

PC20 **Production of egg white protein films for food packaging applications**
Ângelo Luís, Ana Ramos, Fernanda Domingues

PC21 **State of the art of non-conventional treatments for insect control in rice storage**
Inês Sousa, Jorge Oliveira, António Mexia, Graça Barros, Carla Brites

PC22 **Red-legged partridge (*Alectoris rufa*) hunting with lead projectiles results in lead contamination of meat portions, even after the removal of all visible lead projectiles**
Miguel Mourato, Luisa Louro, João Pinto, Mário Quaresma

PC23 **Evaluation of the impact of innovative and sustainable crystal glass bottles during accelerated storage of Port wine**
Cátia Martins, Silvia M. Rocha

PC24 **An Innovate and Rapid Separation Methodology Based on µSPEed/UHPLC-PDA for the Determination of Eight Pesticides in Water Samples**
Laura García-Cansino, María Ángeles García, María Luisa Marina, José S. Câmara, Jorge A. M. Pereira

T6 Tema 6 -Alimentos Funcionais

PC01 **Freeze-dried aqueous extract from sea buckthorn *(Hippophae rhamnoides)* leaves: phenolic composition and application in functional ice creams**
Daniel Granato

PC02 **Functionality assessment of *Scolymus hispanicus* (golden thistle) for its daily-basis incorporation in the Mediterranean diet**
Beatriz H. Paschoalinotto, M.A. Prieto, Compocholi M, Nikolaos Polyzos, S.A. Petropoulos, Isabel C.F.R. Ferreira, Maria Inês Dias, Lillian Barros

PC03 **Characterization of different fractions of fruit products obtained from the SUMOL+COMPAL pilot line to obtain Clean label fruit derivatives**
Madalena Leitão, Joel Santos, Maria João Alegria, Anabela Raymundo, Isabel Sousa

PC04 **Nutraceutical value of *Camellia sinensis* blend with aromatic medicinal plants: Comparison of antioxidant properties, phenolic and flavonoids contents**
Lisete Paiva, Madalena Motta, José Baptista

PC05 **Strategies for reducing the allergenicity of hen egg by treatment with phenolic compounds**
Alcides Vapor, Cândida T. Tomaz, António G. Mendonça

PC06 **Can avocado oil enriched fresh cheese modulate the obesity-related metabolism?**
Manuela Machado, Luís Alcalá, Ana Maria Gomes, Manuela Pintado

PC07 **Extrusion cooking effect on antioxidant activity and total phenolic content in novel gluten-free flours enriched with Fibersol and Passion fruit**

Erika N. Vega, Helayne A. Maieves, Jose de J. Berrios, Montaña Cámara, Virginia Fernández-Ruiz, Patricia Morales

PC08 **Biosorption studies of iodine by brewer`s spent yeast to be used as natural iodine-food-carrier**
Elsa F. Vieira, Andreia D. M. Silva, Sónia A. Figueiredo, Tiago Brandão, Cristina Delerue-Matos

PC09 **Continuous lipase-catalyzed synthesis of MLM-type structured lipids containing capric acid, in packed-bed bioreactors, using crude olive pomace oil as raw material**
Joana Souza-Gonçalves, Agnese Arrigo, Arsénio M. Fialho, Natália M. Osório, Suzana Ferreira-Dias

PC10 **Bioactive properties of *Sorbus aria* (L.) Crantz fruits: antioxidant and antimicrobial activities**
Tamayo Vives, Cristina, Pires T.C.S.P., Barros, Lillian, García Herrera, Patricia, Sánchez Mata, María de Cortes, Morales Gómez, Patricia

PC11 ***Crataegus monogyna* Jacq.: source of antioxidants for functional foods and antifungal activity for natural pesticides**
Tamayo Vives, Cristina, Pires, T-C.S.P., Barros, Lillian, García Herrera, Patricia, Sánchez Mata, María de Cortes, Morales Gómez, Patricia

PC12 **Influence of different cultivation conditions on the biomass growth and protein content of *Chlorella vulgaris***
Maria Silva, Luís Machado, Pedro Geada, José A. Teixeira, Ricardo N. Pereira

PC13 **Study of the antiproliferative and antitumour effects of chia (*Salvia hispanica* L.) seed**
Oliveira-Alves, Sheila, Carvalho J.E., Socca E.R.A., Favaro W.J., Cazarin C.B.B., Maróstica M.R., Serra A.T., Bronze M.R., Vendramini-Costa D.B., Prado, M. A.

PC14 **Phytochemical profile and physicochemical characteristics of Fundão sweet cherries**
Ana C. Gonçalves, Ana Sofia Oliveira, Cristina Garcia-Viguera, Diego A. Moreno, Gilberto Alves, Paula Guedes de Pinho, Luís R. Silva

PC15 **Olive oil-based spread functionalized with an olive pomace extract: antioxidant advantages**
Maria Antónia Nunes, Diana Melo Ferreira, Joana Correia Lobo, Susana Machado, Rita Carneiro Alves, Maria Beatriz Oliveira

PC16 **Cherry stem infusion: a potential beverage to restrain intestinal glucose uptake**
Juliana A. Barreto Peixoto, Nelson Andrade, Anabela S.G. Costa, Maria Beatriz Oliveira, Rita C. Alves, Fátima Martel

PC17 ***Cucumis melo* L. peel flour: Potential as food ingredient**
Mafalda Alexandra Silva, Tânia Gonçalves Albuquerque, Rita C. Alves, M. Beatriz P.P. Oliveira, Carla Motta, Helena S. Costa

PC18 **Olive pâté incorporating olive pomace: a sustainable and functional food product**
M. Manuela Sousa, Joana C. Lobo, Susana Machado, Rita C. Alves, Carla Barbosa, Carlos Pinto, Jorge A. Saraiva, M. Beatriz P.P. Oliveira

PC19 **Cladodes flour and coffee silverskin powder as ingredients for low-carbohydrate diets: is the use of these by-products feasible?**
Liliana Espírito Santo, Diana Melo Ferreira, Thelma B. Machado, M. Beatriz P. P. Oliveira

PC20 **Can coffee silverskin be as promising as green coffee on type II diabetes prevention?**
Juliana A. Barret do, Anabela S. G. Costa, M. Beatriz P. P. Oliveira, Fátir

PC21 **Wild endogeno ous extracts for biological studies.**
Helena Laronha nuel A. Coimbra, Elisabete Coelho

Plenary Communications and Keynote Lecture

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António Augusto Vicente é Professor Catedrático no Departamento de Engenharia Biológica, que já dirigiu, sendo também vice-Presidente da Escola de Engenharia e Diretor do Colégio Doutoral daquela Universidade. É também Membro Sénior e Especialista em Engenharia Alimentar pela Ordem dos Engenheiros, onde é atualmente coordenador-adjunto da Comissão de Especialização em Engenharia Alimentar. É ainda membro da *International Society of Food Engineering* (EUA).

Enquanto investigador, tem dedicado o seu trabalho ao desenvolvimento de sistemas micro e nanotecnológicos para aplicação no setor Agro-alimentar, à avaliação do seu comportamento em sistemas dinâmicos de digestão *in vitro*, ao estudo da influência da aplicação de campos elétricos em células e biomoléculas para alteração da sua funcionalidade e ao desenvolvimento de novos bioreatores e sua aplicação em bioprocessos.

Tem publicados de mais de 350 artigos em revistas internacionais ISI WoS e mais de 30 capítulos em livros de circulação internacional. É autor de mais de 400 trabalhos apresentados em congressos e 5 patentes e editor de 5 livros científicos. Conta com mais de 13 000 citações e cerca de 120 000 leituras no *Research Gate*. É Editor Associado do Journal of Food Engineering e membro do *Editorial Advisory Board* do *Journal of Agricultural and Food Chemistry*. É membro do Júri do Prémio Nacional de Agricultura desde a sua instituição, em 2012. Foi vencedor dos *Food and Nutrition Awards* em 2015 e 2017, na categoria Investigação e Desenvolvimento. Nos quatro últimos anos (2018, 2019, 2020 e 2021) foi distinguido como *Highly Cited Researcher* pela *Clarivate Analytics*. Em 2021 foi distinguido com o Prémio de Mérito Científico da Universidade do Minho e com o Diploma de Mérito Científico da Escola de Engenharia da Universidade do Minho.

Invited Plenary Communications

The future of foods: foods for the future

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There has been a significant shift in the consumers' preferences, acceptance and needs in the last ten years, which has been particularly strong in the last five years. The "top trends" are: Clean claims (e.g. preservatives free); Clean labels; Lifestyle enhancers (e.g. high energetic foods); Functional foods (e.g. with nutraceutical function); Minimally processed foods (e.g. using natural ingredients as much as possible) and the so-called "Green foods" (making use of the benefits of plants - e.g. replacement of animal protein by other protein sources).
Along with this shift, there are two major problems related with the food we eat: I) ensuring people's food, health and wellbeing, and II) ensuring the health of our planet.
When answering to problem I), the future food needs to tackle malnutrition, reduce calorie density, reduce food digestibility, increase micronutrient bioavailability, control gut health, allow personalized nutrition and provide appropriate food for the elderly.
In order to answer to problem II), we need to make use a set of tools for the future: molecular biology, nanotechnology, artificial intelligence, robots & sensors, the so-called "Cellular agriculture" and search for alternative protein sources.
In this talk the latest developments made by our research group towards tackling some of these challenges are going to be presented, together with our vision on what still needs to be done and which partnerships are important to lead us to the future of foods, producing foods for the future.

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PhD in Food Science Univ Nottigham UK. Coordenadora do LEAF. Área de ensino na interface da Indústria: Projecto Industrial e Instalações e Equipamento Industrial; Tecnologia de Cereais. Pioneira nos estudos de Textura e Reologia Alimentar em Portugal. Co-coordenadora da PosGraduação AgriBusinesses no ISEG em colaboração com a Consulai e coordenadora da UC em Tecnologia Alimentar e Inovação na Licenciatura em Nutrição na Fac. de Medicina da ULisboa (FMUL). Foco na sustentabilidade e eficiência desenvolvendo projectos com a Indústria. Uso de ingredientes funcionais, subprodutos da indústria e fontes alimentares subexploradas, em alimentos básicos, com forte impacto na saúde do consumidor. Membro do CoLab4Food e do Lab Associado Terra. Com mais de 100 publicações científicas (http://orcid.org/0000-0001-9384-7646).

Invited Plenary Communications

How urgent is sustainability in food production – rethinking old practices

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The word SUSTAINABILITY may be overused, of course, but that does not take away the urgency of ensuring not to exhaust the planet's resources. We must secure that the next generations will be able to enjoy a planet as extraordinarily wonderful and balanced as the EARTH is, and not a dustbin jammed with plastic and almost lifeless, where the sun does not shine, the rivers do not run clear and the birds do not sing.
Some news are discouraging, it seems that we woke up too late to this reality, so what? Are we going to give up? Can we sleep peacefully knowing that we are not doing our best to reverse this situation?
Food production has an important share in the depletion of resources and the pollution of the planet, therefore I bring this subject to this forum. The general figures are scaring: 30% of total GHGE; 40% of the land; 70% of drinkable water ; pollution with pesticides, fertilizers, plastics. This is the bill for agriculture and cattle raising for food production and we need to add to that food processing and transformation in the food industry.
All Food scientists need to upgrade this idea into the centre of their everyday work. It is not enough to look at the SDGs and the European Green Deal targets, we need to put action into motion. Companies are starting their way, some with impactful and credible actions, others not so much. Some countries are leading the way, those in Northern Europe, the Netherlands. The energy, food and digital transition is going to happen, we need to ease its path.
We are facing the need to increase food production to feed exponentially growing world population. Several strategies are welcomed: i) increasing production yields through precision agriculture; ii) using no land vertical farming and urban farming to produce fresh vegetables, near the consumers, to save land use and distribution transportation; and iii) using non conventional sources of food - from microalgae to alfalfa and white clover, pulses, acorns, etc. and iv) using food industry by-products or agriculture crops leftovers to produce functional foods, reintroducing it into the food value chain to reduce food losses and waste. At present, food waste reaches 1.6 billion tons annually and accounts for the emission of 3.3 billion tons of CO2, consuming about 250 km3 of blue water, and nearly 1.4 billion hectares of land occupied, posing a big challenge with high impact on environment, biodiversity, and climate change.
Chemists, especially food chemists, play a very important role in this transition. Always adopting green chemistry methods, thinking carefully and do the product life cycle analysis exercise, using local indexes and always keeping in mind the economical and social impact. This is an excellent opportunity for us to have real impact and make a difference. With so much knowledge accumulated in food production and analysis, we can put all the tools on the table to ensure the safety of novel foods from alternative sources: e.g. foods from insects, microalgae, by-products from the food industries. The use of white clover and alfalfa for the production of food protein is starting first steps in Denmark. But we can also use less radical alternatives and use the old legumes, i.e. pulses like grasspeas, lupins, beans, chickpeas, fava beans, and peas, to create protein foods that replace those of animal origin, with the advantage of a smoother transition, reviving the old rural recipes and increasing food security.
Food has been spotlighted. Not only for sustainability and transition to the plant-based foods, but also because the ancient wisdom that "We Are What We Eat" has reborn, and the impact of diet on health is now recognized by all.
Therefore, to assemble these two major trends: Sustainability and Impact on Health, has been our major driving force for research. The use of a very old processing/preservation technology like fermentation by lactic acid bacteria has been a powerful tool to reduce the anti-nutrient factors and increase digestibility, as in long fermentation bread, or solid fermented foods from European pulses to produce tempeh and miso; or recovering the green tomatoes left on the fields to produce a fermented product to be used on sauces and condiments. Also using Portuguese pulses to produce beverages alternative to milk with equivalent nutritive values; and using microalgae in cheese, bread, cookies, crackers; or producing staple foods with fruit pomaces from the juice industry to increase their impact on health. These are some of the contributions from our research group to the sustainability of healthy food production, a small input to a greater cause!

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Sílvia M. Rocha is Associated Professor with Habilitation in Biochemistry, at the Chemistry Department of the University of Aveiro (Portugal). Her research is mainly focused on the characterization of plant raw materials and agri-food by-products, for the prospection of bioactive compounds, and understanding them from the analytical, technological, and sensorial point of view, toward the innovation and development of novel formulations and food solutions. Her interests are also devoted to the use of advanced chromatographic methodologies for unveiling complex biological systems based on a metabolomics approach (microorganisms, plant and body fluids metabolomics).
Sílvia M. Rocha published over 165 SCI papers, 2 books, 18 book chapters, 1 interactive CD/book, more than 400 presentations in scientific conferences, and 5 patent applications. She has received 20 national and international awards and distinctions and possesses h-index 39, with more than 4680 citations (http://www.scopus.com/authid/detail.url?authorId=7006653213).

Invited Plenary Communications

Unveiling the chemical nature of food aromas: in the path of the multi-sensoriality

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The human senses shape the life in several aspects, namely well-being, socialization, health status, diet, among others. However, only recently, the understanding of this highly sophisticated sensory neuronal pathway has gained new advances. Also, it is known that each olfactory receptor cell expresses only one type of odorant receptor, and each receptor can detect a limited number of odorant substances. Odorant substances are typically volatile or semi-volatile in nature, exhibit low relative molecular weight and represent a wide variety of chemical families. These molecules may be released from foods, constituting clouds surrounding them and are responsible for their aroma properties[1].

A single natural aroma may contain a huge number of volatile components, and some of them are present in trace amounts, which makes especially difficult their study. Understanding the components of food aromas has become more important than ever with the transformation of food systems and the increased innovation in the food industry. Advanced chromatographic technique such as GC×GC-ToFMS seems to be a powerful technique for the analytical coverage of the food aromas. This technique seems to fulfill the requirements of the innovative strategies in the field of the aroma chemistry such as the smell digitalization and sensomics. Indeed, innovative enhancements, such as smell digitalization and sensomics, that are multi-step analytical approaches, are used to obtain the multipart odor picture of a food, which include the identification and accurate quantitation of odorant molecules. Smell digitalization allows the measuring and chemically revealing of the smells to making them in a digital presentation, which represents cutting-edge research, usually using artificial intelligence to interpret the odor signatures. While sensomics is an approach developed to help in the mapping of both aroma and taste key active molecules, which are perceived by humans' chemosensory receptors, and then integrated by brain.

Furthermore, for a holistic understanding of the aroma of foods, it is crucial to define a broad strategy, involving diverse techniques that can assess the multiple dimensions of the aroma perception, that are intrinsically associated with the multimodal perception concept, i.e., multimodal phenomena concern stimuli that generate simultaneous (or nearly simultaneous) information in more than one sensory modality[2]. Certainly, the chemical aroma data is not enough, but crucial to move forward on this challenging. Thus, this talk will be focused on the advanced tools used to unveil the chemical nature of the food aromas, and their relevance to the quality of life and to the new challenges of society. Due to the huge significance of human olfaction in several fields, namely, to improve nutritional health, diagnose and treat diseases, understand consumer preferences and consumption, measure and chemically reveal the smells represents cutting-edge research with an increase of such a tendency to be expected in the future.

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Nota curricular

Ph D. In Chemistry from the University of Oviedo (2000), currently I develop my teaching and scientific activity in the Department of Physical and Analytical Chemistry of the University of Oviedo (Spain). My previous research, from 2001 to 2019 were carried out in the Regional Institute for Research and Agri-Food Development (SERIDA) of the Principality of Asturias. In SERIDA, I was researcher and Technical Manager of the Animal Nutrition Laboratory. My research topic was focused on the development of on-site and real time methodologies for quality and safety control in food and feed, using spectroscopy as reference technique. The developed methodologies are based on using and developing spectroscopic sensors combined with chemometrics to transform spectroscopic data in a quantitative or qualitative signal. The possibility of being able to carry out my scientific activity in an accredited laboratory (ISO/IEC 17025) and simultaneously in a research center, made possible the development of my scientific-technical skills in a double aspect, research and implementation of developed methodologies in routine analysis of laboratory.

Some results of the research activity include the supervision of 3 Ph.D. Thesis (already defended) and of another 4 Ph.D. Thesis (in progress). Author or co-author of more than 60 articles in refereed (JCR) journals (mainly in the field of Analytical Chemistry, but also in the field of Chemometrics and Food technology) plus 4 book chapter. About 57 % of the publications are indexed by JRC in the highest Quartile, Q1 in the corresponding JCR category. Author or coauthor of more than 100 presentations in national and international scientific conferences. In this context I have been IP of 3 funded research projects (obtained in competitive Spanish public calls) and participated in the research team of 24 other funded research projects, and contracts with industry, to support the transfer of research.

Member of Organising Committee of Feed For Health: International Workshop COST Action FA0802. April 2011 (Gijón, Spain). And Secretary of the International Conferences, 42th Colloquium Spectroscopicum Internationale and 21st ISBC & XIX ISLS held in Gijón (Spain 2022.

It is important to highlight that actually and I am member of the research Group on Analytical Spectrometry from the Department of Analytical Chemistry (GEAB, University of Oviedo) led by Prof. José Manuel Costa Fernández. And I am member of the Work Group 1 of the EU Cost Action, CA19145 - European Network for assuring food integrity using non-destructive spectral sensors (SensorFINT; 2020-2024).

Invited Keynote Communications

Applications of non-destructive strategies for agri-food quality and safety monitoring: trends and challenges

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Currently, with the implementation of quality and safety controls in agri-food industry, a huge number of analysis are demanded at different levels of chain production. On this matter, the determination of quality parameters such as nutritive value (protein, fiber, fat, humidity, energy value, etc.) or safety ones (mycotoxins, microbial load, amines, etc.), are demanded to reduce economic losses in agri-food sector and increase safety of consumers.

However, conventional analytical methods are expensive, time-consuming, environmentally unfriendly and need the collection of samples prior to analyses. Thus, wet laboratory methods are not effective when high throughput of sample analysis are required in field and they also entail sample destruction having a negative economic impact for producers.

An alternative to avoid inconveniences of standard laboratory methods are those based on Near Infrared Reflectance Spectroscopy (NIRS) using portable or on-site instruments. These procedures can be an effective alternative to assess quality and safety of intact food, facilitating their optimal harvest stage and giving information about nutritive value and preservation.

Particularly, as the complete analysis can be performed without previous sample preparation (non-invasive sampling), NIRS allows on-site applications (pre or post harvest) when handheld instruments are employed. Therefore, using this technique and portable NIRS instruments we can move the lab to the food industry or farm. Moreover, the same instrumentation can be employed for quality control of raw materials and final food products.

However, for the establishment of these food controls at the farm, agricultural or industrial levels it is needful to develop robust calibrations to obtain quantitative or qualitative results with adequate accuracy and minimum errors. The development of robust calibrations require the use of chemometric tools able to extract all the relevant information included in NIR spectra.

This lecture will address to evaluate NIRS as a promising alternative for real time, non-invasive analysis in food analysis, exploring the potential of this spectroscopy procedure for specific applications in food. Future advances and challenges using NIR sensors, combined with chemometric tools, will be proposed to monitor food quality and safety, focusing on portable devices, and reporting on the ability of NIRS used as an untargeted methodology.

Keywords

NIR on site, food quality, food safety, traceability, untargeted methods

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Nota curricular

Maria Campos is a Professor at the Faculty of Pharmacy, University of Coimbra - Portugal, where she conducts research on “Drug Discovery” using natural raw materials mainly pollen. In recent years she works directly with patients and Physicians to solve, prevent and/or avoid Drug-Plant Interactions, whose Multidisciplinary Research Group she coordinates at Coimbra University (www.oipm.uc.pt). She teaches several Curricular Units such as Pharmacognosy, Phytotherapy, and also Medicinal Plants. She is the scientific supervisor of various Master's, Doctoral, and Post-Doctoral students. She has given over 170 national and international conferences. Belongs to the Editorial Board of 7 scientific journals and has written 3 books, 17 chapters, and more than 200 publications in Scientific and technical-professional Journals.

Maria Campos works also, as an expert in two International Scientific Committees, at European Medicines Agency and at the International Regulatory Cooperation for Herbal Medicines (IRCH) in the World Health Organization, and in Portugal is a member of five Scientific Commissions at the Ministries of Health, Agriculture and Economy.

Invited Keynote Communications

Food/Herb-Drug Interactions: What we should know.

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Nutrient, Herb, and Drug Interactions are a very hot issue these days. The scientific data is more and more reliable which helps to promote information to patients and clinicians. Both need to be aware of this possibility when therapy is started. The right communication about the possible interactions among the drugs, food, and/or other natural products, including medicinal plants, is the best way to get the most for efficacy and safety, and not compromise the treatment[1,2].

The reason why the intake of certain drugs, should/or should not be taken with food, is exactly to avoid/or to promote these interactions. Nevertheless, sometimes specific interactions are requested when they are necessary to improve the efficacy and safety, or even crucial to increase, for instance, the absorption.

However, depending on the pathology and the therapy apply, some can induce clinical emergencies, very complex to solve and imply hospitalization admission. In the majority of the cases, for instance, with oncologic patients, the decrease of efficacy could cause therapy failure, which is a very problematic situation once allow the tumor grows and, in certain conditions, a high probability to produce metastasis[2]. On the contrary, if the plasmatic concentration of the drug increases, the toxicity will produce more side effects. Added to this, when the natural products involved are toxic or contaminated with toxins the damage could be impredictable[3].

Since drug-herb/food interactions can modify the therapeutic effect of drugs, citizens and health professionals should be aware of these effects and do their best to avoid them. Examples of interactions range from absorption and/or excretion, to distribution and/or metabolization, via various isoenzymes, as for example, CYP 450 (inhibition/induction/substrate) will be presented. Among medicinal plants commonly used, infusions or teas as also other types of extractions from Aloe vera, Camellia sinensis, Chamomile, Curcuma, Dandelion, Flax and Chia seeds, Ginger, Ginseng, Goji berries, Green tea, Fennel, Lemon balm, Linden tree, Melissa and Menthe, are able to inhibit various of these isoenzymes during the metabolization/ detoxification process. This effect can be critical to the plasmatic concentration of the drugs. Even if they are prodrugs the active metabolite will be not produced and a therapeutic failure can happen, otherwise, for the others, the amount of drug can achieve toxic levels and increase the side effects[3]. An opposite event can be produced by St. John's wort or alcohol (this last if consumed in a regular high dose) will contribute to increasing the metabolization of the drugs reducing its plasmatic concentration, which decreases the efficacy or sometimes does not even achieve the therapeutic dose.

Similar effects can occur with relatively high amounts of fruit or legumes juices (ex., Grapefruit, orange or tangerine, blackberries, blueberries, beetroot, etc). The intake of fibers will decrease the absorption of the drugs compromising the dose. During antibiotic treatment, fibers should be avoided from breakfast if the morning dose is made simultaneous. The same care should be done for other drugs.

Elderly Polymedicated Patients require even more detailed care, due to their age and compromised metabolization system. The additional intake, of different natural products, will contribute to a none controlled disease[1,2].

Our research group gives support to clinical cases, based on the data available but also contributes to creating the establishment of more clinical evidence. The empowerment of practitioners and educators will help to develop new tools (and/or adapt the already known) and discuss the most relevant innovations, trends, and concerns as well as the practical solutions and challenges encountered that are investigated in this field. This data, which is important to science and to the health status of society have a reasonable possibility to be included in further guidelines of the various treatment protocols. In different countries, these problems are already screened to assure the best efficacy of the protocols in the future. We will present and discuss some Case reports in Chronic and Oncologic Diseases which

were followed by our research group (Observatory of Drug-Herb Interactions, www.oipm.uc.pt) which will give a broader scenario about these issues.

To a better understanding of this question, we suggest a deep look at some more examples from real clinical cases involving drug-food and drug-herb interactions. On our website we highlighted the most frequent, for instance in Portugal, but also some from the literature available (http://www.oipm.uc.pt/interacoes/index.php?target=casos).

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Nota curricular

Joana Amaral é licenciada em Ciências Farmacêuticas pela Faculdade de Farmácia da Universidade do Porto e doutorada em Química e Nutrição Alimentar pela mesma faculdade. Desde 2000 é Professora no Instituto Politécnico de Bragança, tendo sido membro do Laboratório Associado REQUIMTE (2006-2020) e desde 2018 é membro do Centro de Investigação de Montanha (CIMO-IPB). Foi presidente da Divisão de Química Alimentar da Sociedade Portuguesa de Química (2009-2012) e atualmente é presidente da Food Chemistry Division da European Chemical Society (EuChemS). É autora de mais de 100 publicações internacionais incluindo artigos e capítulos de livro.

Os seus interesses de investigação estão principalmente relacionados com duas linhas de investigação, nomeadamente com a autenticidade de alimentos e plantas medicinais, e com a caracterização nutricional, fitoquímica e bioatividade de alimentos e sub-produtos alimentares.

Invited Keynote Communications

DNA-based approaches for the authentication of complex foods

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In the last decades, several reports on food adulteration and different food scandals such as the "horse meat" and "melamine in milk formulae" have drawn the world's attention and increased consumers mistrust about the food they buy. According to the European Commission, food fraud regards "any suspected intentional action by businesses or individuals for the purpose of deceiving purchasers and gaining undue advantage therefrom, in violation of the rules referred to in Article 1(2) of Regulation (EU) 2017/625 (the agri-food chain legislation)". Food fraud encompasses several different cases such as the substitution of a high-valued food or ingredient by a cheaper one, the addition of undeclared ingredients or species, dilution, mislabeling (including of botanical or geographical origin), unapproved treatments or processing, among others. Therefore, besides raising moral and ethical concerns, it also represents economic losses and unfair competition, as well as possible risks to human health. Among different types of foods, those of high-quality and attaining higher prices on the market, as well as scarce/valuable raw materials, and ingredients acquired in bulk with the loss of some characteristics, are considered the most prone to be targets of adulteration. According to the 2019 annual report on food fraud,[1] within Europe, most cases have occurred in fats, oils, fish, meat, fruits and vegetables, with economically motivated adulteration of food being estimated to create losses around 8 to 12 billion € per year.[2] Moreover, several reports suggest that potentially harmful food supplements may be entering the EU market, a problem that increased in 2020 due to a growing use of e-commerce and the increased focus on personalized nutrition and health that occurred during the pandemic.[3]

Therefore, several works have been proposing the use of advanced methods, including those based on molecular biology, stable isotope analysis, targeted or untargeted mass spectrometry and spectroscopic profiling, to detect food adulterations. However, depending on the matrix and type of adulteration, analytical authentication of foods can be highly difficult and challenging, especially for highly processed and complex food, for which representative chemical fingerprints can be difficult to establish.

Among the different techniques used for food fraud detection, those based on the analysis of DNA molecules have been widely used and are particularly suitable to detect adulterations by the replacement, addition or removing of plant or animal species. In this work, different examples of DNA-based approaches to detect adulteration in complex foods will be presented, including the case of food supplements, honey and argan oil.

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Nota curricular

José S. Câmara is Assistant Professor, with Habilitation, in Food Chemistry and Analytical Chemistry at the University of Madeira. He was President of the Food Chemistry group of SPQ from 2018-2021, and member of the Advisory Board of CQM (https://cqm.uma.pt/).
Published 185+ papers in international journals ((h-index=50; i10:123, >8 000 citations; Google Scholar), 11 book chapters, and presented more than 450 communications in scientific congresses.
Is Associate Editor of Frontiers in Food Science and Technology; Topic Editor of Frontiers in Nutrition; member of the Editorial Board of Molecules, Foods and Beverages, and regular referee of several scientific journals.
His research interests Includes i) microextraction techniques, development and applications, ii) FOODOMICS; iii) volatilomics: iv) food characterization – safety, quality authenticity; v) high resolution separation techniques.
He integrates the list of Top 2% highly cited researchers worldwide (Chemistry, Food Science) (2019, 2020) (Stanford University) and the list of Top Chemistry Scientists in Portugal (research.com)

Invited Keynote Communications

Behind the scenes of agri-food waste - from the health benefits to potential applications

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The exponential increase of world's population (9.8 billion by 2050, UN estimates) combined with the continuous climate changes, water scarcity and decreasing of agricultural areas constitute societal and global problems that challenge food production for the next generations. With current global trends in diets, the exponentially growing population and considering the several millions of tons of foods that are lost and wasted every year in different steps of the food chain, including production, post harvesting, processing and distribution, in 2050 it will be needed 50-60 % more food than today to feed everyone. These overwhelming numbers clearly show that food challenges and planet sustainability are intrinsically bound and must determine urgent measures to mitigate them. It will be important to develop strategies to produce more food and better food with less waste in addition to the implementation of sustainable food production systems through the optimization of food processes to achieve a better environmental footprint, lower production costs and improving quality and nutritional value of food (**Figure 1**). [1] Certainly, with these concerns in mind, the European Commission establish food waste as one of the priority areas of the Action Plan for the European Circular Economy Strategy. [2] This includes a zero-waste strategy envisaging the agrifood wastes valorisation to reduce the environmental pollution. This strategy is based on the extraction of compounds from the food wastes that have a high demand or innovative applications with potential high economic return in different industrial sectors, like nutraceutical, cosmetic and pharmaceutical industries.

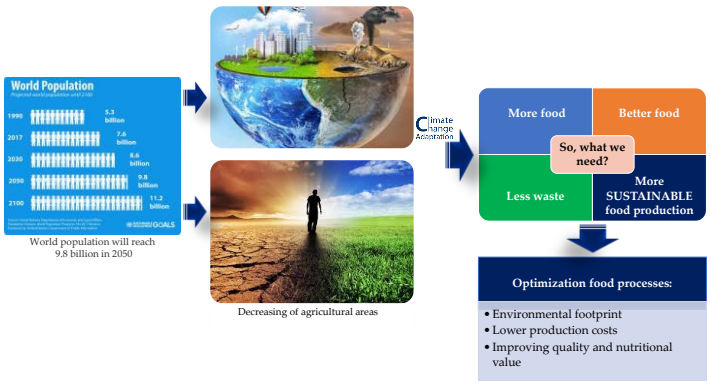

Figure 1: Global and societal challenges in the production of foods for the next generations. [1]

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Nota curricular

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Diretora Executiva da PORTUGALFOODS – Pólo de Competitividade e Tecnologia Agroalimentar, desde junho de 2017.

Entre 2004 e 2017, foi gestora de incentivos, análise e acompanhamento técnico de projetos de I&I. Experiência na interface Universidade-Empresa, nos processos de transferência e valorização de conhecimento e tecnologia.

Vasto conhecimento do tecido empresarial e entidades do Sistema de Investigação e Inovação, com destaque para os sectores da Biotecnologia e Agroalimentar.

Invited Keynote Communications

Collaborative ecosystems addressing priority themes for the Agrifood sector

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Collaborative ecosystems are, by nature, promoters of a closer interaction between the several actors of the innovation system, which together allow the agri-food sector to add knowledge and value, to increase innovation and its performance in the global market. Unequivocally, this engagement among all entities fosters a greater exchange of experiences and the development of solutions. Thus, it is a process of collective overcoming and acceleration of ideas that flows more quickly. PortugalFoods, given its mission, has in recent years acted as a driving force and stimulator of knowledge transfer and economic valorization, adapting Knowledge to the needs of the Portuguese business environment. “Projetos Mobilizadores” are strategic R&D projects which aim is to create new products, processes, or services with high technological and innovation content, which are intended to contribute to the value chain and are configured as driving forces for scientific and technological capacities and competences, bringing significant multi-sectorial impact[1]. Considering this typology of projects, the agri-food sector has implemented them to bring together several complementary skills, to create new products, processes or services that make the sector more competitive and collaborative, as they reinforce strategic synergies and ensure greater differentiation and responsiveness in an increasingly competitive market. MOBFOOD and cLabel+ are examples of this type of projects focused on agrifood sector. MOBFOOD, which ended in 2021, was a project involving 43 entities and was based on three main areas: "Food Safety and Sustainability", "Food for Health and Well-being" and " Quality and Safe Food Production"[2]. cLabel+ is ongoing until May 2023 and aims to explore the clean label concept. This project involves 20 entities: 8 companies with diverse and complementary areas of activity and 12 non-business entities from the Research & Innovation System with a strong experience in project development in the scientific areas of the project[3]. Regarding the clean label concept, this is quite broad, covering environmental and social impacts, which may raise certain contradictions. Thus, it is relevant to explore its different dimensions and invest in consumer literacy[3].

Aknowledgements: cLabel+ partnership
Funding: This project is supported by European Union through ERDF and co-financed by Programa Operacional Competitividade e Internacionalização and Programa Operacional Regional de Lisboa.

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3. cLabel+, available at https://cleanlabelplus.pt/ (Accessed September 19, 2022)

Sponsor Communications

One Step Ahead of Routine Analysis - True Mass Analytical Solutions for Wines

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Analysis of wine has many aspects with food safety and food quality being the most common. Fraud is increasing and this is clearly a major concern, so together with wine adulteration, authenticity has a large impact on both the safety of our food consumption as well as a growing impact on financial aspects for producers.
Wine quality and wine safety issues are well known areas for analysis most of this work is targeted analysis, however more and more labs want to know what else is there and slowly add untargeted workflows.
Another main topic to the wine industry is the capacity to assure the authenticity of the wine, and monitories some markers to differentiate type of wines.

With this presentation, Bruker will try to show the different solutions we provide, using real examples, and demonstrate the add value that any lab can have to work with a versatile high resolution mass spectrometer.

Acknowledgements: Bruker would like to thank to the customers that participate in the different studies and give us the chance to learn and develop differentiated solutions. Special thanks to Cork Industry for the support with knowledge and samples, as well as the Wine industry from Spain and Greece. Special thanks to Professor Nikolaos S. Thomaidis from University of Athens - Lab of Analytical Chemistry
to share with us some results of authenticity in wines.

Perform food analysis with revolutionary 2400 GC - fast, sensitive, sustainable, connected...

Luca Piatti - Environmental Regional Segment Leader EMEA-I, Chrom BLL at PerkinElmer

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Today, industries and laboratories continually seek to improve overall efficiency, optimizing work processes, implementing new technologies to ensure profit in difficult times and delivery quality results.
The new 2400 Headspace GC Platform leveraging on “smart workflows” enables to verify the content of ethanol in fermented food, ensure low detection limits of chemicals with sustainable analysis.

Food safety, quality and authenticity - Agilent solutions for food and beverage characterization and quality control assays

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Ensuring food safety and quality is an increasing challenge. Whether in the analysis of contaminants or the authenticity of label claims, Agilent seeks to find analytical solutions for customers in various applications with excellent equipment with the forefront of technology.

Food safety testing [1] - critical in today's regulated environment. Much of the food we eat and enjoy today is provided through complex global food production, processing and distribution systems. Analytical testing at all stages of the supply chain is essential to ensure food safety and quality. Laboratories face challenges in maximizing efficiency and productivity, while also being able to determine contaminants at ever-lower concentration levels while meeting evolving testing regulations. Agilent supplies chromatography equipment with mass spectrometry detectors (GC-MS and LC-MS) with unique performances for identifying and quantifying diverse contaminants such as pesticides, mycotoxins, dioxins, PAH's and veterinary drug residues in various food matrices.

Food Authenticity [2] - Accidental adulteration or intentional food fraud affects “certified” quality products such as specialty cheeses, premium wines, tea from endemic plants and regional olive oils, as well as staple foods such as rice and milk. Analytical testing is essential to ensure food authenticity and consumer safety and protect industry brands. Agilent has the analytical equipment to help you meet all your food authenticity needs.

Nutrition and food health [3] - The food industry must also adapt to respond to consumers who demand to know more about the products they are buying, and the impact these food products have on their health. The inclusion of food additives (sugars, salts, fats and dye), the nutritional value of foods and diathetic supplements and the metabolic impacts on our health are factors of concern to consumers. Agilent, with its wide range of high-sensitivity analytical equipment, contributes to the development of ever faster, more accurate and sensitive methodologies that allow the complete characterization of the nutritional impact of food on human health.

Figure 1: Agilent *Application Notes* on food safety, quality and authenticity assays.

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PFAS Analysis – Overcoming challenges to meet regulatory limits with a total solution

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The accumulation of PFAS is rapidly becoming a growing concern to the safety of foods intended for human consumption and due to their inherent characteristics possess significant challenges in analysis. As control and monitoring of such substances are evolving, the demands and solutions are becoming more diverse.
In this presentation, we focus on rapid sample method for PFAS analysis on a highly sensitive mass spectrometer to reach necessary performance criteria regulated by the EU. The enhanced negative ion sensitivity of the Xevo™ TQ Absolute Tandem Quadrupole Mass Spectrometer allows for PFAS analysis better sensitivity with a reduced sample injection improving chromatographic performance and increasing column lifetime without compromising on method performance. Next generation Premier™ technology with MaxPeak High Performance Surfaces™ and novel ionization techniques such as UniSpray™ can be further utilized to improve efficiency and robustness by reducing conditioning times and lowering detection limits or injection volumes. Waters is passionate in its continuing and evolving effort to offer total solutions for these notoriously tricky compounds to meet your laboratories specific needs and requirements.

IR & FT-NIR Quality Control of Milk

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Milk is the starting point of any dairy product and is one of the most tightly controlled food products in the world. The composition of milk, however, changes from season to season and even from day to day, making comprehensive quality control essential. FT-NIR and IR spectroscopy are fast and effective tools for the analysis of common parameters in milk like fat, protein, lactose, total solids and solids-non-fat (SNF) content.
Both IR and FT-NIR can be used to address the analysis needs of the modern dairy industry. The choice between the two analysis types is primarily dependent on the specific needs and applications of the customer.

With this presentation, Bruker will try to show the importance of quality control along the dairy production chain and demonstrate that IR and FT-NIR analyses are fast and reliable in the lab or at-line and that are also official methods according to the guidelines of the International Dairy Federation ID

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ICP-MS and GC-MS: an invaluable tool in food analysis

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Access to safe and nutritious food is key to sustaining life and good health. Food safety strategy covers not only the safety of food for human consumption, but also animal feed, animal health and welfare, and plant health. The process must ensure that food is traceable as it moves from the farm to the consumer, especially when transported internationally. Analytik Jena, represented in Portugal by Specanalítica, offers products that meet the needs of the food industry and the regulations that uphold the high standards.[1]
One such product is the PlasmaQuant MS (**Fig. 1**), an ICP-MS (Inductively Coupled Plasma - Mass Spectrometer) instrument with state-of-the-art characteristics, designed to tackle analytical challenges in several scenarios, from day-to-day quality control to research studies in different types of food samples.[2]
Becoming ever more relevant, speciation studies in food samples play an important role in PlasmaQuant MS' analysis portfolio. In fact, High Performance Liquid Chromatography (HPLC) coupled to ICP-MS is the preferred system configuration for the determination of arsenic species in foods and beverages, such as rice and apple juice. The HPLC offers fast separation of the main arsenic species in less than 10 minutes while the Analytik Jena PlasmaQuant MS ICP-MS provides ultratrace detection, with limits around to 0.4 µg of As species per kg of rice and 3 ng of As species per L of apple juice. [3]

Figure 1: The PlasmaQuant MS.

Furan is an aromatic heterocyclic compound of four carbon atoms and one oxygen atom. It has been found not only in heat processed foods, but also one of numerous compounds, including dioxins, produced during incineration of waste. Because of its association with dioxins, the National Toxicology Program has been evaluating furan for potential carcinogenetic properties and found it to have cytotoxic and carcinogenetic effects in laboratory animals. This study presents the data for performing the current FDA static headspace standard addition method for the detection of furan in food along with a dynamic headspace method[4]. Other volatile organic compounds can also be determined simultaneously with the furan assay. A HT3 Headspace Analyzer[5] (Fig.2) was used in the both the static and the dynamic mode along with an 8700 GC/MS[6], from Scion instruments, for the quantitation of furan in food. An environmental column was used for the quantitation of furan, and to assist in the detection and preliminary identification of additional volatile organic compounds (VOCs) released from the samples. These VOCs, including benzene and toluene can be quickly identified with the environmental column and confirmed when an USEPA Method standard is included in the analysis.

Figure 2: HT3 Headspace and 8700 SQMS from Scion Instruments.

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Oral and Flash Communications

Química Alimentar: Estrutura, Composição e Qualidade Alimentar

Effect of linseed (*Linum usitatissimum*) as an alternative to xanthan gum, over the physicochemical and sensory properties of pasta fortified with macro and microalgae

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Pasta is an important food in the nutrition of people all around the world, being one of the most frequently consumed cereal based products[1], for its ease of transportation, cooking, handling, and storage, as well as its low cost and low glycemic index[2]. Due to the use of refined wheat semolina, dried pasta composition is nutritionally poor, mainly in vitamins, minerals, natural antioxidants and dietary fibre. However, it can be used as a carrier for health-promoting substances providing specific physiological functions by the inclusion of nonconventional ingredients, such as algae that contains various nutritious and bio-active compounds. The partial addition of high-fibre raw materials, can weaken the protein-starch matrix, affecting textural and sensory properties[3]. To maintain the pasta texture and properties it is also necessary to consider the partial addition of structure builders as guar gum and xanthan gum. Nevertheless, the use of whole products as an alternative to extracted or synthesised products is increasingly preferred.

This research aimed to study the linseed behaviour in pasta fortified with algae as a substitute for a common structure builder, xanthan gum.

Pasta was produced using a domestic extruder and half of the samples were dried (68 °C for 42 min, followed by 5 h, 30 min at 76 °C) and half were analysed in fresh. Durum wheat pasta was made using commercial wheat semolina and water (control). *Fucus spiralis* (4 %) and *Chlorella vulgaris* (5 %) were incorporated into the recipe by replacing durum wheat flour (control with algae). Additional samples with structure builders were made. Structure builders (linseed flour and xanthan gum) were incorporated into recipes, with algae, by replacing durum wheat flour at the following proportions (w/w): 0.95%, 2.37% and 4.75% (w/w). All samples (**figure 1**) were evaluated for optimal cooking time, cooking loss, colour variation, texture profile analysis, sensory evaluation, total phenolic content and radical scavenging activity.

The addition of algal biomass increased the cooking time of pasta (> 4 min) and changed its colour, giving the pasta a green tone. The algal biomass had a significant effect on the pasta texture for almost all of the textural parameters analysed in both fresh and dried samples. The enrichment of pasta did not cause a reduction in total sensory score. By adding xanthan gum to the enriched formulation, the optimal cooking time was increased, and the formulation with the highest quantity of xanthan needed much more time to achieve optimal cooking than the rest of the formulations (13 min more than the control in dried pasta), a situation mirrored by the high quantities of weight loss during cooking (64% more weight loss). The presence of linseed flour in the formulations did not decrease the cooking time needed of the algal enriched formulation. The addition of these structure builders affected the colour parameters of pasta with high variability between fresh and dried samples. The phenolic content and antioxidant activity were higher in the samples with linseed flour (0,12mg GAE/g dried pasta; 0,21mg GAE/g fresh pasta) mainly due to the high content of these phytochemicals present in *Linum usitatissimum*. The textural profile analysis showed that the addition of algal biomass can affect textural parameters (increase in hardness by at least 17% and decrease in cohesion (>61%) springness (>49%), gummines (>41%) and chewiness (>45%)) due to the lowering of gluten content in the formulations and that by adding linseed flour, these parameters can be improved. Neither the enrichment of the pasta with *Fucus* and *Chlorella* biomass, nor the addition of xanthan gum or linseed flour change the total score given by panellists in the sensorial test, however the panellists did feel a significant decrease in the textural palatability of the formulation with xanthan, and between X3 and L1 the latter was the most accepted (scores of 4.0 and 5.3 for dry mass and 3.9 and 4.7 for fresh mass, on a scale of 0 to 5).

The differences observed in the biochemical composition and physical parameters of pasta enriched with 5% algal biomass and the addition of xanthan gum and linseed flour observed in this study could lead to the formulation of pastas with differentiated nutritional contents and possibly to the development of nutraceutical pasta with important bioactivity against highly reactive molecules that may cause damage to DNA, protein and lipids (antioxidant activity); all by also maintaining the cooking and palliative characteristics of the regular pasta that is so widely consumed around the world and that is one of the consumers favourite.

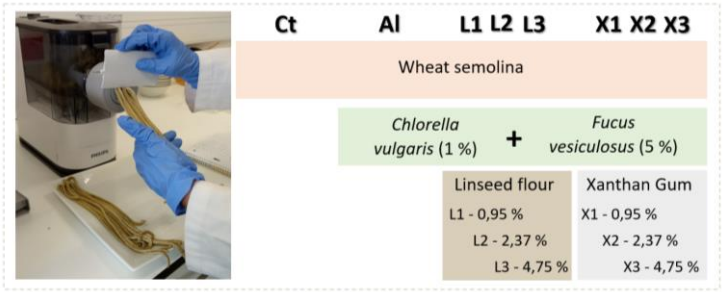

Figure 1: Pasta extrusion to obtain the fresh mass (left side). Sampling scheme (right side): Ct- control; Al- algae control; L1, L2 and L3 – samples with semolina, algae and 0.95 %, 2.37% or 4.75 % of linseed flour; X1, X2 and X3 – samples with semolina, algae and 0.95 %, 2.37% or 4.75 % of xanthan gum.

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The unsung ingredients of salt pan waters: sulfated polysaccharides

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Marine environments are the warehouse of a variety of novel bioactive compounds prone to be explored. Polysaccharides, that are excreted by marine organisms, are among the main materials, particularly the sulfated ones. Sulfated polysaccharides have been proposed as bioactive agents, regarding their potential antiviral, anticoagulant, antioxidant, and immunomodulatory activities[1]. Along these functional properties, some sulfated polysaccharides have already been explored as food additives due to gelling, emulsification, and thickening behaviours[2]. The growing interest in sulfated polysaccharides has led the search for new sources and their structural features. Seawater is the most available source of carbohydrates and polymeric material, which can be naturally concentrated in salt pan waters due to its evaporation by wind and sunlight[3]. Therefore, in this study polymeric material and polysaccharides were analysed along salt production in the evaporation ponds and in the crystallizer water. Along salt production, polymeric material, isolated by dialysis, of seawater (13 mg/L) accumulates in the evaporation ponds (9-73 mg/L) and in the crystallizer (133-144 mg/L). The polymeric material accumulated was composed by 29% of polysaccharides, with 45 mol% of sulfate esters, 23 mol% of uronic acids, 12 mol% of galactose, and 1 to 6 mol% of glucose, mannose, xylose, fucose, rhamnose, arabinose, and ribose. Markedly, these polysaccharides provided viscous solutions above 0.1% (w/v). These results highlight salt pan waters as a worth exploring source of highly sulphated polysaccharide ingredients for functional food and feed applications.

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Chemical properties and microbiological quality of three hazelnut varieties cultivated in Portugal

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Hazelnut is a dried fruit that stands out for its organoleptic and nutritional characteristics and therefore is highly appreciated globally. Worldwide, there are several hazelnut varieties, and their characteristics are dependent on the genotype, agricultural techniques, post-harvest practices, geographic location and climatic conditions. In addition, the physicochemical characteristics influence the quality and shelf life of this fruit
. Therefore, the aim of this study was to analyse some chemical properties and also the microbiological quality of three hazelnut varieties (Grada de Viseu, Tonda de Giffoni and Butler) cultivated in Portugal. For that purpose, hazelnut kernels were analysed for moisture, water activity, fat, protein, ash and the specific extinction coefficients (K_{232}, K_{268} and ΔK which is calculated from the values of the specific extinction coefficients at 264 and 272 nm). It was also performed a quantification of microorganisms at 30 ^{o}C and of moulds and yeasts at 25 ^{o}C. The results showed that fat was the major component for all the varieties, ranging from 70.14±1.75 g/100 g (var. Butler) to 66.46±5.33 g/100 g (var. Tonda), with no significant differences between the varieties. All analyses were done in triplicate. The fruits of the variety Grada had a higher moisture content (6.01±0.26 g/100 g), while the fruits of the var. Tonda (4.78±0.40 g/100 g) presented the lowest value, but in this case with statistically significant differences (p=0.004). According to the recommendations of the European Union, moisture content of hazelnut kernels should not exceed 6.0% [2] Regarding the water activity, the lowest value was observed for the var. Tonda (0.54±0.01), followed by the var. Butler (0.55±0.01) and finally the var. Grada (0.56±0.01), with no significant differences between them. The var. Grada was the one with the highest ash (2.73±0.08 g/100 g), fibre (6.35±0.25 g/100 g) and protein content (18.00±0.34 g/100 g), with statistically significant differences between the varieties in the three cases. Specific extinction coefficients are related to oxidation processes and provide information about the quality of the oil extracted from hazelnuts, as well as its state of conservation. The fruits from the var. Grada presented the lowest value of K_{232} (0.54±0.01), followed by the var. Butler (0.59±0.01) and in last the var. Tonda (0.80±0.08), which means that the fruits of the var. Tonda had more primary oxidation products, being the results for this var. statistically different from the others. As for the K_{268}, all the varieties presented values close to zero (Var. Grada=0.03±0.00, var. Tonda=0.04±0.00 and var. Butler=0.03±0.00), meaning that all the samples had low levels of secondary oxidation compounds. Furthermore, there were no significant differences between the varieties (p=0.373). The values of the ΔK ranged from 0.004±0.00 for the varieties Grada and Butler and 0.003±0.00 for the var. Tonda, again with no statistically significant differences, which means that it is a fresh and well-preserved oil. Regarding the microbiological results, as it can be observed in **Table 1**, all the varieties presented a satisfactory microbiological quality in accordance with the limits established for the count of microorganisms at 30 ^{o}C and mould and yeast by the National Health Institute Doutor Ricardo Jorge [3] (<10^6 CFU/g for microorganisms at 30 ^{o}C, <10^5 CFU/g for yeast and <$5x10^2$ CFU/g for moulds). Moreover, there were found statistically significant differences among the varieties under study. It is important to highlight that var. Grada was that one that presented more microorganisms at 30 ^{o}C and also more moulds and yeast, when compared to the other varieties. This may be due to the higher moisture content of this variety.

Table 1: Mean count (log CFU/g±standard deviation) of total microorganisms at 30°C and molds and yeasts of the samples under study (n=3).

Sample	Microorganisms at 30 °C[1]	Moulds and Yeast[1]
Grada de Viseu	2.86±0.03[c]	2.42±0.07[b]
Tonda de Giffoni	2.62±0.03[b]	2.37±0.06[b]
Butler	2.28±0.04[a]	1.67±0.13[a]
p-value	<0.0005	<0.0005

[1] Mean values in the same column with the same letter are not statistically different (p>0.05)

The results of this study provided important information about the chemical properties and microbiological quality of the most representative hazelnut varieties cultivated in Portugal.

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Study of the interaction between lysozyme and chlorogenic acid by fluorescence spectroscopy

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Lysozyme (Lyz, E.C.3.2.17, N-acetyl-muramic-hydrolase) is a globular, ubiquitous and thermolabile protein, with high enzymatic and bacteriostatic activities commonly found in nature. Lys has a low molecular weight (14.3 kDa) and high isoelectric point (pI = 11.16). Egg white is a natural source of lysozyme, which constitutes about 3 to 4% of the total proteins.[1]

Phenolic acids are a class of phenolic compounds widely distributed in the plant kingdom. The interactions between phenolic compounds and proteins can form soluble or insoluble complexes, changing the protein's native structure and its properties.[2]

Thus, in this work, the effect of the interactions of egg Lyz with chlorogenic acid (CHA) was evaluated on the protein tertiary structure as a conceivable way to promote changes in Lyz functional properties.

The binding mechanism between Lyz and CHA was investigated by fluorescence spectroscopy with the emission spectrum recorded from 295 nm to 450 nm at an excitation wavelength of 280 nm. The concentration of Lyz was fixed at 2 µM, while the solutions of CHA ranged from 10 to 72.5 µM, in 50 mM phosphate buffer and pH 3.50 and pH 7.40, as they were incubated for 10 minutes at 310 K. The fluorescence extinction constant (K_{SV}) was obtained using Stern-Volmer equation and the binding constant (K_b) was given by Lineweaver-Burk equation.[3]

The results showed an increase in fluorescence suppression with increasing concentrations of CHA, thereby quenching protein intrinsic fluorescence (**Figure 1**). The extinction of fluorescence in proteins occurs when aromatic residues (mainly tryptophan) are exposed to the solvent, which means that changes in the tertiary structure of the native protein occur. The quenching (K_q) for CHA at pH 3.50 and 7.40 are higher than the maximum scatter collision quenching constant of the biomolecule (K_q = 2.0 x 10^{10} M^{-1} s^{-1}), which indicates that a static quenching mechanism is the main responsible for Lyz fluorescence quenching, leading to the formation of a complex. Moreover, K_{SV} values indicate that the fluorescence decrease is proportional to the CHA concentration, observed in the Stern-Volmer plots (**Figure 2 - A**) and the calculated parameters (**Table 1**). The values obtained for K_b (**Figure 2 – B**) for 310 K and pH 3.50 and 7.40 reveal strong interactions due to high binding stability ($K_b \geq 10^4$ M^{-1}) (**Table 1**).

Therefore, the interactions of Lyz with CHA give rise to changes in the tertiary structure of the protein with consequences on the protein properties.

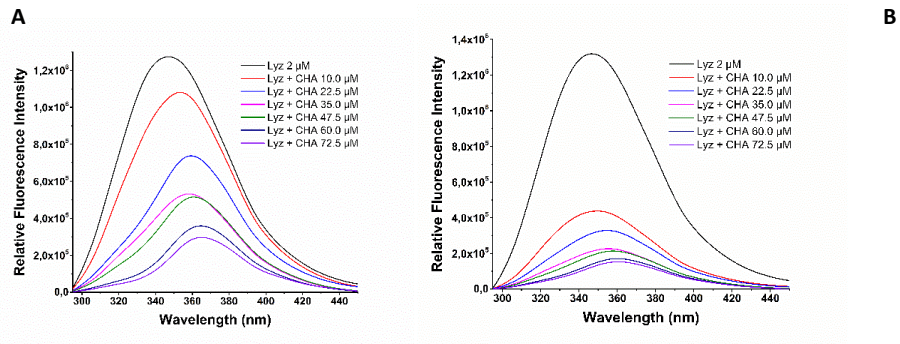

Figure 1: Fluorescence spectra of Lyz (2µM), and 310 K with CHA at pH 3.50 (**A**) and pH 7.40 (**B**) solutions.

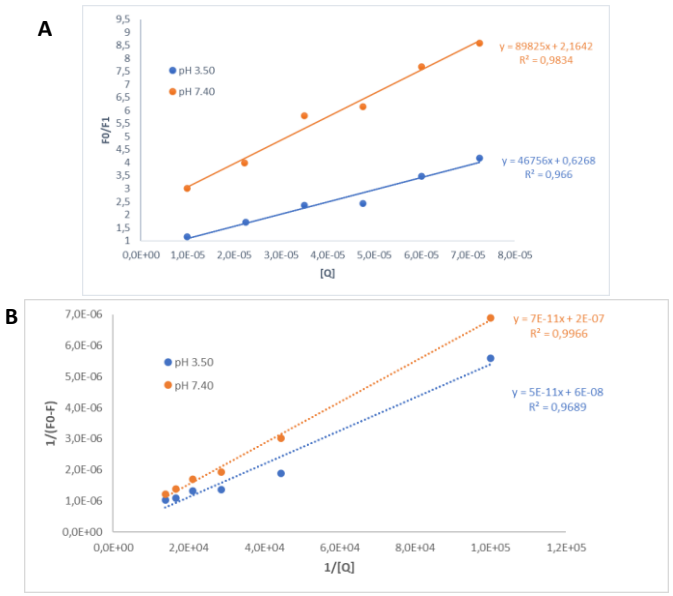

Figure 2: Stern-Volmer plot (**A**) and Lineweaver-Burk plot (**B**) of Lyz (2µM), and 310 K with CHA at pH 3.50 and pH 7.40 solutions.

Table 1: The Stern-Volmer (K_{sv}), quenching (K_q) and binding (K_b) between Lyz and the CHA.

Phenolic Acid	pH	K_{sv} (M^{-1}) x10^4	K_q (M^{-1} s^{-1}) x10^{12}	K_b (M^{-1}) x10^4
CHA	3.50	4.67	4.68	1.47
T (310 K)	7.40	8.98	8.98	1.51

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Taste properties of Royal Gala apple fruit: a combination of molecular and sensory analysis

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The apple (Malus domestica) is one of the most produced fruits and worldwide consumed due to its sensory and nutritional quality characteristics (natural source of phytochemicals with the beneficial effects on health) [1]. Apples, mostly composed of water (85% of fresh fruit), are a significant source of phytochemicals, namely phenolic compounds (PC) such as flavonoids, phenolic acids, and di-hydrochalcones. While this phenolic composition impacts health outcomes of apple intake, it can also impact the organoleptic properties of apples, namely regarding astringency (AST), bitterness and sweetness. Astringency (AST) is characterized by a sensation of puckering, drying and constriction felt in the oral cavity. Different mechanisms are behind this complex tactile sensation, the most accepted one is the precipitation of salivary proteins (SP) by interaction with PC. However, the involvement of oral epithelia and the activation of trigeminal ganglia have been reported [2]. Different studies have explored the association between phenolic content and perception of AST or bitterness on fruit. The results allowed the association of bitterness to fractions rich in oligomeric procyanidins and AST to fractions with high molecular weight procyanidins [3]. In this framework, efforts have been made to design procedures that combine sensory and molecular studies to achieve a robust way of evaluating the organoleptic properties felt during a fruit sensory test. In this work, it was intended to analyze which apple PC interact more with the different families of SP and cross this data to panelist evaluation. So, through a sensory test where 9 tasters analyzed sequentially 5 apples of the Royal Gala variety from different origins, the SP families that suffer greater modification as well as which PC have more interaction during oral processing (chewing) of apple was evaluated. For that, saliva was collected before the beginning of sensory test (control saliva) and after each apple chewing. For PC evaluation, a piece of apple equal to the one distributed to the tasters (PC control) as well as the food bolus of each apple portion were analyzed. Additionally, the influence of the α-amylase content of tasters, which SP are adsorbed to the food bolus and the effect of polysaccharide content were also analyzed.

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Valorization of strawberry, blueberry, and raspberry bioresidues for application infood industry

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Food waste has become a global concern over the past few years, becoming a social, economic, and global problem. About 14%of the world's food production is wasted between harvest and sale, 17% is wasted between retail and final consumption by the consumer, and the rest becomes food itself. Therefore, the reduction of waste and finding ways to reuse it have become a common goal, accompanied by debates, public policies, and research to find solutions. [1,2]

In this context, when specified to the fruit industry, specifically of red fruits, the waste during the logistic process is even more accentuated due to the fragility of these fruits and the ease and speed in which they can be crushed, destroyed, etc. In addition,even if the fruit reaches the shelves for consumption, any physical change (color, size, appearance) can also turn them into bio- waste, contributing to the increase of the percentage of lost and unused food. Therefore, the use of bioresidues generated by these fruits as raw material for the development of new natural products that add value in relation to the destination already associated with these bioresidues has shown to be of academic and industrial interest, since they already present a good acceptance by the consumer public. Therefore, the objective of this work was to analyze the nutritional and chemical profile of strawberry, blueberry, and raspberry biowastes to be used as a basis for the development of new products with high added value. Regarding the nutritional value, it was found that the strawberry residues presented a higher percentage of moisture, followed by the raspberry and blueberry samples. Raspberry presented a higher percentage of protein than the rest of the fruits, corresponding to twice the value obtained for blueberry and three times the value detected for strawberry. Raspberry also had a higher percentage of carbohydrates and a higher energy value. On the other hand, the blueberry had relatively lower contentsof ash and lipids than the other fruits, which had a similar percentage of these compounds. Regarding the chemical composition, a total of five to seven organic acids were identified, and raspberry stood out for the total number of compounds identified, with a predominance of fumaric acid, while strawberries and blueberries presented high amounts of citric acid and shikimic acid. Regarding fatty acids, twenty-five different molecules were identified, with linoleic acid as the most abundant one, followed by α-linolenic and palmitic acids. Raspberry presented the highest levels of tocopherols, in a concentration two times and six timeshigher than those found in blueberry and strawberry samples, respectively, with a prevalence of α-tocopherol. These results demonstrate the suitability of the studied residues to be used as raw materials for the development of new products with interesting nutritional and chemical characteristics.

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On the quest for low calorie carbohydrate-based sweeteners

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Over the last years, industries have been making efforts to replace sugar in foods by using sweet tasting molecules. However, in addition to off-taste profiles, these compounds usually result in the loss of texture perceived by the consumer as negative.[1] Besides sucrose, fructose and glucose, there are other sugars able to provide sweetness and texture to foods but without the caloric contribution. These include fructooligosaccharides (FOS) that, given their backbone structure composed of a sucrose unit extended by 1)→Fru*f*-(β2→ chains, are unable to be metabolized by human digestive enzymes.[2] As FOS might be produced from inulin depolymerization, inulin rich foods represent a potential source of these non-caloric carbohydrate-based sweeteners. Based on this hypothesis, this work aimed to obtain FOS-rich syrups by taking advantage of yacon, an inulin-rich tuberous root characteristic of the Andean region[3] and efficiently cropped in Portugal. To achieve this, different citric acid concentrations were added to the extracted juice allowing to promote inulin hydrolysis while performing water evaporation until obtain a syrup of 73 °Brix. Citric acid promoted the thermal depolymerization of inulins, with an initial degree of polymerization up to 40, into different FOS structures as 1-kestose, nystose and 1,1,1-kestopentaose. The proportion of these compounds (3-20% of syrups dry weight) in relation to the amount of free sugars (glucose+fructose) decreased with the increase of citric acid concentration. The more acidic syrups were also less sticky and presented lower stringiness than those of higher pH, but still are comparable to the texture properties of commercially available syrups from inulin rich sources as yacon and agave. The sweetness index of FOS syrups was also equivalent to the commercial samples and competitive to that of sucrose. In this way, yacon inulins can be used to produce sweet tasting FOS-rich syrups of low caloric value and with distinct textures, suitable for tailored sugar reduction in foods.

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Red tomato vs. yellow tomato: which is healthier? A comparative study of nutritional and antioxidant traits of tomato farmer's varieties

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The health benefits of tomato (*Solanum lycopersicum* L.) are unquestionable. It is a key component of the Mediterranean diet rich in vitamins and antioxidants able to protect against degenerative diseases associated to oxidative stress and inflammation.[1] Today, there is a large number of tomato cultivars and varieties with a wide range of morphological and sensorial characteristics. In Trás-os-Montes, Northeastern Portugal, local populations still prefer to consume tomato farmer's varieties for their distinctive taste and health-promoting effects, as they are grown using extensive farming techniques.[2] Therefore, this study was carried out to characterize the nutritional composition and *in vitro* antioxidant activity of two tomato farmer's varieties known as 'round' and 'yellow', which are shown in **Figure 1**. The analyzed components included proximate constituents (protein, fat, and ash, which were determined by official methods of food analysis, and carbohydrates were calculated by difference), free sugars (quantified by high-performance liquid chromatography (HPLC) with refractive index detection), fatty acids (analyzed by gas chromatography coupled to flame ionization detection), lipophilic antioxidants (carotenoids and tocopherols, determined by a spectrophotometric method and HPLC-fluorescence detection, respectively), and hydrophilic antioxidants (vitamin C was quantified by redox titration with 2,6-dichloroindophenol and phenolic compounds were characterized by HPLC with photodiode-array detection and mass spectrometry (HPLC-DAD-ESI/MS). In addition, tomato methanolic extracts were screened *in vitro* for their reducing power, DPPH radical scavenging activity, β-carotene bleaching inhibition capacity, and thiobarbituric acid reactive substances formation inhibition capacity. The 'yellow' tomato variety revealed an interesting nutritional composition, characterized by higher levels of fructose, glucose, α-linolenic acid, and tocopherols. In turn, the so-called 'round' tomato contained higher amounts of lycopene, β-carotene, and phenolic compounds and proved to be the most powerful in antioxidant activity.[2,3] A *cis p*-coumaric acid derivative was identified as the most abundant phenolic compound in both fruit, while quercetin pentosylrutinoside was the major flavonoid.[3] Overall, both tomato farmer's varieties proved to be rich in nutrients and health-promoting compounds.

Figure 1: Transverse and longitudinal sections of the 'yellow' and 'round' tomato farmer's varieties.

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Nutritional and physicochemical analysis of quince from Cova da Beira region: similarities, differences and particularities

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Quince, a fruit from the autumn season of the quince tree (*Cydonia oblonga* Miller), has a considerable nutritional value (e.g., pectin, vitamins C and B complex, minerals or chlorogenic acids). On the other hand, this fruit is characterized by having a hard, rough-looking pulp with a bitter and astringent taste. Since quince is not consumed raw, is mainly used in the production of jams and marmalades. Despite being easy to grow and weather resistant, its production is often neglected and undervalued. Thus, the study objective was to value quinces from the Cova da Beira region by the physicochemical and nutritional characterization, substantiating by how different production years and localization affect quince varieties properties.

In this study, a nutritional analysis was made (moisture, protein, fat, ash, fiber, sugars – sucrose, glucose, fructose, and minerals – calcium, copper, iron, phosphorus, magnesium, manganese, potassium, sodium, zinc, n= 10) and quality/physicochemical (weight, size, color (CIE L*a*b*), total soluble solids, pH, acidity and texture – hardness, elasticity, resilience, cohesiveness and chewability, n=100) of the three quince varieties (Gigante de Vranja, Portugal and Galega) harvested in 2020 and 2021 in the Cova da Beira region (two different locations).

Overall, Cova da Beira Quinces had a composition 75.3-82.4% of water (g/100g), 14.8-21.6% of carbohydrates, 1.7-2.4% of fiber and 0.3-0.5% of ashes, considering the different varieties and production years. However, the studied varieties showed significant differences in most parameters depending on the variety, location and production year. The principal components analysis allowed to highlight the composition of minerals as well as the sugar content and profile, as differentiating parameters. On the other hand, the sample grouping with identical nutritional composition does not seem to be related to the fact that they come from the same growing place or variety. The analysis of the two years' cultivation showed that the samples continued to be grouped in a similar way, revealing a lower influence of this variable. As for the physicochemical analysis, quinces had significant differences mainly in the color, in the soluble solids content and in the texture. Thus, here were able to show that Cova da Beira Quince's present variability in terms of composition depending on the variety, place and year, which can be explored for new quince product applications.

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Extraction of chlorophylls from the aerial parts of carrots (*Daucus Carota* L.) for the development of alternative natural colorants

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The food industry has a great interest in the use of wastes and by-products that are not used by the industrial food sector from which they are originated, since they cause economic losses and high environmental pollution. For being a rich source of nutrients and bioactive compounds, such as chlorophylls, some bio-residues can find application as natural food colorants. These molecules have potential to be used in the development of functional foods and nutraceuticals, allowing the enrichment of a product with benefits for human health. In this context, two types of extraction were performed from the aerial parts of carrot, where the parameters involved in each extraction method were varied to maximize the extraction yield of chlorophylls: in maceration, the extraction time and solvent, and in ultrasound-assisted extraction, the power and solvent. On the other hand, in order to prioritize environmentally friendly processes, green solvents (water, 90% ethanol, and hexane) were used. Extractions were performed protecting the samples from light and the results were obtained using a newly developed chromatographic method through high performance liquid chromatography (HPLC) coupled to a diode array detector (DAD) and mass spectrometry (MS).

Ultrasound-assisted extraction allowed a higher recovery of chlorophylls than maceration (**Figure 1**), with ethanol revealing a higher extraction capacity than the other solvents. The extractions using 400, 200, and 100 W of power allowed concentrations of 110.4±0.4, 36.60±0.02, and 29.7±0.1 µg/mL, respectively. Chlorophyll a was common to all extracts, being the only compound detected in the 100 W extraction. With increasing power, a higher extraction of compounds was evidenced, specifically chlorophyll b (at 200 and 400 W) and pheophytin a (at 400 W). Chlorophylls were not detected in quantifiable concentrations in the hexane extracts with the US technique. As for the extractions with maceration and ethanol as solvent, the 60 min extraction allowed the extraction of a total amount of chlorophyll of 12.11±0.03 µg/mL, while the 120 min extraction extracted 10.77±0.03 µg/mL. Chlorophyll b and pheophytin a were more abundant in the extraction performed for 60 min, while the chlorophyll derivative was detected in a higher concentration in the extraction performed for 120 min. Compared to water and hexane, ethanol allowed the extraction of higher amounts of chlorophylls. This study can serve as a basis for further research on the best conditions for the extraction of chlorophylls with high coloring capacity; on the other hand, it was demonstrated that the aerial parts of carrots have great potential to be used as sources of natural pigments not only in the food industry, but also in other industrial fields.

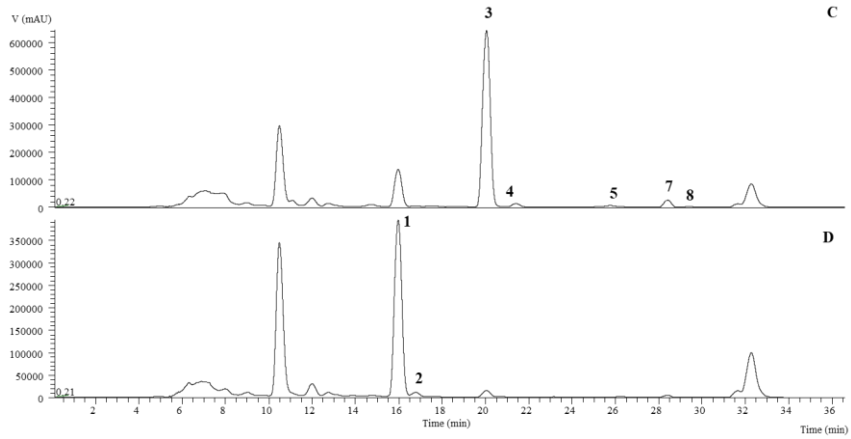

Figure 1: Chromatogram of the phenolic profile, recorded at 430 nm (A) and 470 nm (B), of the ethanolic extract of the aerial part of carrot, obtained from the US extraction using 400 W.

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Chemical and bioactive characterization of *Euterpe oleracea* Mart.

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Besides the economic factor, açaí has been highlighted for its nutritional value, but also for its richness in α-tocopherol (vitamin E), phenolic compounds and for having interesting bioactive potential[1]. Therefore, the objective of this work was to perform the chemical and bioactive characterization of freeze-dried açaí pulp. Organic acids were determined by HPLC-DAD, tocopherols by HPLC-fluorescence, and phenolic compounds using HPLC-DAD-ESI/MS. The antioxidant potential was assessed through cellular antioxidant activity (CAA), thiobarbituric acid reactive substances (TBARS), and radical scavenging activity (DPPH) assays; the antimicrobial capacity was evaluated using the microdilution method against pathogenic microorganisms, cytotoxicity against tumoral and non-tumoral cell lines by the sulphorhodamine B assay, and the anti-inflammatory potential using RAW cells.

Citric acid (3.36 ± 0.07 g/100 g DW) was the most abundant organic acid, being fumaric acid found in the lowest concentrations (0.0117 ± 0.0001 g/100 g DW). Alpha and beta tocopherols were identified, with contents of 0.046 ± 0.001 mg/100 g DW and 0.17 ± 0.12 mg/100 g DW, respectively. Regarding the phenolic composition, taxifolin-*O*-deoxyhexylhexoside was the main compound (4.34 ± 0.03 mg/g of extract), followed by sinapoyl hexoside (2.27 ± 0.04 mg/g), quercetin-3-*O*-rutinoside (2.21 ± 0.06 mg/g) and finally isorhamnetin-3-*O*-rutinoside (0.54 ± 0.02 mg/g), presenting a total of non-anthocyanin phenolic content of 9.36 ± 0.15 mg/g of extract. Also, five anthocyanin compounds were identified, totaling 11.99 ± 0.22 mg/g of extract, where the compounds cyanidin-3-*O*-glucoside (4.72 ± 0.20 mg/g) and cyanidin-3-*O*-rutinoside (4.54 ± 0.03 mg/g) presented the highest levels, with lower, but significant, contents for pelargonidin-3-*O*-rutinoside (0.60 ± 0.01 mg/g).

For the antioxidant potential, EC_{50} values of 270 ± 5 µg/mL in the DPPH and 61 ± 2 µg/mL in the TBARS assays, and 61% of oxidation inhibition at 2000 µg/mL in the CAA assay were obtained. Concerning the antimicrobial capacity, tested against food borne and clinical pathogens, the açaí extract was more active against Gram positive bacteria, with inhibition concentrations ranging from 1.25 to 10 mg/mL. As for cytotoxicity, the extract revealed activity against breast carcinoma cells (MFC-7) with IC_{50} values of 255 ± 22 µg/mL, while no significant effect was found against lung carcinoma cells (NCI-H460) and the normal cell culture (PLP2), for which values above the maximum concentration tested were found (>400 µg/mL). The extract also revealed anti-inflammatory activity with EC_{50} values of 384 ± 11 µg/mL.

Taking in consideration the results obtained in the range of analyses performed, it can be concluded that the açaí pulp is an interesting source of bioactive molecules, highlighting its the antioxidant activity. Therefore, this fruit could be exploited for the development of functional formulations, besides being a candidate to explore as a natural source of colouring or preservative agents.

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The effect of the drying process on the composition of two varieties of prickly pear (*Opuntia ficus indica*)

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Opuntia ficus-indica (L.) Mill (OFI) is a species of cactus that grows in arid and semi-arid areas. Its fruits, known as prickly pears, are part of the diet of several countries, such as the Mediterranean countries and Mexico due to its richness in nutrients, containing various compounds with beneficial effect for human health [1,2]. After harvest, prickly pears present a relative short time to be consumed and when preserved as fresh under refrigeration condition, can suffer chilling injuries leading to a faster degradation. The rise in the production of OFI raises the need for better preservation techniques to increase the shelf life of figs. Drying is a processing option to help preserving the fruit and to increase its shelf-life, keeping it available for a longer period than compared with the fresh product, so that it can reach different markets [3].

Thus, the main goal of this work was the study the effect of the drying process on the composition of two varieties of prickly pear. Different temperatures were tested, 50, 60, 70 ºC. Drying the figs at 60ºC demonstrated to have less damaging effects, with less changes observed on the chemical profile of the pulp. The results of the figs dried at 60ºC showed lower acidity and higher content in ascorbic acid than the figs dried at 50ºC and 70ºC, and also the results obtained in terms of phenolic content and antioxidant activity were better or similar to the ones presented by the figs dried at 50ºC and 70ºC. Therefore, this temperature was chosen to dry sliced OFI fruits from two different varieties (orange and red), with or without peels. Dried fruits were kept at room temperature for 6 weeks, and chemically analysed every week in terms of pH and total acidity, ascorbic acid, and bioactivity (total phenolic compounds, antioxidant activity-DPPH assay, betanin and indicaxanthin content).

During the storage period the fruits acquired moderately acidic characteristics with a pH below 7 (pH variation of 5.2 - 5.8). Although with some loss of antioxidant activity, it appears that the dehydrated fruits were stable throughout this period, in physical-chemical terms. The drying process is presented as a low budget technology, accessible and able to promote the increment of the shelf-life of the fruits.

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Wheat-based canned products nutritional properties

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The importance of a varied diet for health, preventing disease and/or nutrient deficiency is unquestionable.[1] At their base, bread and cereals provide valuable protein and complex carbohydrates (particularly starch and fiber), as well as important B vitamins and iron, and are generally cholesterol-free and rich in essential amino acids. However, the inclusion of vegetables, fruits, and meat in the diet is a common way to enhance it by ensuring the intake of other valuable nutrients.[2] Although the consumption of fresh foods is highly recommended, it is often not possible, and canned foods are an option for their replacement, presenting a longer shelf life and being a more economical and accessible option, thus assisting in ensuring the necessary intake of nutrients that should be daily ingested.[3]

In the present study, two organic canned sprouted wheat products were characterized in terms of nutritional value according to AOAC procedures and chemical composition, namely in free sugars (HPLC-RI). One of the products contained organic sprouted wheat, braised organic sprouted wheat, water, olive oil, black garlic, and salt, and the other contained organic sprouted wheat, braised organic sprouted wheat onion, pumpkin, carrot, red bell pepper, turnip, dehydrated garlic, and vegetable broth (**Figure1**).

Regarding nutritional value, the distribution of macronutrients was similar in both samples, with carbohydrates standing out, followed by fiber, and proteins and lipids with similar values. The samples also showed high moisture content, with canned wheat and vegetables having the highest content, and the energy value was higher for canned wheat and black garlic. In terms of free sugars, fructose, glucose, sucrose, maltose, and raffinose were identified in both samples, with maltose being the most abundant sugar, and sucrose and fructose showing the lowest contents for canned wheat and black garlic and canned wheat and vegetables, respectively. In conclusion, these organic sprouted wheat preserves showed an important nutritional and chemical composition for the maintenance of a varied and balanced diet, and their inclusion in the diet can help prevent possible nutritional deficiencies.

Figure.1 Canned wheat and black garlic (left), canned wheat and vegetables (center). Photo on the right is an example of the canned wheat and vegetables before adding vegetable broth.

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Interactions between beer phenolic compounds and human salivary proteins: insights toward astringency and bitterness perception

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Phenolic compounds attract special interest due to their health benefits by having antioxidant activity and preventing cardiovascular and neurodegenerative diseases. [1] Transversely, some of these compounds are responsible for several -organoleptic features of plant-based foodstuffs, such as color and taste properties (*e.g.*, astringency and bitterness). Regarding consumers preferences and choices, color is one of the first and most significant quality parameter in which a certain food product could be rejected by a depreciative visual perception. Astringency and bitterness are commonly perceived as unpleasant, however there are a few differences on the physiological mechanisms toward these two taste properties perception. [2]

Astringency refers to a puckering, drying, and rough sensation in the oral cavity. While, bitter taste perception occurs through activation of specific membrane G protein-coupled receptors, namely bitter taste receptors (TAS2Rs), the molecular mechanism of astringency is still a debated topic. The interaction and precipitation of salivary proteins (SP), mainly proline-rich proteins (PRPs), by PC is often believed as the major mechanism toward astringency perception. [2]

During the last decades, researcher have been attempting to find a relationship between instrumental and sensory data, to better understand the actual human perception. In fact, there are few research papers that could relate sensory data with instrumental data, and several correlations were reported between taste perception and instrumentally measured taste molecules. In fact, Luna *et al.* (2002) established a positive correlation between phenolic compounds and the perception of bitterness, astringency, and green notes in cocoa samples. [3] This work is focused on deepening the knowledge on astringency and bitter taste perception toward phenolic compounds interaction with salivary proteins. To achieve this, this study was divided in two major aims: *i*) to study the interaction of human salivary proteins with beer phenolic compounds; and *ii*) to identify and characterize the phenolic compounds present in different types of beer. This work will provide more insights about the putative influence of these compounds on taste properties of beer.

For this purpose, a beer sensorial study was firstly performed with a well-trained panel for these two taste modalities. For this study, sensorial and instrumental data were collected to attempted to corelate them with the actual human taste perception.

For the instrumental analysis, the total concentration of salivary proteins non-precipitated was determined, in mg/mL, at each time of the sensorial test. Regarding to experimental data analysis, the panelists were divided into three groups, according to their total salivary protein concentration: *i*) group with low salivary proteins content (control saliva with less than 1 mg/mL of SP), and *ii*) group with medium salivary proteins content (control saliva with between 1 and 3 mg/mL of SP), *iii*) group with high salivary proteins content (control saliva with more than 3 mg/mL of SP).

The interaction between PC and SP was assessed through the determination of non-complexed SP after PC-SP interaction and consequent precipitation. It was possible to establish that these two taste modalities depend also on the levels of complexed SP precipitation and on the SP production (saliva stimulation), through if it occurs only partial precipitation of SP; total precipitation and consequent production of SP. A plethora of possible case-scenarios for the differences among astringency and bitterness perception was observed by the studied panelists, and a positive correlation between sensorial and instrumental analysis was established.

Overall, the obtained results suggest that the different groups of salivary proteins could be related to the astringency and bitterness perception. Therefore, the precipitation and/or production of three different salivary proteins, precisely, bPRPs, aPRPs and Statherin/P-B peptide, suggested that these proteins could affect bitter taste perception. Moreover, a positive correlation between sensorial and instrumental analysis were performed, indicating that the instrumental analysis used in this study are a feasible and reliable method for study the astringency and bitterness perception of beers.

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Application of edible coatings, supplemented with extracts of macroalgae and halophyte plants, in fillets of mackerel (*Scomber scombrus*) to reduce fat content in frying processes

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Fried foods have high acceptance worldwide due to its attractive sensory properties, namely crispy texture, golden crust, and pleasant taste. However, frying is a complex process, encompassing numerous degradative reactions (oxidation, hydrolysis, polymerization, and isomerization) due to the action of oxygen, high temperature and the water released by the food [1,2]. Also, during frying a significant oil uptake by the food products is observed, increasing their fat content with deleterious consequences on consumer health. Therefore, green technological approaches are required to minimize these constraints. This study aimed at developing edible coatings for application in pre-frying Atlantic mackerel fillets (*Scomber scombrus*), in order to (i) reduce oil uptake, (ii) minimize water loss, preserving the succulence of fried fish and (iii) preserve the quality of fish fat by reducing its oxidation. For this purpose, edible coatings based on alginate or carrageenan were prepared with ethanolic extracts of the brown macroalgae *Pelvetia canaliculata* or with aqueous: ethanolic extracts (1:1, v/v) of the invasive halophyte plant *Carpobrotus edulis,* that were used as source of antioxidant compounds. Antioxidant activity of coatings was evaluated by the total phenolic content, following the Folin-Ciocalteu method and by the DPPH method as described by Neves et al [3]. The frying fillets were characterized regarding color, texture, moisture, water activity, ash content, lipid content, thiobarbituric acid (TBA) index, and fatty acid profile. Fried fillets with coatings supplemented with *C. edulis*, particularly those made of alginate, showed to be the most efficient in reducing oil absorption to about 30%, in comparison with control sample (fried fish without coating), and in minimizing water loss, allowing to obtain juicy fillets with lower lipid content. No significant differences were observed in the texture and color of the fillets, compared to the control sample, as well as in the TBA index, which indicates a preservation of fish fat quality.

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Valorisation of halophyte plants produced in Portugal: from nutritional value to bioactivity

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Salinity stress is one of the major abiotic problems affecting agriculture (30% of the irrigated crops and 7% of dry land crops)[1]. The osmotic stress and the ionic toxicity caused by the soil salt content impairs the water and the essential nutrients absorption by the plant root system which consequently imposes restrictions to the plant development[1]. The halophyte plants have the ability to survive and complete their life cycle in a soil salt concentration ≥ 200 mM of NaCl. These salt-tolerant crops, with environmental advantage in soils characterized by high salt content and reduced water quality can be used as viable crops in adverse environments for the majority of traditional crops. Despite of the reduced number of studies reporting the nutritional and phytochemicals composition of these plants[2], the food market increased their demand for these plants. More than a potential alternative source of salt in the diet, the halophyte plants have phytochemical added value, with interest for the development of novel food products but also with interest for the dermo-cosmetic industry, pharmaceutical industry, and agricultural sector.

For the present study seven different halophyte species produced under hydroponic conditions by RiaFresh® in Portugal (*Salicornia ramosissima*, *Sarcocornia fructicosa*, *Mesembryanthemum cristallinum*, *Mesembryanthenum nodiflorum*, *Carpobrotus edulis*, *Inula crithmoides* and *Chritmum maritimum*) were characterized in terms of their nutritional and phytochemical compositions. From the seven different species, *S. fructicosa* highlighted as the one with the highest protein and total ash contents (4.44 ± 0.17 and 5.70 ± 0.23 g/100g FW), as well as the one with the highest salt content (2.80 ± 0.36 g/100g FW), being characterized not only by the highest levels of sodium (1120 ± 145.60 mg/ 100g FW) but also by the highest levels of potassium (400 ± 84.00 mg/ 100g FW).

Using colorimetric spectrophotometric assays and liquid chromatography coupled to mass spectrometry it was possible to characterize the phenolic composition of the ethanol: aqueous extracts prepared from the halophyte plants as described by Oliveira et al., 2021[3]. Data showed that *Salicornia ramosissima* had the highest total phenolic content (TPC), 410 µg GAE/g FW, followed by *Sarcocornia fructicosa* (330 µg GAE/g FW). In *S. ramosissima* 78% of the phenolic compounds were derivatives of caffeoylquinic acid and in *S. fructicosa* there were a diversity of compounds, including derivatives of rhamnetin (24%), isorhamnetin 3-O-robinobioside (18%), caffeoylquinic acid derivatives (17%), and p-coumaric acid derivatives (16%). The higher phenolic content and phenolic compounds' diversity in these halophyte plants could explain their higher antioxidant activity measured by ORAC (Oxygen Radical Antioxidant Capacity) and HOSC (hydroxyl radical scavenging capacity). Regarding the antihypertensive activity, *C. maritimum* showed the highest antihypertensive activity with ACE (angiotensin converting enzyme) inhibition values of 561.50 ± 21.35 mg/mL.

Based on the phenolic composition, *Salicornia ramosissima* and *Sarcocornia fructicosa* were selected for the bioaccessibility study and the phenolic composition, in the oral, gastric, and intestinal fractions further analysed, for the first time, after applying the static INFOGEST *in vitro* digestion model. The acidic hydrolysis promoted by the acidic conditions during the gastric phase contributed to increase the release of phenolic compounds and therefore the phenolic compounds' bioaccessibility (e.g., 33% and 169% of the corresponding content determined before digestion for the caffeoylquinic acid, and for the gallocatechin, respectively). Future analysis with dynamic models of digestion, including colonic fermentation, should be conducted to further investigate the impact of gut microbiota on phenolic compounds bioaccessibility and bioactivity.

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Impact of origin on the nutritional evaluation of dark chocolates from Africa and America

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Dark chocolate can be defined as a dispersion of sugar and cocoa solids in a fat continuous phase where the physicochemical and microstructural properties of chocolate depend on several factors such as genetic, agricultural practices, postharvest procedures but also the geographical origin. Previous studies have reported such influence of geographical origin of cocoa on peptide profile, bacterial diversity, volatile compounds, fatty acid content and proximate analysis [1,2,3]. Hence, the aim of this work is to study the influence of the geographical origin in the nutritional characterization of single origin dark chocolate. Seven samples of dark chocolate were acquired locally, namely Cuba (70% cocoa), Brazil (66.8% cocoa), Madagascar (67.4% cocoa), Mexico, (66% cocoa), Dominican Republic (70% cocoa), São Tomé (70% cocoa) and Venezuela (73.5% cocoa). Chemical analysis included nutritional profile, fatty acids, total phenolic content (TPC), methylxanthines, sugars, organic acids, β-carotene and vitamines. Results were subjected to statistical analysis where the significance level was set to 1%. In order to use one-way analysis of variance, assumption of normality was previously tested by using Shapiro-Wilk's test[1]. The homogeneity of variance was tested by performing Levene's test. When assumptions had been met, post-hoc single-step multiple comparison Tukey's HSD test was employed to investigate significant differences between mean values, while in case of heterogeneity of variances, Welch's test was used, along with Dunnett's T3 test for determination of all possible pairwise contrasts. A Principal Component Analysis (PCA) was used to study the inter-sample and inter-variable (nutritional attributes) relationships. The obtained results presented an energy content from 473.67 to 520.33 kcal/100g, while fat content ranged from 30.6% to 35.9% (w/w) and were composed mainly by saturated fat, similar to previous works[3]. In a smaller content, PUFA presented values between 2.93% and 3.62% of total fat content, higher in Brazil, São Tomé and Madagascar ($p < 0.01$). Brazil presented the lowest thiamine and the highest riboflavin values. The results of γ-tocopherol ranged between 1.41 and 2.57 mg/100g, higher in Brazil, while α-tocopherol ranged from 0.19 to 0.61 mg/100g, lowest in Venezuela ($p < 0.01$). The highest TPC value was observed in Madagascar, around 1290 mg GAE/100g, whereas the lowest were observed in Dominican Republic (719 mg GAE/100g) and Cuba (673 mg GAE/100g). Theobromine presented values between 1.11 mg/100g and 1.73 mg/100g, with no influence from geographical origin. Caffeine presented lower values than theobromine, ranged from 0.09 mg/100g to 0.30 mg/100g, higher in Venezuela. As expected, sucrose presented higher values than fructose, consequence of the production process, where the highest value was observed in Mexico (33.81%). Fructose ranged from 2.22% to 2.89%, highest in Mexico and lowest in Dominican Republic. Consequence of the post-harvest biochemical changes, samples exhibited a predominance on citric acid, between 0.73 and 2.30% (w/w), higher in Brazil, Madagascar, and Dominican Republic. Acetic acid presented values between 0.57 mg/100g and 1.76 mg/100g, whereas lactic acid values ranged from 0.58 mg/100g to 0.72 mg/100g. Also, oxalic acid and malic acid were detected, in an amount lower than 1.05 mg/100g, being associated with the development of sensorial properties. The PCA analysis included the attributes PUFA, sucrose, caffeine, TPC, citric acid, thiamine, α-tocopherol, γ-tocopherol, β-carotene and theobromine (**Figure 1**). It can be observed that cocoa from Brazil presented a higher score of theobromine and γ-tocopherol, while Madagascar is associated with higher scores of PUFA, TPC and α-tocopherol. Dominican Republic and Venezuela are associated with higher scores of caffeine. Cuba is associated with a higher score of thiamine. Finally, São Tomé and Mexico are associated with higher scores of citric acid, sucrose and β-carotene[1].

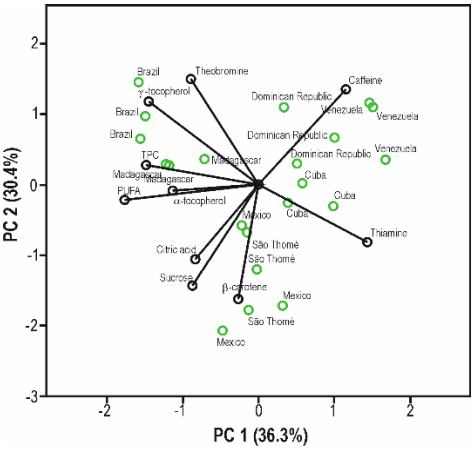

Figure 1: PCA biplot showing the objects scores (countries) and component loadings (chocolate nutrients).

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Nutritional characterization of gilthead seabream (*Sparus aurata*) fed with *Pelvetia canaliculata* supplemented diet: a biorefinery approach for seaweed biomass valorization

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Feed can represent up to 50% of the overall production costs in intensive aquaculture regime. The use of by-products from the food industry may be a sustainable way to increase the nutritional value of fish feed while reducing production cost. This study was carried out within the scope of the Ocean2Oils project that, following the concept of biorefinery, aims to implement a sustainable and integrated process for exploring edible seaweeds as a source of natural antioxidants, hydrophilic biopolymers, and other components for specific purposes. *Pelvetia canaliculata*, a brown seaweed widely scattered in the Portuguese coast was used as source of bioactive compounds for edible oil supplementation, aiming at increasing its nutritional quality and oxidative stability. The freeze-dried seaweed was added to sunflower oil for ultrasound (US) assisted extraction of antioxidants directly into the oil and was recovered by centrifugation [1]. It is expected that the residual seaweed biomass, left after the extraction of antioxidant-rich fraction, still possesses a good nutritional value, namely as protein and fiber sources, that may be applied as ingredient in aquaculture feed formulations. The use of *Pelvetia canaliculata* powder (1, 5 and 10%, m/v) and algae waste obtained after sunflower oil supplementation (1 and 10%, m/v) was evaluated in aquafeeds for gilthead seabream (*S. aurata*) juveniles fed for 44 days. Several parameters were analysed in the fish muscle, namely: (i) moisture (AOAC, 2016; method 930.15); (ii) ash content (AOAC, 2016; method 955,04); (iii) total protein by the Kjedahl method using a conversion factor of 6.25 (following AOAC, 2016; 940.25), (iv) total lipid content by gravimetry according to Folch et al [2] and (v) fatty acid profile by GC-FID analysis according to Fernandes et al. [3]. Furthermore, some healthy fat quality indices were determined, such as: atherogenic (AI), thrombogenic (TI), nutritive value (NVI), peroxidizability (PI) and Hypocholesterolemic/hypercholesterolemic (h/H) indexes and n6/n3, UFA/SFA and PUFA/SFA ratios. The results attained in this study showed that the different diets for gilthead seabream juvenils didn´t change the proximate composition of fish muscle, in comparison with the control sample (isoproteic and isolipidic standard diet without *P. canaliculata*). However, the diet supplemented with 10% (m/v) of algae waste clearly influenced the fatty acid profile of fish muscle, leading to a decrease in saturated fatty acids and an increase in monounsaturated fatty acid content of fish muscle. This diet also influenced UFA/SFA and n6/n3 ratios and h/H, AI, NVI and PI indices, showing to be a healthier formulation for gilthead seabream aquafeed. Nevertheless, none of the remaining diets changed the nutritional composition and fatty acid profile of fish muscle in comparison with control sample. Therefore, *Pelvetia canaliculata* can be used as supplement in gilthead seabream diets, being a sustainable strategy for waste algae application following a circular economy approach.

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Chemical characterization of almond varieties natives from Algarve region

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Almond, *Prunus dulcis* (Miller) D.A. Webb, syn. *Prunus amygdalus* Batsch is an important crop due to its fruits with high comercial value. In Portugal, there are traditional orchards in which almond trees grew on marginal soils, rainfed and with consequent low productivity; more recently huge investments in modern orchards with varieties originally from France and Spain such as 'Ferraganés', 'Ferraduel', 'Guara', 'Marcona', among others, are replacing the Portuguese varieties[1]. So, it is important to preserve the traditional almond varieties and the purpose of the present study was to compare the chemical composition of 18 traditional cultivars maintained in the field collection with those commercial cultivars from Europe.

A Principal Component Analysis (PCA) was performed considering moisture, protein, ash, fat content, carbohydrates of each cultivar studied. Factor 1, which explained 56% of the total variability, explained the variation observed between energy, lipids, carbohydrates and ash content, with samples with higher lipid content and energy having lower carbohydrate and ash content. Factor 2, which represented 23% of the total variation, was explained by the variation in moisture and protein content. The cultivars Bonita, Lourencinha, Zé de Oliveira and Bonita do Caliço, all Portuguese cultivars formed the group of samples with the highest values of lipids and energy and lower levels of carbohydrates and ash. An inverse relationship is observed for the sample Convento, which has the highest carbohydrate content and nutritional composition that differentiates from the other samples.

The results of the PCA for acidity value (VA), peroxide value (PV), K_{270} and K_{232} of almond oils showed that K_{270} and K_{232} were the ones that most contributed to the discrimination of samples along the factor 1, which explained 53% of the total variability. Factor 2, which explained 30% of the total variability, discriminated the samples mainly by the PV. The profile of monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs) in almond oils showed that MUFAs are the most important group of fatty acids ranging from 66.23 to 82.55%.

The differentiation of the cultivars was also accessed by RAMAN spectroscopy, and the differentiation observed with the PCA performed with spectral data are quite similar to those obtained for the chemical composition.

As main conclusion, the different varieties of Algarve almond present a higher quality and different characteristic according to the different cultivars. In some cases, it is possible to distinguish by RAMAN spectroscopy, some cultivars according to their chemical composition.

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Effect of moisture on the characteristics of hazelnut kernel during storage

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The organoleptic and nutritional quality of hazelnuts during storage can be greatly influenced by environment conditions. The aim of this study was to evaluate the effect of moisture on the physicochemical characteristics of hazelnut kernels when stored under controlled conditions of temperature (25 °C) and different relative humidities (RH) (57.6, 68.9, and 78.6%). To obtain these RH in the hazelnut storage atmospheres, the salts NaBr, KI, and NH4Cl were used, respectively. The following properties were evaluated: moisture, water activity (a_w), color, texture, and fat oxidative stability, both in the initial sample and after 1, 2, 4 and 6 months of storage. The results showed that there was a progressive increase over time in moisture and water activity, with growth being most pronounced from 4 months onwards. Moreover, hazelnuts stored at a RH of 78.6%, at 6 months of storage were completely spoiled, making their evaluation of impossible. The hardness and friability of the hazelnuts decreased over the storage time, with the differences being more pronounced for the samples subjected to higher RH. In general, the color of the skin and core of the fruits showed little variation over the storage time for all parameters evaluated (L*, a*, b*) and for all RH conditions tested. The hazelnuts stored at a RH of 78,6% could only be preserved until the 4th month, but presented low oxidative stability, being comparable to the hazelnuts with 6 months at RH of 57,6%, meaning that with a reduction of about 10% of RH it is possible to increase the oxidative stability about 2 months. Thus, it can be concluded that high relative humidities lead to a lower conservation of the quality characteristics of hazelnuts. Moreover, during the 6 months of storage the color of the fruits was little affected for the different RH tested, however all the other evaluated characteristics presented significant alterations, namely the texture and the oxidative stability of the fats.

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Hydrolysable tannins in aged wine spirits: a fresh perspective using alternative ageing technology and high-resolution mass spectrometry

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Wine spirits (WSs) are usually aged in wooden barrels, but using wood pieces instead of barrels, with or without micro-oxygenation, is a technological alternative that has been investigated by our team. The current research was focused on identifying ellagitannins, their derived species, and the evolution of degradation pathways. For this propose, in this study, the behavior of hydrolysable tannins in a wine spirit aged in 50 L demijohns with chestnut wood staves and three levels of micro-oxygenation or nitrogen, was examined.[1,2] Gallotannins and ellagitannins were identified by LC-ESI-HRMS/MS using a Q-TOF in samples collected at 8, 21, 60, 180, 270, and 365 days of ageing, and their relative abundances compared according to the ageing technology. The studied compounds derive from the wood and have a significant sensory impact in the aged wine spirits due to their association with astringency, which is closely related to the quality of these beverages. For the first time, the importance of oxygen in gallotannins and ellagitannins formation/degradation pathways in WS was established, and results aided to explain the steady increase in gallic and ellagic acid contents on WS during ageing. The results also highlighted the presence of penta-O-galloyl-β-D-glucose, tetra-O-galloyl-β-D-glucose, tri-O-galloyl-β-D-glucose, di-O-galloyl-β-D-glucose, and mono-O-galloyl-β-D-glucose, 2,3-(S)-hexahydroxydiphenoyl-β-D-glucose, pedunculagin, isomers vescalagin/castalagin, as well as two products stemming from ethanol-promoted oxidation of castalagin/vescalagin and vescalin/castalin, in the WS aged with chestnut wood (**Figure 1**).

This study provided a better understanding of ellagitannin derivatives and determined their presence on WSs, which are associated with an increase in gallic and ellagic acid concentrations during ageing.[2]

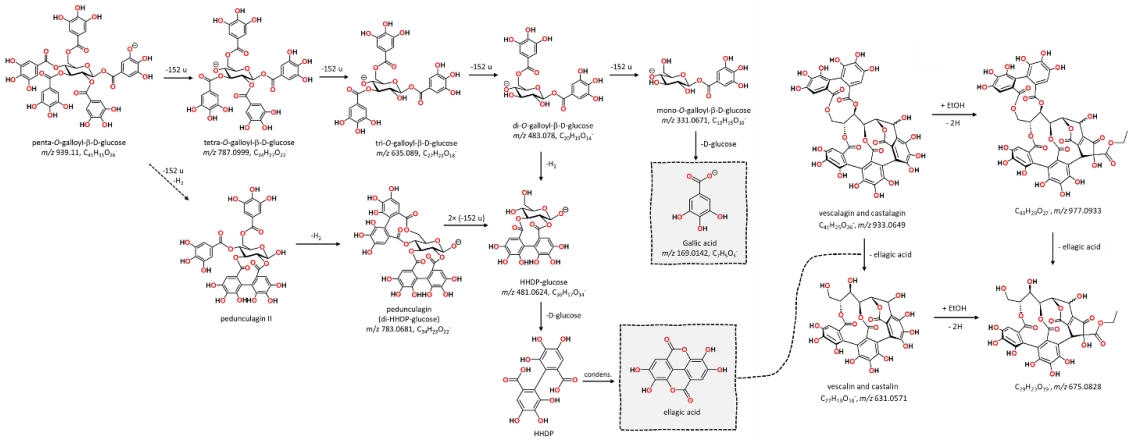

Figure 1. Proposed fragmentation patterns and degradation pathways of gallotannins and ellagitannins. Structures of vescalagin/castalagin isomers and their degradation pathway for the two derivatives that were tentatively assigned to the ethanol-promoted oxidation products of vescalagin/ castalagin and vescalin/castalin.

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Rodrigues from AZ3Oeno Portugal, Sílvia Lourenço, João Amaral, Deolinda Mota from INIAV—Polo de Dois Portos, and A. Pedro Belchior for their technical support.
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Olive oils from the Douro valley: influence of the different sub-region on the quality and composition

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The Douro region, located in the northeast of Portugal, in the Douro watershed, surrounded by mountains that give it particular mesological and climatic characteristics, the region extends over a total area of about 250,000 ha. This region is traditionally known for its wines and landscapes. This region is divided into three naturally distinct sub-regions, not only due to climatic factors but also socio-economic factors: Baixo Corgo, Cima Corgo and Douro Superior. In the last decade, the olive oils produced in this region gained expression due to their distinguished sensory characteristics. Nevertheless, the knowledge about the olive oils produced in this region is scarce, and any relationship was established with the different subregions. In this sense, the present work aimed to study the quality and composition of the olive oils from different locations in the Douro region and to observe if the sub-regions affect the studied attributes. Thus, in the crop season of 2020/2021 a total of 134 samples were collected in the different sub-regions. For each sample, the quality parameters (free acidity, peroxide value, specific coefficients in ultraviolet and sensorial analysis); chemical composition (fatty acids, tocopherols and individual phenols) evaluated by chromatography; oxidative stability determined by the rancimat method; and antioxidant activity, were evaluated. The descriptive sensory profile was determined for the samples classified as extra virgin olive oils. The obtained results show that of the 134 oils collected; only 56 were classified as extra virgin olive oil, 31 as virgin olive oil and 46 as lampante olive oil. These results indicate that the region, from the point of view of quality, needs to train agents linked to olive cultivation in order to improve the quality of the oils since only 42% of the oils are of superior quality. Regarding fatty acids, MUFA were the most predominant acids, varying from 72.6% (Douro Superior) to 73.5% (Baixo corgo); nevertheless, no significant differences were observed between sub-regions. Concerning tocopherols, there was a variation of α-tocopherol of 185.7 mg/kg of olive oil (Baixo Corgo) and 233.4 mg/kg of olive oil (Douro Superior) and no significant differences were observed between the sub-regions. In the determination of the phenolic alcohol, it was found that the oils from Douro Superior are those with the highest concentration of hydroxytyrosol and tyrosol, while Baixo Corgo showed the lowest amounts. Significant differences were observed for stability between sub-regions, ranging between 4.54 h and 12.81 h for Baixo Corgo, 6.11 and 15.64 for Cima Corgo, and 7.28 and 14.15 for the Douro Superior. Regarding antioxidant activity, among the three sub-regions, Cima Corgo was the one that presented, overall, lower DPPH values, with values between 38.59 % and 93.40 % of inhibition, while Baixo Corgo presented values between 64 .52% and 93.16% inhibition and 50.94% and 94.08% inhibition for Douro Superior. The total phenols content showed the same trend as the DPPH, being the sub-region of Cima Corgo, the one that presented, in general, lower values, varying between 104 and 727 mg of gallic acid/kg. The Baixo Corgo and Douro Superior sub-regions presented values between 149 – 537 mg/kg and 137 – 620 mg/kg, respectively. The descriptive sensory profile of extra virgin olive oils showed some variations according to the sub-region. A predominance of ripe fruity was observed in all sub-regions. Nevertheless, in the Douro Superior sub-region a greater amount of olive oils with green fruitiness occurs. In the fruit sensation, tomato, apple, banana and nuts sensations were observed in all sub-regions. Concerning herbaceous sensations, the predominant one was that of tomato leaves in all regions, while cabbage only in the Douro Superior sub-region. Other minor attributes also occur, such as pistachio and kiwi, nevertheless, these attributes appear in less than 15% of the oils, so they are not considered attributes with an important role in the sensory profile of Douro olive oils.

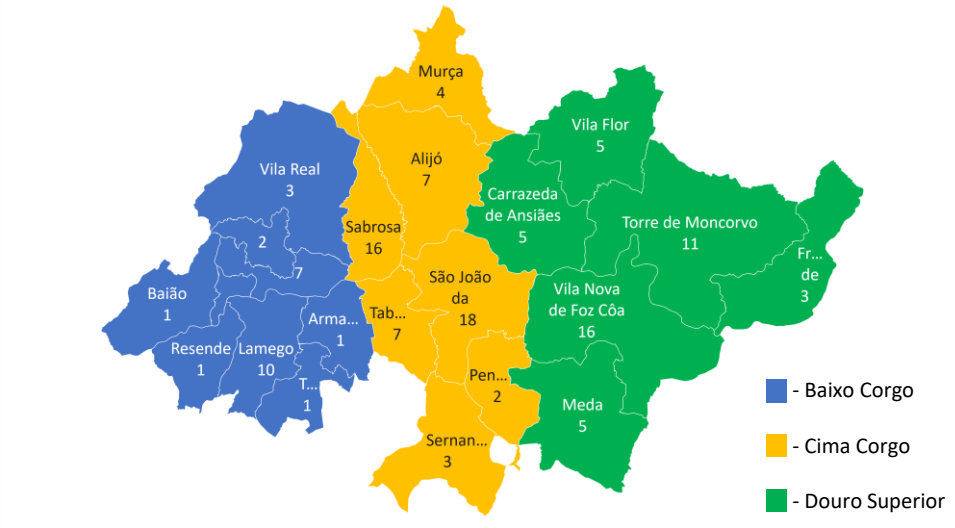

Figure 1: Distribution of olive oil samples collected by different municipalities according to the sub-regions delimited for Port Wine.

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Effect of heat treatment on the quality and composition of canned tuna coating liquid

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Canned tuna is one of the most popular products on the market with great commercial value.[1] It is a product rich in protein, and fat that is mainly unsaturated fatty acids, namely omega-3 as an essential fatty acid. Tuna can be presented and commercialized with different covering liquids. Olive oil is a vegetable oil rich in monounsaturated fatty acids with different organoleptic properties from other vegetable oils.[2] According to the intensity of fruitiness, extra virgin olive oil can be classified as ripe fruity and green fruity, the latter being classified as mild, medium and intense, depending on the intensity. Temperature is one of the factors that degrade the organoleptic characteristics of olive oil.[3] In this sense, the present work aims to study the effect of the application of heat treatment on tuna coating liquids. To this end, different coating liquids were studied (sunflower oil, refined olive oil, mature olive oil, soft green and intense green), and the behaviour with the application of heat was verified. Fifty cans were filled with coating liquid, closed in a crimping machine and pasteurized following the usual procedure in the industry. The filling liquids were analysed before and after heat treatment, namely at time 0 (T0) and at the end of six months (T6), having been stored at room temperature and at 50ºC. Quality parameters (free acidity, peroxide value, specific extinction coefficients), oxidative stability by the Rancimat method and antioxidants (DPPH and total phenols) were evaluated for each sample. Regarding the quality parameters at T0, it was found that most of the values are within the legal limits established by regulation 2568/1991, except for the acidity parameter in mature olive oil, which presented an average value of 0.98%, and also for the sunflower oil in K_{232}, K_{268} and ΔK that exceeds the established value. Regarding the covering liquids in T6 with heat treatment at 50ºC, it was verified in the free acidity that they all exceed the legal limit. In the case of peroxide value, all meet the legal limits and for K_{232} only sunflower oil exceeds the legal limit. In K_{268} the limits are exceeded by all hedging nets. In the case of ΔK, only sunflower oil exceeds the legal limit. There was a clear increase in the parameters: free acidity and peroxide value from T0 to T6 50ºC, where as in the specific extinction coefficients, this increase was not registered. Regarding the oxidative stability, comparing the T0 with the T6 50ºC, it was found that both in the T0 and in the T6 50ºC the sunflower oil was the one with the lowest oxidative stability. At T0, refined olive oil was the one that showed the highest oxidative stability, while at T6 50ºC, the mature green olive oil showed the highest stability. Regarding the antioxidants, namely DPPH, it was found at both times that sunflower oil had the lowest percentage of inhibition and intense green olive oil the highest. The same happened in the total phenols. With this work, it is possible to see that sunflower oil is clearly the liquid coverage that has the lowest qualities for consumers since it was the one that presented the lowest values in all parameters, even not respecting the legal limits of some parameters. It was found that olive oil is, from the quality and benefit to the consumer point of view, the best topping liquid to adopt to accompany tuna, given that even with the application of temperature, there are drastic drops in the proportion of antioxidants. Adopting olive oil as a vegetable covering will make it possible to have a combination of nutritional and organoleptic properties of better quality for the consumer.

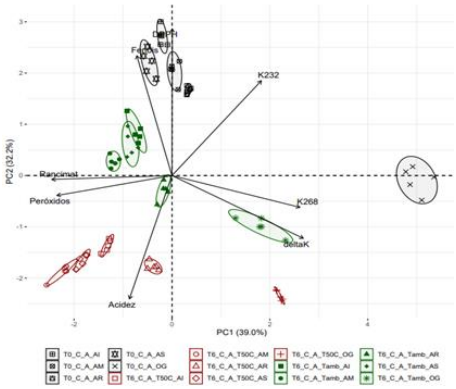

Figure 1. Principal component analysis discrimination of the different canned tuna samples according to the covering liquid at different storage times using quality parameters, antioxidant activity and Rancimat for samples in contact with the coating liquids.

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A modified high-performance liquid chromatographic method for simultaneous quantification of skatole and androstenone in pig's backfat

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Boar taint is an unpleasant odour found in the carcasses of entire males typically present in the meat of 5 to 10% of uncastrated male pigs, mainly caused by the accumulation of androstenone (AND) and skatole (SKA). These compounds accumulate in the adipose tissue of growing animals in relation to pubertal development [1], causing off-odour and off-flavour remarks that create an unpleasant perception of pork quality in the consumer. SKA (3-methylindole) is formed from the amino acid L-tryptophan in the large intestine of pigs from tryptophan by anaerobic bacteria in the animal intestinal tract. AND is a testicular steroid with a urine-like smell produced in the Leydig cells and regulated by the hypothalamic-pituitary-gonadal axis, similarly to the synthesis of the gonadal hormones, androgens and oestrogens [1]. Redistribution of SKA and AND from pig's blood to fat tissue occurs easily due to the lipophilic characteristics of these compounds. Boar taint quantification has been developed over the years using a variety of techniques and instruments. However, this dispersion of methods leads to a need for validation of existing methods and a need for standardisation of methodology to quantify boar taint compounds [2]. This study aims to validate a method for extraction and simultaneous determination of SKA and AND using an HPLC with fluorescence detection.

The proposed method in this paper is based on the work of Hansen-Moller [3], which achieved the simultaneous determination of SKA and AND for the first time using high-precision liquid chromatography (HPLC). Sample preparation consisted in extracting liquid fat from adipose tissue after microwave heating. Methanol was added, and samples were sonicated and centrifuged before passing through a 0.2 µm filter. Chromatographic separation was achieved using a ThermoScientific UltiMate 3000 HPLC system equipped with a AkzoNobel Kromasil 100-5C18 5µ 250x4.6 mm column operating at 40 ºC. Composition of mobile phase eluents was as follows: (A) acetic acid 0,1%, (B) Acetonitrile, (C) Tetrahydrofuran, (D) Methanol 95%, with the following gradient profile:0.0-5.0 min: 45% A, 55% B; 5.0-6.0 min: 40% A, 55% B, 5% C; 6.0-6.1 min: 20% A, 30% B, 30% C, 20% D; 6.1-12-0 min: 40% B, 40% C, 20% D; 12.0-12.1 min: 45% A, 55% B; 12.1-13.0 min: 45% A, 55% B. Fluorescence detection was performed with excitation at 285 nm, emission at 340 nm (0–6.0 min), followed by excitation at 346 nm and emission at 521 nm (6.1–13 min). Manual derivatisation of standard solutions and samples was performed at room temperature for 5 min before injection with dansylhydrazine in 2-fold stoichiometric proportions. An external standard calibration method was applied, using various concentrations of standard solutions of SKA and AND. For this method, limits of detection and quantification (LoD and LoQ) were calculated. Additional validation parameters such as precision and robustness were determined. Also, the recovery percentage was assessed by spiking pig's fat containing low background concentrations of the analytes of interest.

A good chromatographic separation was achieved. SKA and AND eluted at 4.37 and 9.94 min., respectively (**Figure 1**). Eight standard solutions were used for SKA (0.3-37.6 ng/g) and 6 for AND (6.0-451.1 ng/g). The calibration curves correlation coefficient was 0.9999 for both analytes. LoD values were 1.53 and 16.02 ng/g and LoQ 4.64 and 48.54 ng/g for SKA and AND, respectively. SKA recovery was 99.72±2.34% (2.34% RSD) and 102.84±1.62% (1.57% RSD) for AND. Results showed good precision values (repeatability <2.46% RSD for SKA, <6.85% RSD for AND; intermediate precision <2.87% RSD for SKA, <6.98% RSD AND). Method robustness was tested by applying small variations in HPLC parameters, and the obtained values were within the reference values [4], with peak asymmetry <2, N>2000, and a small HETP number.

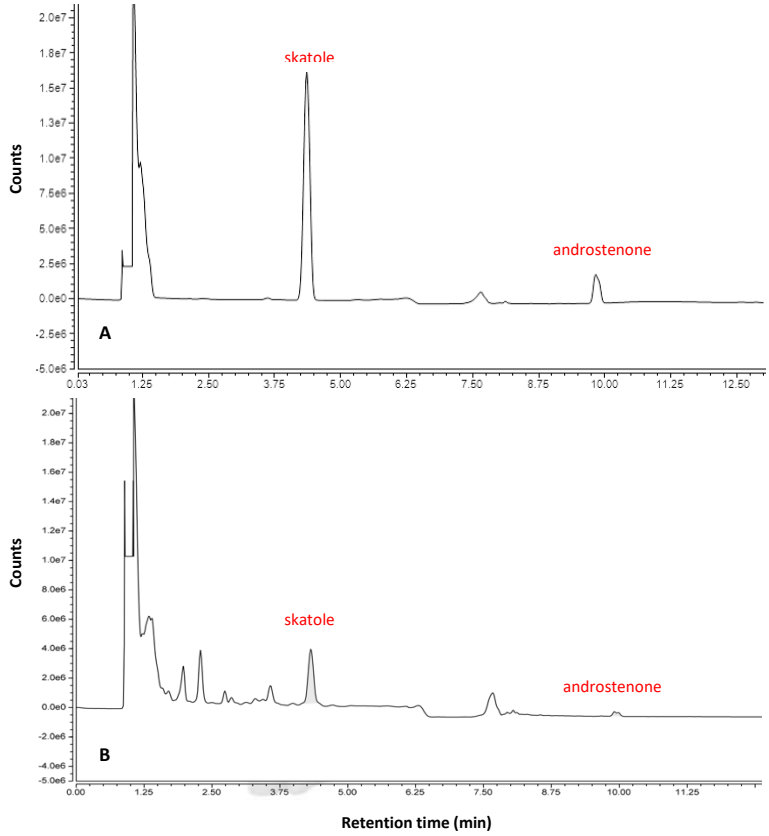

Figure 1: Standards (A) and fat sample (B) chromatograms for skatole and androstenone

The need to accurately measure the compounds responsible for boar taint leads to the re-evaluation of existing methods and adaption of them to the equipment available. The results obtained with this method provide relatively fast simultaneous detection of SKA and AND, with low LoD and LoQ, and also with simple sample preparation. The validation results proved the method to be accurate and robust, with excellent percentages of analyte recovery. This method is currently being applied to a boar taint screening study in Portuguese slaughterhouses.

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The phenolic profile for the discrimination of honeydew honey with origin in *Quercus pyrenaica oak*

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Honey is a natural food highly valued by consumers for its nutritional and therapeutic properties. Produced by honey bees from the nectar of flowers, called nectar honey, or from plant secretions or excretions produced by plant-sucking insects, and called honeydew honey.[1] Plants produce honeydew secretions, as a result of injury by insects or through phloem sap exudate in its acorns. In the north of the Iberian Peninsula, the latter secretion type can be observed in black oak (*Quercus pyrenaica*) forests during summer, specially in mountain areas with moderate humidity. Palynological characteristics of honeydew honeys are dependent on the production area, season and the meteorological conditions in the area where the honey was produced.[2]

Honeydew honeys are darker than most blossom honeys. They are also usually described as having higher values of several physicochemical variables evaluated in the routine quality control of honeys. It is an intensely mineralized honey and rich in phenolic compounds.[3]

Given the growing interest in honeydew honeys, especially for their superior therapeutic properties, this work aimed to contribute to the valorization of honeydew honey with origin in *Quercus pyrenaica* by the characterization of their phenolic profile. For that, 42 honeydew honey samples obtained, in September of 2021, from four experimental apiaries located in areas of black oak forest in Montesinho Natural Park, were characterized by the pollinic and phenolic profile. The phenolic compounds were extracted with amberlite XAD-4® and analyzed by liquid chromatography with diode array detection and coupled to electrospray ionization mass spectrometry in negative ion mode (LC-DAD-ESI/MS). The analysis of the UV spectra together with the molecular ion identification $[M-H]^-$ and MS^n fragmentation allowed the identification of about 15 phenolic compounds, among which phenolic acids, isoprenoids and flavonoids. The phenolic profile of the samples was similar, presenting variations in the quantities which were correlated with the geographical and climatic conditions.

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Chemical characterization and bioactive potential of coffee pulp, a by-product of coffee industry

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The processing of coffee, from cherries to roasted beans, generates a number of bioactive and nutritionally valuable by-products. In the wet method, the pulping of the cherry generates the "coffee pulp", which consists of the skin and pulp.[1] Coffee pulp contains mostly fiber (61%), sugars (14%), protein (12%), and also contains caffeine (1.3%).[1,2] Secondary metabolites with antioxidant properties, such as chlorogenic acids, caffeic and ferulic acids, are also present.[2] Considering that for every two tons of coffee, one ton of coffee pulp is produced, there is a substantial amount of this by-product each year.[1] However, coffee pulp has not been utilized in the most effective way, as it is usually deposited in landfills, where its caffeine and polyphenols content causes environmental concerns.[1,2] Hence, it is necessary to add value to this coffee by-product and make the coffee chain more sustainable.

Colombia's coffee is famous worldwide for its flavor and mild but rich aroma, being this country one of the world's top coffee exporters. The main objective of this study was to perform a detailed chemical characterization of the Colombian coffee pulp (from *Coffee arabica*) to better assess its potential applications. Dried samples were kindly provided by a Colombian producer through a national coffee importer and roaster company (JMV-José Maria Vieira, SA). The following parameters were determined: moisture, protein, lipids, fiber (total, soluble, and insoluble), and ash contents by AOAC methods; chlorogenic acids profile and caffeine by RP-HPLC-DAD; fatty acid profile by GC-FID and vitamin E profile by HPLC-FLD-DAD; total and free amino acid profile by RP-HPLC-DAD; sugars profile by HPLC-ELSD. FRAP and DPPH$^{\bullet}$ inhibition tests were also performed to assess antioxidant activity.

Overall, the results indicate that coffee pulp is an excellent source of fiber, with total, insoluble, and soluble fiber contents of 45.99, 36.89, and 9.10 g/100g dw, respectively. The predominant chlorogenic acid in coffee pulp was 5-caffeoylquinic acid (2.82 mg/g dw), followed by 5-feruloylquinic acid (0.17 mg/g dw). High levels of caffeine were also found in the sample (13.64 mg/g dw). In terms of total amino acids, aspartic acid (7.36 mg/g dw), hydroxyproline (5.65 mg/g dw), and glutamic acid (5.63 mg/g dw), were the three most abundant.

In conclusion, coffee pulp can be used to extract bioactive compounds, such as chlorogenic acids, caffeine, and valuable amino acids, which can then be used, for example, in dermocosmetic and food industries. In addition, since this by-product is high in fiber, it would be interesting to explore its potential for application in functional foods. In sum, discovering alternative uses for coffee pulp can reduce environmental pollution and also contribute to food security.

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Inovação de Produtos e Tecnologias

Raspberry fruit stabilization for its valuation in the development of muffins

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Raspberry is characterized by its beneficial effects inherent to its anthocyanin profile. [1] Due to its high perishability, 1/3 of its production is wasted, making its valuation essential. In this work, freeze-drying and convective drying at 30 and 40 ºC were used for fruits stabilization and subsequent incorporation into muffin formulations.
The two red raspberry varieties under study, Pacific Deluxe and Versailles had similar colour (CIELab parameters) and similar profile and content in phenolic compounds. When evaluating the impact of dehydration techniques on the two varieties, freeze drying was the one that did not show significant differences in terms of structure, colour, composition of phenolic compounds and antioxidant activity, when compared to fresh raspberry fruits. On the other hand, dried raspberries through both convective drying conditions showed significant differences in these parameters, with a significant reduction in the content of phenolic compounds as well as on their antioxidant activity. Besides, no significant differences were observed among the composition of 2 varieties and drying at 30 °C revealed a higher impact in the colour of the dried fruits.
The incorporation of Versailles raspberry fruits in fresh, freeze-dried, and dried at 40 °C forms into muffin formulations resulted in products with different colours: fresh raspberries showed a green colour, which was attenuated in muffins made with dried raspberries and non-existent in muffins with freeze-dried raspberries. This greenish colour, resulting from the impact of alkaline pH of the dough, was enhanced by the syneresis phenomenon when using fresh raspberries. Sensory analysis revealed good acceptance of all muffin formulations, however, those containing freeze-dried samples proved to be the most appreciated in terms of fruit appearance and sweet/acid balance. Thus, showing a promising approach for developing a new product, promoting the valorisation and sale of raspberry fruit waste under a circular economy concept.

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Extending Shelf Life of Fresh Red Raspberry (*Rubus idaeus* L. cv. 'Kweli') Using an Eco-friendly Antifungal Active Package

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Raspberry commercialization has recently undergone significant changes due to increasing customer quality requirements, sustainable consumption concerns, and demand throughout the year. Such changes require producers and traders' to develop new strategies to improve fruits shelf-life in a sustainable way, which can be achieved by innovation in packaging technologies. The main objective of the present study was to develop a new packaging strategy, based on novel active film-pads inside commercial compostable packages, envisaging to extend shelf life of fresh red raspberry (*Rubus idaeus*. L. cv. 'Kweli'). The active film-pads were developed by immerging prepared chitosan (Ch) films by casting, into green tea (GTE) and rosemary (RSME) ethanolic extracts, and let do dry at room temperature in the dark. Both active film-pads presented a good capacity to inhibit *P. expansum* growth. Furthermore, they also presented an important water absorption capacity (around 1400%, dry basis) and good mechanical properties. From the properties studied, the active film-pads showed to be good candidates for application in active packages for small fruits prone to fungal infection, as carriers of antimicrobial compounds and moisture absorbers.

As such, the developed active pads were placed on the bottom of commercial fruit trays, underneath fresh red raspberry fruits, and the trays were heat sealed with a compostable polylacid lactic (PLA) film. Preservation studies of packaged fruits were carried out during 14 days of storage at refrigerated conditions (4 ºC). Raspberry samples were periodically analyzed throughout storage, in terms of quality attributes (fungal decay, weight loss, firmness, surface colour, pH, total soluble solids, total phenolic content and antioxidant activity). The gas composition inside the packages was also analyzed over time. The packaging systems with active film-pads Ch+GTE and Ch+RSME were highly effective in reducing fungal growth and decay of raspberries during storage. In fact, while fruits packaged with active film-pads did not show any visual fungal infection after 7 days, 40% of the fruits packaged without film-pads presented fungal growth. Furthermore, only 5 - 13% of spoiled fruits were detected after 14 days in packages with active film-pads, in contrast to the amount of 80% of spoiled fruits observed in packages without active pads. In addition, fruits preserved using packages with the Ch+RSME active film-pads showed much lower mass loss (6%), firmness decrease (16%) and antioxidant activity reduction (around 9 and 15% for DPPH and FRAP methods) after 14 days, when compared to fruits preserved without active film-pads (mass loss: 10%; firmness decrease: 60%; antioxidant activity reduction: around 40 and 55% for DPPH and FRAP methods). The sustainable packaging strategy studied presents an excellent potential for the preservation of raspberries and other highly perishable small fruits.

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Microalgae as natural colorants in pastry products

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As the trend toward cleaner, healthier labels consistently gains momentum, the need to find alternative sources increases. Color is the calling card of food products and can be the deciding factor in purchasing a food product. Nevertheless, the use of synthetic colorants has been associated with the surge of health problems, specifically in children, that have tarnished their reputation and led to the need to find natural alternatives. Additionally, eggs are important components of cakes, providing color and many other properties, however, there is growing interest in egg substitution due to several reasons. In this regard, this study aimed to evaluate the potential of microalgae to be used as natural food colorants in bakery products. Two different commercial strains of *Chlorella vulgaris* (*Chlorella vulgaris* White and *Chlorella vulgaris* Honey) dried biomass and dried ethanolic extracts (80 %) of *Tetraselmis chuii* were incorporated into two different cakes (eggless brioche and fondant, respectively). In Brioche, three incorporation levels were tested: 1 % (w/w) of *Chlorella vulgaris* White, 1 % (w/w) of *Chlorella vulgaris* Honey, and 1 % (w/w) of a mixture of *Chlorella vulgaris* White and *Chlorella vulgaris* Honey (1:1). The impact of microalgae addition was evaluated in terms of color using instrumental analysis (colorimeter) and sensory characteristics (texture, aroma, taste).

The results showed that, the eggless brioche containing 1 % of the *Chlorella vulgaris* mixture (1:1 *Chlorella vulgaris* White and *Chlorella vulgaris* Honey) was found to be close to the control traditional brioche in terms of color (Figure 1). Moreover, the use of whole biomass represents an added value due to the fact that not only provides color, but also nutritionally enriches the product with proteins and polyunsaturated fatty acids. A preliminary sensory analysis of the brioche using a panel of untrained consumers (n = 43) suggested positive results with most people (72 %) responding that they would "probably buy" and "definitely buy" the product. The use of some microalgae species as food colorants may be hindered by their potential taste, making it necessary to use extracts for these purposes. *Tetraselmis chuii* extracts were applied in fondant formulation as green colorant and the provided color evaluated instrumentally. Four levels of the incorporation of *Tetraselmis chuii* extracts were assessed: 0.5 % (w/w), 0.3 % (w/w), 0.1 % (w/w), and 0.05 % (w/w). At a concentration of 0.05 %, the extract provided a pastel green color to the product with minimal algae flavor (Figure 1).

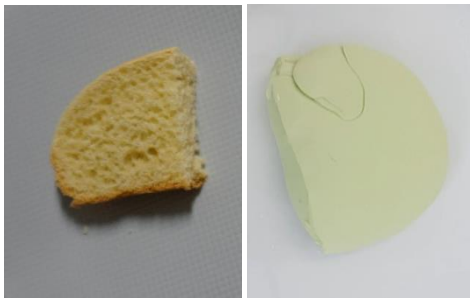

Figure 1: Brioche with *Chlorella vulgaris* as an egg substitute (left) and fondant with *Tetraselmis chuii* extract (right).

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Bioplastics based on chitosan and micro/nanocellulose loaded with sage essential oil for extending shelf-life of fresh poultry meat

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Packaging is fundamental to guarantee the correct functioning of the global food system and related value chains, and it is expected that this key sector keeps being responsible for the production of millions of tons of fossil-based plastic waste that will be accumulated in ecosystems for hundreds of years[1,2] and contributing to the acceleration of global warming[3]. Thus, it is crucial to develop new technologic research into biological and renewable packaging that can preserve food in the same way that common plastics do, preventing the rapid deterioration of food.

This laboratory project aimed to develop active chitosan (Ch)/sage essential oil (SEO) bioplastics reinforced with micro- (MCC) and nanocrystalline cellulose (CNC) particles to obtain a bioplastic with improved active properties. To our knowledge, very little is known about the combined effect of MCC and CNC as reinforcement and as encapsulation agents of natural antioxidant compounds, like SEO, and the associated effect as active packaging to perishable food matrices. This novel procedure adopts the circular economy concept, reusing biomass, to produce a novel high added-value bioplastic with the objective of being incorporated as a fully biodegradable and active element in food packaging. Chitosan was chosen as the bio-based polymeric matrix, while the strengthen fillers were the MCC/CNC isolated from *Arundo donax* L. (also known as Giant Reed, an invasive lignocellulosic plant in the Mediterranean area). The biobased polymers were produced by the casting method[4]. Chitosan (1.5% w/v) film form solutions (FFS) were incorporated with MCC/CNC (2.5% w/w chitosan) and SEO (1% v/v FFS).

The fresh poultry meat samples were wrapped in the different bioplastics (pristine chitosan, chitosan with SEO, chitosan with SEO and MCC/CNC) for 13 days in a refrigerated environment. Unwrapped fresh poultry meat was chosen as the control. Physicochemical tests were performed at 0, 3rd, 6th, 9th, and 13th day (measuring pH, total titratable acidity, color, volatile basic nitrogen and thiobarbituric acid reactive substances index).

Over the evaluation times, the unwrapped meat showed a higher state of degradation compared to the meats packaged in bioplastics. This degradation was not only visually observed and felt by the strong characteristic odor of petrification, but was also confirmed through parameters such as pH, which increased from 6 to 7.3, or through the quantification of volatile basic nitrogen, which passed from 14 mg/kg of meat to 88 mg/kg of meat. The meat packaged in biofilms had a much lower microbiological growth, in the three parameters evaluated, compared to the unpackaged meat, thus demonstrating the antimicrobial activity recognized to chitosan. The introduction of SEO has been shown to have a pro-oxidant effect on meat, as evidenced by the increase in malonaldehyde (MDA) concentration over time. This activity decreased with the introduction of MCC/CNC, demonstrating that these micro and nanoparticles had the ability to encapsulate the essential oil. In general, fresh poultry meat protected with bioplastics exhibited an extension in its shelf-life time, proving that this technology has an encouraging potential to be applied by the food industry.

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Biobased polymers of chitosan incorporated with *Aloysia citrodora* and *Cymbopogon citratus* essential oils for packaging fresh poultry meat

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Aloysia citrodora, Palau, commonly known as Lemon Verbena, or Lúcia-Lima in Portugal, is a medicinal plant that belongs to the *Verbenaceae* family, well known for its characteristic odor and taste, but also for presenting many useful properties, such as antioxidant, and antimicrobial, among others[1]. *Cymbopogon citratus*, commonly called Lemongrass, in Portuguese, Erva Principe, is an aromatic plant, member of the *Poaceae* family, originated from West India, now used worldwide for many applications, such as antibacterial, antifungal, anti-inflammatory and antioxidant [2].

These essential oils, rich in phenolic compounds, with antioxidant properties, can act as safer food preservatives, natural and free from synthetic chemicals, and can serve to increase the shelf life of foodstuff. Therefore, the main goal of this work was to evaluate the effect of biobased polymers of chitosan incorporated with 2% of *Aloysia citrodora* and *Cymbopogon citratus* for packaging poultry meat. The biobased polymers were produced by the casting method[3] and chitosan film form solutions (1.5% w/v) were incorporated with the oils (2%) and with CNC (cellulose nanocrystals) as reinforcement of the biopolymer. To evaluate the potential of the produced biobased polymers for packaging of fresh poultry meat, physicochemical properties, such as moisture, color, basic volatile nitrogen, pH, total titratable acidity and thiobarbituric acid reactive substances index were analyzed at days 0, 3, 6, 9 and 13, stored in a refrigerator and using unwrapped meat and wrapped meat with pristine chitosan as controls. Results indicate that when used as primary packaging of the meat, the samples protected with the biobased polymers presented a decrease on the deterioration speed, which was represented by the preservation of the initial pH of the meat and reduction of the oxidation process. Results indicate that the pH of the unwrapped meat increased from 6 to 7.5, and that the quantification of volatile basic nitrogen reached 150 mg/100g of meat, at 13th day of storage. In contrast, the meat wrapped with the biobased polymers maintained the pH at around 5.5-6 and the maximum volatile basic nitrogen observed at 13 days was in the range 20-25 mg/100 g meat. Unwrapped meat also showed a higher lipid oxidation at 13 days. Meat wrapped with chitosan helped to preserve the oxidation of the meat, and the inclusion of CNC reduced the oxygen permeability, which contributes to deaccelerate meat oxidation. Compared with the chitosan with CNC, the incorporation of the oils did not improve the action of the packaging in terms of antioxidant effect, after 13 days. But helped to slower this oxidation. The migration of those oils from the polymers to the meat, changed the color of it, turning the meat more yellowish. The different polymers tested contributed to maintain or to dry a bit the wrapped meat when compared to the unwrapped meat and the acidity was maintained or even increased. In general, fresh poultry meat protected with bioplastics exhibited an extension in its shelf-life time, and the incorporation of the oils helped to retard the oxidation processes, in a more significant manner than chitosan with CNC. It will be interesting to see the effect of the oils incorporation in the chitosan towards the microbiological growth of the wrapped meat.

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Is it possible to prepare coffee infusions resembling espresso coffee brews? The role of carbohydrates

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All coffee brews are prepared with roasted coffee and water, giving origin to espresso, instant, or filtered coffee, exhibiting distinct physicochemical properties, depending on the extraction conditions. The different relative content of compounds in the brews modulates coffee body, aroma, and colour.

Carbohydrates are the major group of compounds in green and roasted powder, as well as in coffee brews, having a considerable impact on brew properties. Galactomannans (GM) and arabinogalactans (AG) are the main carbohydrates in coffee brews. GM, a linear polysaccharide composed mainly by mannose residues branched with single residues of galactose, are related to the viscosity verified in coffee brews, and the amount of carbohydrates is associated to espresso coffee (EC) foam stability[1], evidencing their importance in EC. In instant coffee, AG assume a preponderant abundance due to the extreme extraction conditions applied, which consequently lead to a relative decrease in the content of other compounds, such as caffeine and chlorogenic acids.

Carbohydrates content and composition were the target compounds as they are organoleptically important for EC due to their association to foam stability and viscosity. Instant coffee powders can be obtained with chemical overall composition to EC in many of the parameters analysed (carbohydrates, caffeine, chlorogenic acid, pH, foamability, and colour), resembling espresso, although with lower lipids content. Their redissolution at espresso concentration allowed a viscosity, foamability and volatile profile representative of an espresso coffee, opening new exploitation possibilities[2].

The infusion process results also in a high fraction of unextracted compounds, mainly carbohydrates. Under a circular economy, the residue (spent coffee grounds) can be extracted under more drastic conditions to produce instant coffee, leading to the total use of the coffee powder in two distinct products, espresso coffee and instant coffee by a two steps extraction process[3].

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Yeast polysaccharides as food ingredients for clean label dressings and sauces

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Yeast polysaccharides may be obtained from brewer's spent yeast cell walls, an abundant by-product from brewing industry. Yeast cell wall polysaccharides are mainly composed of mannoproteins and glucans, polysaccharides of a high added value which have a wide variety of applications. In the food industry, one of their applications include the use as bio-emulsifiers[1] which allows the use of clean label ingredient with the advantages of biodegradability, selectivity, and environmental compatibility over artificial products.[2]
To evaluate the yeast fibers potential as emulsifiers, brewer's spent yeast polysaccharides were extracted using alkali and subcritical water (200 °C) and tested by measuring the emulsifying capacity of each sample for one month. The emulsifying capacity was measured by adding the different samples to 4 mL of water and 6 mL of vegetable oil and shaking (30s, hand shaking or by Ultraturrax), resulting in a water in oil emulsion. The volume of the emulsion, and the volume of the water and oil phases that had already been separated from the emulsion were measured, enabling the calculation of the emulsifying capacity percentage. The texture of the emulsion was also measured using a back extrusion rig calculating the emulsion firmness and cohesiveness.
The results showed that the different soluble extracts have different performances: some have a good performance when applying low energy (hand shaking) while others needed high energy (Ultraturrax) to have emulsifying capacity. The emulsions with high emulsifying capacity showed small water bubbles size in the oil emulsion. Using a response surface methodology (RSM) study, pH is the variable that most affects the emulsion capacity of the samples followed by sample concentration. Nevertheless, the extract which contained mannoproteins with lower amount of carbohydrates, when used in a 2.0% (w/v) concentration at pH=11 (pH emulsion=5.23) and 0.5% of NaCl, when applying low energy has the best emulsifying capacity (85%) for one month, higher than the commercial emulsifier Xanthan Gum, with emulsifying capacity of 33% at the same conditions. However, this occurs when applying high energy, since low energy is not an option for Xanthan Gum. The firmness and cohesiveness of the emulsions prepared with yeast polysaccharides showed values similar between them (7.9 to 86 g for firmness and -6.7 to -6.0 g for cohesiveness) and similar with those of egg yolk (7.9 and -6.0 g in firmness and cohesiveness, respectively) but much lower values than Xanthan Gum (35.1 and -34.4 g in firmness and cohesiveness, respectively).
Brewer's spent yeast polysaccharides seem to be a competitive alternative to the commercial emulsifiers, namely egg yolk, with promising uses in dressings and sauces for vegans, in addition to the low price of the raw material, thus valorising this by-product as food ingredient.

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Grass pea sweet miso as a clean label ingredient for innovative vegan emulsions

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Food commodities are nowadays regarded as a way to promote one-health[1], boosting consumer's health (both physical and mental), and contributing to a more sustainable world. Simultaneously, flexitarian[a], vegetarian and vegan diets have been increasing their popularity over the past years, one sign of the higher awareness of consumers about environmental, health and ethical issues. Hence, if companies want to keep competitiveness, they must change the way they regard new product development, investing in reinventing traditional products and adapting them to new trends.

This work, part of the cLabel+ project, aimed to evaluate the effect of the incorporation of miso in plant-based Casa Mendes Gonçalves' emulsions properties.

Miso, a fermented paste widely used in oriental cuisine, is the product obtained from the fermentation of soya beans with koji, an *Aspergillus spp.* inoculated rice, together with a consortium of yeasts and lactic acid bacteria. In general, fermented foods are known for having higher content in organic acids, antimicrobial metabolites and other bioactive compounds produced during fermentation, which increase potential health benefits and the shelf life of these products[2]. For this work, grass pea (*Lathyrus sativus L.*) miso was used, since grass pea is a more sustainable raw material than soy in the Portuguese context. The combination of the nutritional and technical impact of this innovative miso in food emulsions might positively influence human nutrition, simplify formulations, and therefore improve consumers' perception of the developed products.

Miso's nutritional and chemical composition was assessed: total protein (DUMAS method), total fat (Soxtec method), mineral content (ICP-OES), pH, total phenolic compounds (Folin-Ciocalteau method), and antioxidants (DPPH and FRAP methods). Regarding bioactivity, miso's antimicrobial activity against pathogenic and contaminant microorganisms was tested by spot-tests. Two assays were performed: 1) using miso-based plates to determine microorganism's ability to grow in this ingredient; and 2) using adequate nutritious media plates added of miso, to determine miso's potential inhibitory effect. Plates were produced with 1, 5 and 10 % (w/w) of miso. To discard salt's inhibitory effect, salt plates with equivalent NaCl content to miso were also tested. Results showed that miso is not nutritive to pathogenic bacteria (*E. coli*, *S. aureus*, *Salmonella enterica typhimurium*, *Bacillus cereus* and *Listeria innocua* – equivalent to *Listeria monocytogenes*), and presents an inhibitory effect against these microorganisms as concentration increases.

Considering previous results from the referred assays, different proportions of previously produced[3] grass pea miso (5, 7.5, 10 and 15 g/100 g – **Figure 1**) were incorporated in a vegan mayonnaise recipe, as a substitute for salt and other flavouring, colouring, and preservative agents. The vegetable protein and oil contents were also adjusted according to miso proportion.

Texture (namely adhesiveness and firmness), viscoelastic behaviour (SAOS), microstructure (SEM), colour (CIELAB method) and pH were evaluated for the obtained products. The products were evaluated regarding microbiological parameters, namely total mesophiles, total lactic acid bacteria and total yeast.

A preliminary sensory analysis was conducted to select the most promising formulations for potential prototyping.

[a] A flexitarian is a person who follows a generally plant-based diet, occasionally consuming animal products.

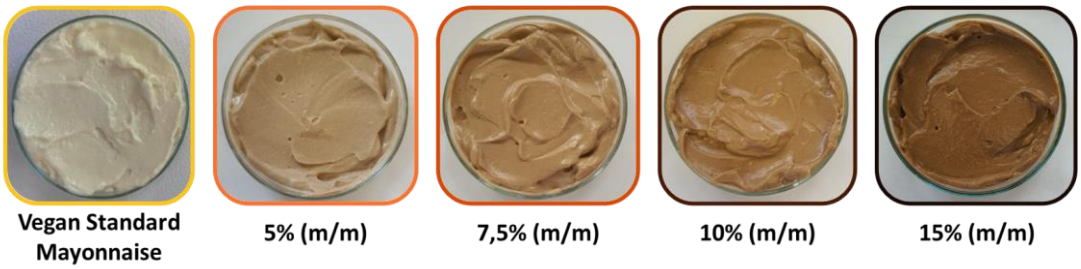

Figure 1: Emulsions produced with different proportions of grass pea sweet miso.

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Development of a clean label meat free alternative to deli ham

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Since ancient times it has been established that meat consumption is essential in a healthy diet, providing high quality protein. However, livestock production for meat industry requires numerous resources and is a key driver to the increase of greenhouse gas emissions which is one of the contributors to the environmental impact. In addition, recent studies have pointed out the negative effects of meat consumption on well being[1, 2]. To minimize environmental impact, reduce the development of health-related problems and make food production sustainable, the number of people who choose to completely exclude meat from their diets has increased[1]. Therefore, we need to develop meat analogues or meat substitutes. The concept of "meat analogues" refers to high-protein food products, devoided of meat, but which have similar texture, appearance, nutritional value and taste to traditional meat products and are produced through equivalent processes[1]. However, meat analogues are generally highly processed products that include several food additives in their composition. An exploratory study revealed that most consumers are concerned about the use of food additives in processed foods[3]. So, the objective of this work was to develop a clean label meat free product alternative to deli ham without any food additives, for a Portuguese company. The main categories of food additives used in commercial deli hams plant-based analogues are the following: i) colouring agents, ii) preservatives and iii) thickening agents. Therefore, to create a clean label deli ham meat free analogue, the referred categories were substituted by the following alternative ingredients: i) a clean label plant-based colouring, ii) spices, since they are important natural food preservatives, and iii) a natural texture agent. The main focus of these work was to identify a promising texture agent to replace the additives incorporated in the original formulation. Over 9 natural hydrocolloids agents with gelling properties were tested: pregelatinized banana starch, psyllium flour, cassava flour, inulin, fructo-oligosaccharides, rice flour, quinoa flour, chia flour and chestnut flour. A concentration of 10% of each hydrocolloid was tested each time. From these tests, it was possible to establish that the most promising hydrocolloid was the psyllium as it forms a homogenous strong and elastic gel. Compared to the other tested prototypes, the psyllium incorporation prototype was significantly the best performant since it has proved to be a non-friable succulent gel. Regarding the flavour, the incorporation of psyllium is neutral. Subsequently, the concentration of psyllium, the ingredients adding sequency and the cooking time were tested. To evaluate and compare these new prototypes, more objective tests were performed such as the assessment of colour, pH, moisture, texture and rheology parameters. It should be noted that these results were also evaluated by comparing them to commercial hams. The colour and pH were analysed using, respectively, the CR 300 Minolta colorimeter (Osaka, Japan) and the pH-Meter Crison - Model Basic 20 and the moisture content was estimated by gravimetric methods. Regarding the textural parameters, a Texture Profile Analysis in penetration mode was performed, using a TA-XT plus texturometer, with a 10 mm diameter cylindrical probe (load cell of 5 kg) and a penetration of 5 mm at a penetration speed of 1 mm/s. The samples were prepared by placing slices in a pile of 1 ± 0,2 cm height. The rheology measurements were carried out on a HAAKE MARS III controlled-stress rheometer using a 20 mm serrated parallel plate sensor and a gap adjusted to the slice thickness. Small Amplitude Oscillatory Shear measurements (SAOS) were performed, namely a frequency sweep test at 20 °C, after a stress sweep to determine the stress value for linear viscoelasticity behaviour. The results showed that the best prototype was the one who evidenced to be the most similar to the targeted commercial product.

Research on this area is essential to a clean and sustainable food production and to offer alternatives to meat without compromising the use of natural ingredients.

Figure 1: Plant-based deli ham analogue with psyllium incorporation as a substitute for texture agent food additives

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3D Priting of snacks containing *Tenebrio molitor* flour

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Mealworm flour [*Tenebrio molitor* L. (Coleoptera, Tenebrionidae)] is a good source of protein and essential amino acids - 45% (w/w) - as well as essential fatty acids -29,5% (w/w) - and fiber[1]. With the socio-economic changes that arise from climate change and consequently cause the rising prices of meat products, a viable and sustainable alternative to protein originated in livestock must be found, and entomophagy is only one option[1,2]. Additive manufacturing process, or 3D printing, came as an appellative alternative, reliable and repetitive, producing snacks with surprising textures and shapes, able to attract the consumer's attention.

In the present work, the original recipe of the snacks was obtained from Letras *et al*. (2021)[3]. Attempts were made to improve the recipe due to the excessive hardness of our control. By altering the ratios between wheat, rice and maze flour a better and softer texture was obtained, although the result of the control printing had less detail than the initial formulation (**Figure 1(A), 1(B)**). When the mealworm flour was added to the mix, at 8% (w/w), the dough changed textures, becoming more like bread dough instead of a liquefied, which is the intended profile for the printing. At this stage, the printer was unsuccessful, most of the time resulting in obstruction of the cartridges due to the sheer size of the particles of the mealworm flour. To solve this problem the mealworm flour was grinded and sifted until obtained a similar granulometry to wheat flour.

It was empirically observed that mealworm flour absorbs most of the water present in the dough, giving it a hard and bread-like texture. MicrodoughLab was used to measure the torque applied to the dough, and the results gave the information about the water to be added (40% w/w to a 45% w/w) to obtain a dough printing with similar behavior to control. The results nearly matched with the torque applied to the control dough, and after a trial in the printer the result can be seen in **Figure 1 (C)**. More tests need to be performed on the Microdough Lab to achieve ideal water retention for a better flow of the dough during the printing process.

After the successful printing of the snacks containing mealworm, the initial recipe was optimized to the biochemical characterization of the dough and snacks. As one of the claims for the mealworm flour presented by Djouadi *et al.* (2022) was to increase protein and mineral content, the same procedures were adopted for both nutrients. According to the AOAC International procedures, the protein content was determined using a DUMAS protein/nitrogen analyzer, Total fat content was measured through the Portuguese Norme - NP4168. The total phenolic compounds and antioxidant capacity of our snacks were also determined. Djouadi *et al.* (2022) procedure was followed by using the Folin-Ciocalteu reagent method for the phenolic compounds, and for the antioxidant activity DPPH and the FRAP method were used, and finally the mineral component of the recipe was determined gravimetrically through incineration at 550ºC in a muffle furnace, for 24 hours.

From results, the protein and mineral contents of snacks with insects were higher than the control snacks, although the fat content had not a significant increase. *T. molitor* flour is, indeed, an alternative animal protein of the future, considering the resources of animal protein that we are used to consume will be very scarce.

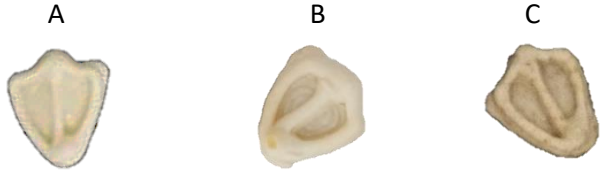

Figure 1: 3D Printing snacks A: Control (Letras *et al.* (2022) ; B: Control improved formulation; C- Snack Containing *T. molitor* (8% w/w).

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Bioactivity, rheology, texture and chemical characterization of Halloumi cheese fortified with *Chlorella vulgaris* biomass

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The use of microalgae as healthy ingredients has been studied in several food products. Microalgae are potential sources of bioactive compounds, which can affect the antioxidant properties and the mechanical behavior in food[1,2]. The addition of microalgal biomass in dairy products has been of increasing interest to obtain more sustainable and nutritive fermented products. Halloumi is a traditional semi-hard cheese originated from Cyprus, currently produced everywhere, and it can be made from a mixture of goat and sheep milk, or just with cow milk. This cheese, with unique sensory characteristics, has a high melting point, so it can be easily fried or grilled[3] and used as a meat substitute. In this sense, the nutritional composition and rheology properties are crucial for sensory acceptance. In the present work, we intended to evaluate how the incorporation of C*hlorella vulgaris* affects the nutritional composition, rheological characteristics and bioactivity of the cheese.

Nutritional composition (namely lipids and ash) was significantly ($p<0.05$) influenced by the addition of different microalgal biomass levels showing an increase of 6.60 % in lipids and 20.66% in ash for 5% *Chlorella*-fortified cheese when compared to the control sample. Moreover, a boost in the mineral profile was observed ($p<0.05$) in this treatment, namely manganese (Mn) and sodium (Na) with 0.014 and 10.24 mg/100g respectively. Regarding the bioactivity of the cheese, the antioxidant activity analysis and phenolic compound determination were carried out using the FRAP and Folin-Ciocalteu methods respectively. The results indicated an increase ($p<0.05$) in the antioxidant activity and phenolic compound concentration, specifically in treatments fortified with 3% and 5% of algae as shown in **Figure 1 (A-B)**.

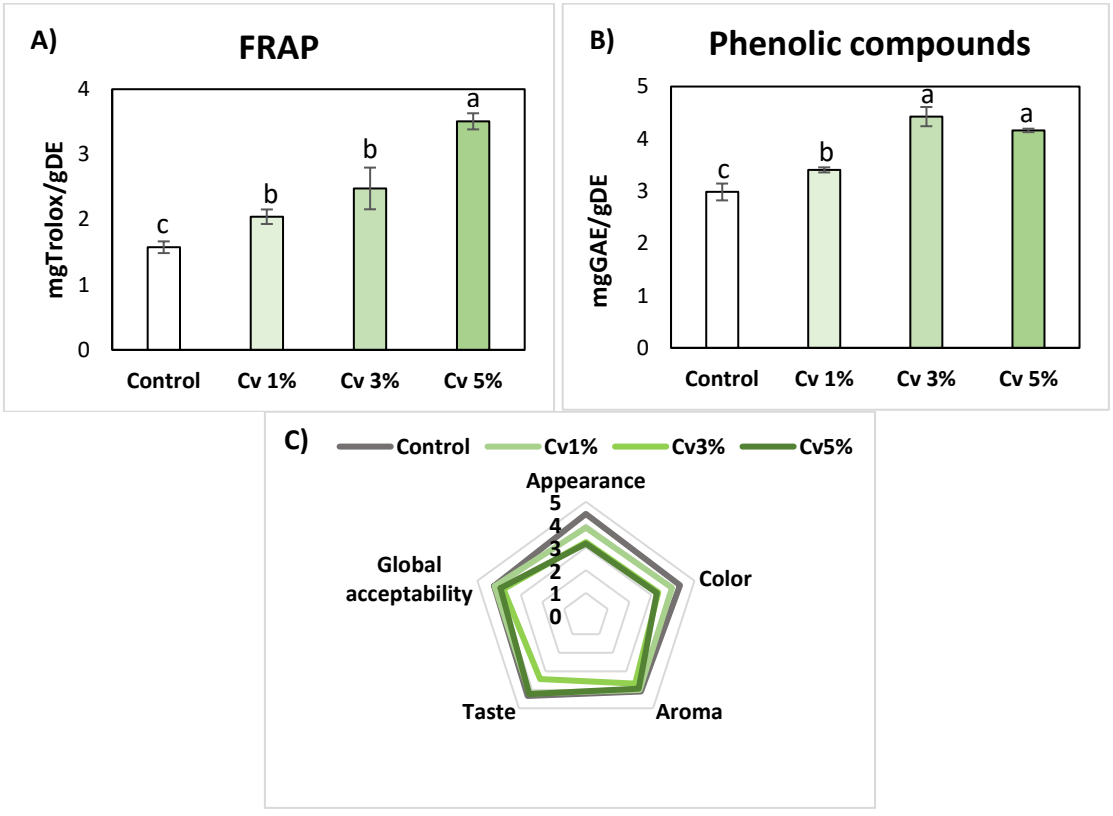

Figure 1: Antioxidant activity (A), phenolic compound concentration (B) and sensory profile (C) of 1, 3 and 5% *Chlorella*-fortified cheeses and the control sample.

Temperature and frequency sweep tests (SAOS) were performed at 20 °C and 90 °C, the impact of adding microalgae was not noticed for the studied levels (1% to 5%). the cheeses presented G´> G´´ despite a higher frequency or temperature dependence. Texture analysis of the cheeses performed by using a puncture test revealed a hardness reduction when algae levels were increased ranging from 23.20 N in the control sample to 15.70 N in 3% *Chlorella*-fortified cheese. Despite de color changes with algae addition, instrumentally evaluated by a colorimeter, sensory analysis of Halloumi cheeses showed that panelists preferred the control and 1% *Chlorella*-fortified cheese, both with an overall acceptability of 4.20 according to **Figure 1C**. In addition, it was observed that 53% and 30% of the tasters "would probably buy" the cheese supplemented with 1 and 3% of *C. vulgaris* respectively, indicating a high probability of consumption of these innovative and hybrid cheeses, made from animal and vegetable sources.

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Study on the incorporation of seaweed in fresh cheese

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One of the most appreciated dairy products by consumers is fresh cheese due to its mild taste and pleasant texture. This type of cheese is obtained by coagulating the milk by adding rennet (renin) and without adding microbial cultures (starter cultures). The formation of the curd is influenced by a number of external factors, which in turn affect the cheese yield. However, maximizing cheese yield is a critical factor for the producer, so the addition of ingredients such as polysaccharides to the curd can influence this process. Marine algae are attractive food resources with high content of polysaccharides, but also proteins, ω-3 fatty acids and micronutrients that can play an important role in human health[1]. In some islands of the Azores, seaweeds have been traditionally exploited for human consumption and commercial purposes[2]. More recently, seaweeds are only occasionally used as food and only a few species are mentioned in food preparation. The objective of this work was to study the incorporation of dried macroalgae collected in the Azores in the technological, nutritional and sensory characteristics of fresh cheese. Two brown algae collected and dried on the island of Faial were used, namely *Fucus spiralis*, commonly known as sea bean, and *Petalonia binghamiae*, known as lobster weed. These algae were added to the fresh cheese curd in two dosages: 0.5 g/100 g and 1 g/100 g. The nutritional composition, pH and antioxidant activity, measured by the ability to scavenge the DPPH radical and by the ferric thiocyanate method (FTC), of these cheeses were analysed. Syneresis was also evaluated by measuring the water-holding capacity (WHC) and weight loss of the cheeses during storage at 4 °C. The cheeses were also evaluated in organoleptic tests by a panel of 22 semi-trained tasters. The chemical composition did not change significantly ($P > 0.05$) with the addition of the algae to the cheeses, except for the cheese with the higher dose (1 g/100 g) of *Fucus spiralis*. No significant differences ($P > 0.05$) were observed in WHC, although a slight increase was observed in the cheeses with *Fucus spiralis*. Notably, the addition of dried seaweed significantly reduced the weight loss of fresh cheeses during storage ($P < 0.05$). A significant increase ($P < 0.05$) in antioxidant activity, as measured by the ability to scavenge the DPPH radical and total antioxidant activity (FTC), was also observed in cheeses with both algae (*Fucus spiralis* and *Petalonia binghamiae*). The *Fucus spiralis* at a dose of 0.5 g/100 g showed the best results in sensory tests, preference and purchase intention.

In conclusion, the incorporation of dried algae in fresh cheese, especially the algae *Fucus spiralis* at a dose of 0.5 g/100 g, reduced the loss of whey during storage, improved the antioxidant activity of the cheese, and was well accepted by consumers. This is a pioneering study on the introduction of algae into this type of cheese. The mixture of algae and milk produced in the Azores combines the sea and the land, resulting in a different product with improved technological properties and potential health benefits.

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Moderate Pressure Pasteurisation at Room Temperature as a new *quasi*-energetically costless nonthermal preservation methodology – a case study on milk

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In the last years several studies proved that hyperbaric storage (HS) at pressures above 75 MPa have the capacity to inactivate microorganisms.[1] These results led us to test HS with the specific aim to exacerbate microbial inactivation, as a new nonthermal pasteurisation approach (to achieve at least 5.0 logs reduction in bacterial vegetative pathogens), tentatively called moderate pressure pasteurisation (MPP) by hyperbaric inactivation (HI).[2] This way, foods would be pasteurised at room temperature, while being stored, which represents a huge advantage to the food industry since reduces the deteriorative effects of heat on foods. Therefore, to enhance microbial inactivation, slightly higher pressures (above 100 MPa) compared to HS (up to around 100 MPa) would be used during the storage period or part of it (the period in which MPP would occur), since after MPP, pressure could be reduced to atmospheric pressure or to HS pressure levels, if subsequent storage is to be carried out by RF or HS, respectively. Thus, MPP would occur at naturally variable room temperature, what is an innovative feature for a pasteurisation process *per se*. Additionally, the whole process, MPP followed by preservation by HS, could occur at RT and it would be a *quasi*-energetically costless process, since energy (in a minor amount) would be only needed to compress the pressure vessel.[2]

So, to test this novel pasteurisation process, MPP by HI was carried out in milk at three different pressure levels (150 MPa, 200 MPa and 250 MPa), over 24 h, at naturally variable uncontrolled room temperature (≈ 20 °C) and compared with high pressure pasteurisation (HPP) at 600 MPa (one cycle for 90 s and a second cycle of 120 s) followed by storage under refrigeration for 21 days.

According to the first results, MPP by HI at 250 MPa over 24 h caused higher microbial reduction on total aerobic mesophiles (TAM), lactic acid bacteria (LAB) and Enterobacteriaceae (of at least 2.2, 1.7 and 1.3 log CFU/mL, respectively) than HPP (of 1.1, 1.0 and 1.2 log CFU/mL) for the same microorganisms. As MPP was more effective to inactivate endogenous microorganisms than HPP, MPP by HI was then applied to inoculated microorganisms, namely two pathogens surrogates (*Listeria innocua* and *Escherichia coli*) and one pathogen (*Salmonella enterica*). In this context, MPP showed a clear reduction of inoculated microorganisms to below the detection limit, in only 16 hours for all pressures applied with reductions of at least 5.7, 5.4 and 5.5 log CFU/mL for *L. innocua* (**Figure 1**), *S. enterica*, and *E. coli*, respectively. Additionally, after MPP by HI (at 200 MPa and 250 MPa), the samples preserved under refrigeration maintained lower TAM, LAB and Enterobacteriaceae counts compared to HPP samples, being the counts below the quantification/detection limit (< 2.0/< 1.0 log CFU/mL) for at least 21 days. For inoculated microorganisms, MPP by HI (at 200 MPa and 250 MPa) resulted also in counts below the detection limit (< 1.0 log CFU/mL) up to at least 21 days under refrigeration.

Summing up, the results show that MPP by HI is very promising as a new nonthermal food pasteurisation technique, since over 5.0 log reduction of vegetative bacteria were achieved, with counts maintained below the quantification/detection limit (< 2.0/< 1.0 log CFU/mL) for at least 21 days under refrigeration. Thus, this work points for the possibility of pasteurising foods by MPP followed by storage by HS, tentatively called moderate pressure pasteurisation and storage (MPPS), being a methodology *quasi* energetically costless with a lower carbon footprint.

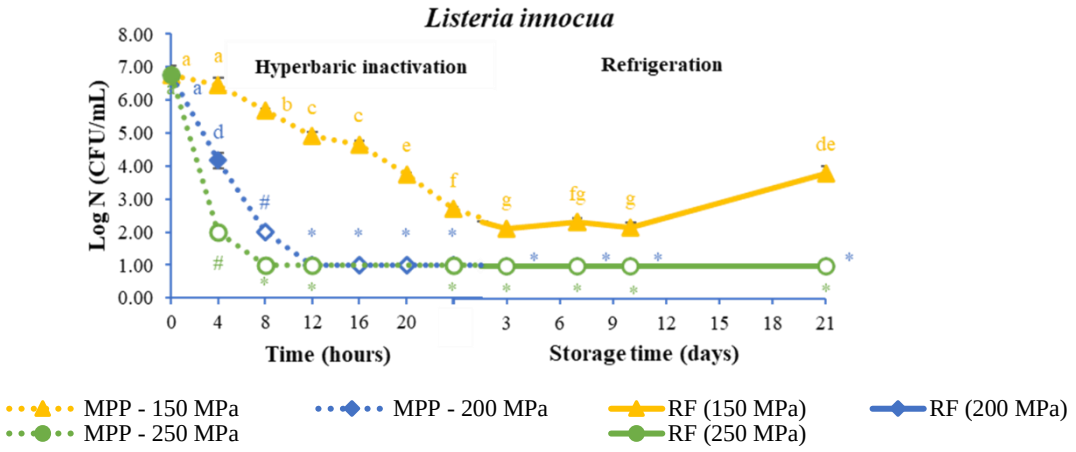

Figure 1: Effect of moderate pressure pasteurisation (MPP) by hyperbaric inactivation (HI) of milk at 150 MPa, 200 MPa, and 250 MPa and ≈ 20 °C, up to 24 hours (dashed lines), followed by storage under refrigeration at 4 °C (RF), up to 21 days (continuous lines), on milk inoculated with *L. innocua* ATCC 33090. Unfilled symbols with # and * indicate microbial counts below the quantification or detection limit (< 2.0 log CFU/mL or < 1.0 log CFU/mL, respectively), while different letters denote significant differences ($p < 0.05$) (a-g) for the same microorganism and storage condition along time.

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Funding: This work was financially supported by FCT (Fundação para a Ciência e Tecnologia).

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Opuntia spp. cladodes: Can this be a source of pectin for the food industry?

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Opuntia ficus indica (OFI) is a plant of the *Cactaceae* family and is well adapted to arid and semi-arid lands such as the ones in the Mediterranean area. The pads, denominated cladodes, are widely used for food and animal feed. Also, they can find application on cosmetic, pharmaceutical, and nutraceutical industries. Cladodes are rich in water, carbohydrates, and pectin. Pectin is a complex polysaccharide that is commonly used as gelling and stabilizing agent by the food industry. Usually, pectin is extracted from citrus peel fruits, and cladodes emerge as an alternative feedstock to this polysaccharide. Factors that affect pectin extraction are pH, time, temperature, solvent, agitation, solid/ liquid ratio. Extraction under acidic conditions and high temperatures are favourable for pectin extraction due to the promotion of the hydrolysis of protopectin [1,2]. Thus, the aim of this work was to optimize the pectin extraction from OFI cladodes and to compare the characteristics of this pectin with the pectin obtained from citrus peel fruits.

Firstly, the cladodes were characterized and then the extraction procedures were executed. Pectin extraction procedures comprises the general steps of washing/cutting; mixture with solvents; centrifugation; precipitation in ethanol; and drying. For this work, different solvents were tested (water, acetic and citric acid), different pH (1.5 – 7), different extraction temperatures (70-90ºC), different extraction time (40-60 min) and different L/S (Liquid/solid ratio) (5-15). Extraction procedures were tested in pulp and peel of cladodes. Lastly the pectin extracted was compared with commercial pectin in terms of the characterization of the main chemical functional groups through ATR-FTIR. Through the ATR-FTIR spectra it was also possible to calculate de degree of esterification of pectin.

Cladodes demonstrated slight differences between peel and pulp in terms of characterization. Both parts showed a high moisture content (90 and 95% wet basis, respectively), ash content of 20 and 16.5% dry basis (db), and a substantial total sugar content, higher in the pulp (30% w/w db) than in the peel (14.6% w/w db). In terms of total fiber content, both parts of the cladode presented values around 28g/100g db, proven to be a major fraction present in the vegetative part of the plant. Also, both peel and pulp demonstrated to be a good source of bioactive compounds in terms of phenolic compounds and antioxidant activity.

Based on the study of the solvent effect, an extraction yield was obtained in a range of 3.5% to 15% (in dry basis). The use of organic acids such as, citric acid and acetic acid solutions improves the extraction yields once they avoid pectin depolymerization and emerge as an alternative to mineral acids commonly used in pectin extraction [3]. The study of the pH demonstrated that a higher extraction yield can be obtained under acidic conditions (1.5- 4) obtaining values in a range of 2.5 -19 %w/w db. The ATR-FTIR spectra of the resulting materials revealed similarities to the commercial pectin on the chemical groups that are characteristic of this polysaccharide. Commercial pectin presented a high degree of esterification (85.35 %DE). The degree of esterification of OFI pectin showed low degree of esterification (≤50 % DE), which is characteristic of pectin extracted from this biomass [2].

Optimized yield of pectin extraction was obtained using citric acid as solvent, in the proportion of 10 L/S at a pH of 2, extracting at 80ºC for 50 min. Moreover, the pectin obtained will further be evaluated in terms of its characteristics to produce films, namely film forming ability. The use of the extracted pectin over the use of fossil-based plastics to produce a new packaging material is aligned with targets of the European Green Deal and of the Sustainable Development Goals of the United Nations.

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Post-harvest conservation of chestnut (cv. *Martaínha*), comparison of two controlled atmospheres during 60 days.

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According to Instituto Nacional de Estatística, during 2021, Portugal produced 38 thousand tons of chestnuts. The northern region of mainland Portugal is the largest concentration of chestnut trees is found and accounts for about 88% of the volume of national production. Portugal is the seventh-largest producer of chestnuts globally, although this value only represents approximately 2% of world production[1].
The quality parameters of chestnut are defined by colour, flavour and texture; however, this optimal status is only maintained for a short period of time. Chestnuts have a high moisture value that is quickly lost during conservation compared to other nuts. The significant factors in post-harvest depreciation are moulding or rotting caused by larval development of insects on the tree and later by fungi. Infections often start in the larval galleries of insects by the contact of the fruits with the ground before picking[2].
According to Cecchini (2011)[3] the best storage conditions are -1/-2°C and 90% relative humidity but the range of CO_2 and O_2 in a controlled atmosphere are yet to be optimized.
This study aims to increase the storage time of chestnuts. All of them were selected by immersion, and those that floated were rejected. For the control of the evolution, sixty chestnuts for each treatment were marked (numbered), weighed, and colour evaluated. Half of the fruits were submitted to a pre-treatment for seven days with CO_2 (40%), and another group did not undergo any pre-treatment. The chestnuts were conserved in two types of controlled atmospheres, a first with CO_2 (5%) and O_2 (3%) and a second with CO_2 (15%) and O_2 (3%). The numbered chestnuts were evaluated for each combination in weight, colour, texture, TSS, and sensory analysis.
According to the results obtained, we concluded that there are losses over 60 days in terms of quality, reaching a maximum difference of 10,5% in texture and 18,2% in weight loss. Regarding the sensory analysis, the chestnut maintained acceptability throughout the whole study.

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Cowpea immature pod purée: an innovative functional food product for elderly

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The demand for high-protein vegetal products has increased over the last few years, being grain legumes an excellent dietary source of protein, fibre, micronutrients, and phytochemicals. Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important grain legumes, widely growing in tropical and subtropical regions and in some Mediterranean countries.[1,2] Furthermore, it is a crucial crop in the face of scenarios of climate change since it is a heat and drought resistant crop and it improves soil fertility by fixing 80% atmospheric nitrogen in agricultural ecosystems.[1] Although dry seed is the highest economically relevant part of the crop, young leaves and green pods and seeds are also consumed worldwide.[3] Cowpea has a high protein and carbohydrate contents with low fat level. It is a valuable source of diverse health benefits components such as soluble and insoluble dietary fiber, resistant starch, phenolic compounds, minerals, such as potassium, magnesium and selenium, and B-complex vitamins.[2] Cowpea protein is rich in essential amino acids, particularly lysine and histidine. The ratio of essential to non-essential amino acids in cowpea seed is over 50%, indicating that it has the capacity to fulfill human nutritional needs. The nutritional composition of cowpea has been related to several health benefits, such as prevention of diverse metabolic and cardiovascular diseases.[3]

Previous works had shown that cowpea immature pods exhibited higher phenolic composition and antioxidant activity as compared to immature and dry seeds.[1] Thus, the present work aims to develop a cowpea immature pod ready-to-eat purée for elderly people, which fit their physiological limitations, to promote the maintenance of their muscle mass and the synthesis of neurotransmitters implicated in depression disorder and in sleep quality.

In a preliminary approach, this study intends to assess the phenolic content (total phenols, flavonoids and *ortho*-diphenols) by spectrophotometric methods and the antioxidant capacity, through ABTS•$^{+}$, DPPH• and FRAP assays, of the cowpea at two different growth stages, namely immature pods and green seeds. Additionally, their nutritional composition was also analyzed – the content in crude protein, crude fat, total starch, the dietary fibre, both soluble and insoluble, following the procedures described by Association of Official Analytical Chemists, as well as essential and non-essential amino acids by high-performance liquid chromatography with fluorescence detection (HPLC-FLD).

As expected, it was verified that immature pods revealed a significantly higher content of total phenols (11.73 ± 0.43 mg Gallic Acid (AG)/g dry weight), *ortho*-diphenols (13.18 ± 1.26 mg GA/g dw) and flavonoids (6.04 ± 0.51 mg Catechin (CAT)/g dw) as compared to the green seeds (3.32 ± 0.01 mg GA/g dw, 2.58 ± 0.17 mg GA/g dw and 2.92 ± 0.08 mg CAT/g dw, respectively). *The higher antioxidant activity* was also displayed by the immature pods (ABTS•$^{+}$: 0.05 ± 0.00 mmol Trolox/g dw, DPPH•: 0.04 ± 0.00 mmol Trolox/g dw, FRAP: 0.04 ± 0.00 mol Trolox/g dw) in contrast with the green seeds (0.01 ± 0.00 mmol Trolox/g dw, 0.01 ± 0.00 mmol Trolox/g dw and 0.01 ± 0.00 mol Trolox/g dw, respectively). Furthermore, immature pods demonstrated *low* crude fat content (1.74 ± 0.03%), such as green pods (1.95 ± 0.10%), and higher content of crude protein (27.48 ± 1.05%) and of insoluble dietary fibre (35.63 ± 0.53%) when compared to the green seeds (22.69 ± 1.20% and 12.37 ± 0.56%, respectively).

The results suggest that cowpea immature pods have remarkable potential to be included in the development of a new functional food product, which could contribute significantly to the improvement of sleep quality, to reduce depressive symptoms and to improve the quality of life and autonomy in activities of daily living of the elderly. To our knowledge, here we present the first study concerning the nutritional composition of cowpea immature pod, suggesting that it is a great asset, allowing farmers to make their business more profitable and diversified.

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Obtaining nutritionally enriched emulsified alternative - mayonnaise with *Tenebrio molitor* flour

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Tenebrio molitor is an insect of the family Tenebrionidae, which is a viable food source for human consumption due to its high nutrient content [1]. Recently, flour from this insect has been used in the production of products such as bread and biscuits [2,3]. This study aims to increase the nutritional quality of a factory-made mayonnaise sauce by incorporating *Tenebrio molitor* flour (a sustainable source of protein and bioactive compounds) and eliminating egg, modified starch, and, in some formulations, part of the chickpea. For this purpose, the impact of different concentrations of *Tenebrio molitor* (0% - Control, 1.7%, 3.4%, 5.1%, 7.5%, 10% and 15%) in the different mayonnaise formulations was studied. The microstructure of the samples, the texture profile (TPA), and the rheological properties (SAOS and frequency sweep tests) were analyzed. Different nutritional analyses were also carried out, such as the calculation of the total phenolic content (TPC) and the evaluation of the antioxidant capacity (DPPH and FRAP) of the mayonnaise extracts (**Figure 1**). Finally, a sensory analysis was used to determine the acceptability of the product on the market. At low concentrations (up to 7.5% of *Tenebrio molitor* flour) the structure remains practically unchanged. However, for higher additions of *Tenebrio molitor* (10% and 15%), a loss of firmness, stickiness, and viscosity is observed. The G' and G''(1Hz) values of these mayonnaises (10% and 15%) were significantly lower than those of the control mayonnaise, indicating an instability effect. Although the formulation with 7.5% *Tenebrio molitor* flour was not the best rated in the sensory analysis, it showed a higher complexity in the microstructure and a higher antioxidant capacity compared to the control sample. In addition, this attempt also presented the highest concentration in total phenolic compounds (16.25 mg GAE/g) and significantly increased the content of proteins (7.97%), lipids (14.68%), and minerals (Na, K, Mg, P, S, Fe, Cu, Zn), compared to the control sample, maintaining a similar appearance to this sample.

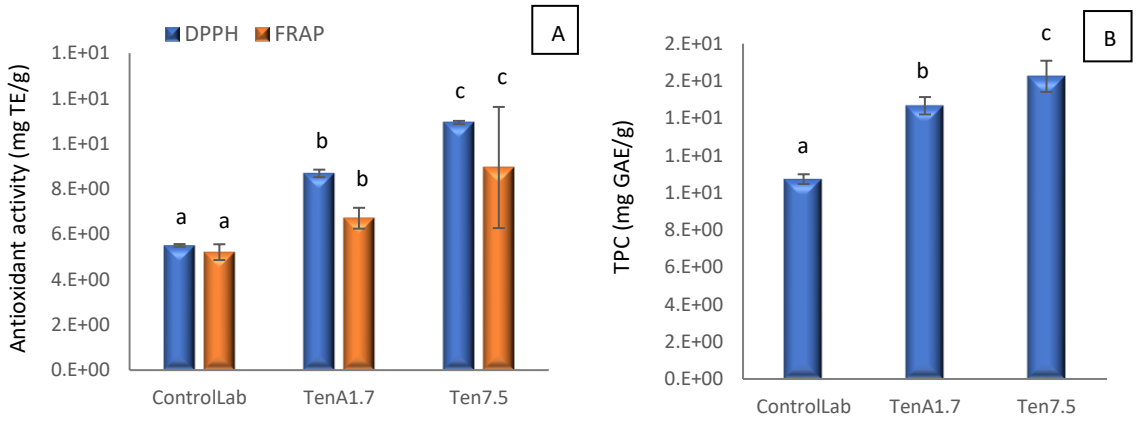

Figure 1: Antioxidant activity (A) and phenolic composition (B) of mayonnaises with the incorporation of different proportions of *T.molitor* flour (1.7% and 7.5%) and comparison with control samples (0%), n=3. The results of the DPPH (diphenyl-1-picrylhydrazyl radical) and FRAP (ferric reducing antioxidant power) analyses are expressed in mg Trolox equivalents (TE)/g. The results of the TPC analysis (total phenol content) are expressed in mg gallic acid equivalents (GAE)/g. Means with different letters are significantly different (Tukey test, $p < 0.05$).

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Design of "Pera Rocha do Oeste" structured fruit with agar and locust bean gum: nutritional, antioxidant, textural and sensorial properties

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Structured Fruits (SF) are food products prepared by mixing fruit pulp with small concentrations of hydrocolloids. These convenient food products present a longer shelf life compared to fresh fruit and are a good alternative to post-harvest fruit waste with low commercial value. The aim of this study was to develop a "Rocha" pear SF using the hydrocolloids Agar and Locust bean gum (LBG). "Pera Rocha do Oeste" has a Protected Designation of Origin and is characterized by a firm pulp and higher contents of dietary fiber and ferulic acid compared to other varieties of pears.[1] Experiments were performed to *(i)* evaluate the best hydrocolloid composition of SF and the *(ii)* effects of cooking the fruit and the inclusion of the peel (pectin source) on the sensorial performance of SF. Five SF were prepared using 100 g of fruit and two concentrations of agar and LBG: 0.2% and 0.8% of LBG in relation to 0.75% agar (**Figure 1).** For each formulation, 100 g of puree were transferred to a food processor (Thermomix ®, model TM5) and mixed with the specific composition of hydrocolloids. The mixtures were kept at 90 ± 2 °C for 8 minutes and under stirring (rotation speed 6) and then poured into spheric silicone molds (W x H x L = 25 × 15 × 30 mm), where they remained at room temperature (± 25 °C) for 30 min. After remove from the molds, SF were placed in a hermetically sealed container and stored under refrigeration at 5 ± 1 °C for 12 h to complete the gel maturation of the SF. Samples A-E were evaluated regarding some physicochemical (pH, moisture, water activity), nutritional (carbohydrates, reducing sugars, dietary fiber, and ash) and antioxidant (total phenolics, DPPH, FRAP and ABTS assays) parameters and were sensorially evaluated by a group of 50 untrained tasters of both genders (aged from 18 to 56 years old). SF with higher scores of overall acceptability were used for texture studies. Samples with 20x20x20 mm were placed on the plate and a 2-cycle compression test was performed. The texture parameters Firmness (N), Springiness, Resilience, Adhesiveness (N), Cohesiveness, Gumminess (N) and Chewiness were calculated as described by [2] and [3].

Results showed that samples A-E were similar in terms of pH, moisture, and ash. Although the SF prepared with cooked peel+pulp, agar and 0.2% LBG (sample C) presented significantly ($p < 0.05$) higher contents of dietary fiber, total phenolic compounds and antioxidant activity, the SF prepared with cooked pulp and a mixture of agar and 0.2% LGB (sample D) or 0.8% LGB (sample E) were scored with higher ($p < 0.05$) "Global Acceptance" and "Intention of Purchase" (**Figure 2**). Both formulations (samples D and E) presented a water activity of 0.970, suggesting that these food products must be kept in cold storage and consumed within three or four days to maintain a sense of freshness close to the one observable in the pulp. They also display a similar textural behavior, with non-significative differences in what concerns of most texture parameters, exception made for a significative difference ($p = 0.013$) in firmness. However, although sample D was firmer than formulation E, both structures respond in a similar way ($p > 0.05$) after the first bite (first compression cycle).

In conclusion, the mixture of agar and locust bean gum at 0.2% in relation to 0.75% agar was the most promising hydrocolloid composition for the formulation of "Pera Rocha do Oeste" structured fruit. Within this composition, the formed network was strong enough to guarantee good acceptance in the texture attribute and allowed the release of compounds that are associated with the flavor of the product. The developed product shows gastronomic potential, it could be applied by the food industry as a filling for chocolate and confectionery products, for example. This innovative food product could also be an excellent alternative to valorize low quality "Pera Rocha do Oeste", being a good way to increase the consumption of this pear variety through an appealing product with promising nutritional properties.

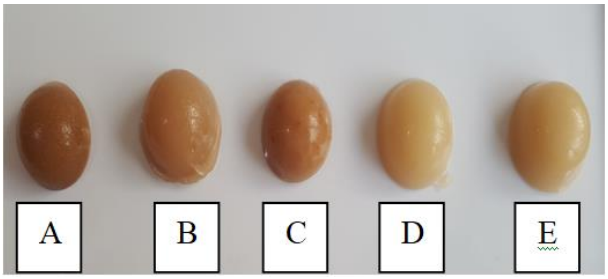

Figure 1: "Pera Rocha do Oeste" structured fruits (samples A-E).

Sample A - SF prepared with raw fruit (peel + pulp) and Agar + 0.2% LBG, Sample B - SF prepared with raw pulp and Agar + 0.2% LBG, Sample C - SF prepared with cooked fruit (peel + pulp) and Agar + 0.2% LBG; Sample D - SF prepared with cooked pulp and Agar + 0.2% LBG; Sample E - SF prepared with cooked pulp and Agar + 0.8% LBG.

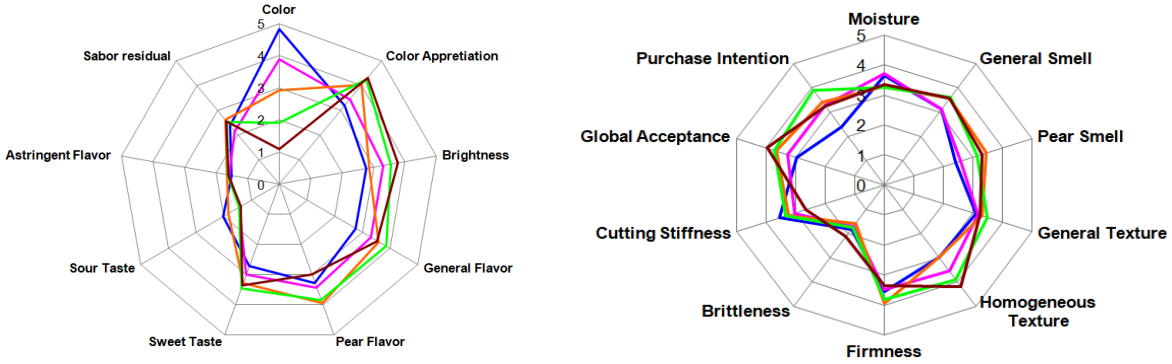

Figure 2: Sensory profiles of "Pera Rocha do Oeste" structured fruits based on averages of ratings by n= 50 panelists. Scale: 1-dislike extremely; 2-dislike slightly; 3-neither like nor dislike; 4-like slightly; 5-like extremely. Sample A, **Sample B**, Sample C, Sample D and Sample E.

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Production of a sustainable, healthy and plant-based food under controlled conditions: Innovative miso

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Consumers' awareness of the food chain's impact on the environment and the well-being of society has contributed to a huge demand for more sustainable, nutritious and eco-friendly foods.
In recent years, a clear change in consumers' diet has been observed, towards the valorisation of plant-based foods; responsible/authentic food production; sustainable/regenerative agriculture; of pleasure meals (new *flavours*, aromas, textures and colours) and food that benefits their gut microbiota[1].
Miso is a great example of the type of food that consumers are looking for. This traditional fermented food from Japan is a paste, consumed in soups and used as a seasoning/*flavouring* agent. Usually, miso is produced by fermenting cooked/smashed soybean with salt, koji and back-slopping inoculation[2]. Koji (steamed rice inoculated with *Aspergillus oryzae*[2]) is currently considered one of the 12 biggest global food trends in 2022, used by *Top Chefs* from New York and London, as a star ingredient[3].
Health benefits are associated with miso consumption, due to microorganisms involved in the fermentation process, such as, lactic acid bacteria (LAB) and halophilic yeast, many of them with probiotic potential[2].
The foremost goal of this PhD project is to develop and characterize innovative, sustainable and healthy food products, including miso. In miso, the main raw material (soybean) will be replaced by traditional Portuguese crops (chickpea, lupine and cowpea). To produce miso, besides *A. oryzae* coming from koji, other microorganisms will be inoculated, like yeasts (*e.g., Zygosaccharomyces rouxii* and *Debaryomyces hansenii*) and LAB (*e.g., Tretragenococcus halophilus*, *Lactiplantibacullus plantarum*), to improve organoleptic proprieties and enhance health benefits. Furthermore, to ensure food products' quality and safety, miso will be produced under controlled conditions regarding the number and viability of the fungus spores inoculated via koji and of LAB and yeast cells, the amylolytic, proteolytic and lipolytic activity of the fungus, and fermentation temperature and time.
In order to choose the best *A. oryzae* strain for miso and standardize its inoculum (koji), qualitative analyses were performed on *Aspergillus* fungi strains to study their ability to grow and sporulate and their hydrolytic activity potential. One laboratory (LK, isolated from koji) and two commercial (KK and BK) strains were tested. The strains were plated in potato dextrose agar (PDA) and incubated until visible sporulation. Cells were collected; resuspended in sterile water and 10-fold serial dilutions were prepared. The total spore number was determined in a hemocytometer. To select the best media to grow the fungus and determine relative cell viability (RCV%), dilutions were inoculated in three different media (malt extract agar (MEA); yeast extract peptone dextrose agar (YPD) and PDA). Amylolytic activity potential was tested in a medium supplemented with 0.2% starch (pH = 6), after 4 and 8 days of incubation at room temperature, followed by the addition of 1% (w/v) iodine solution. The enzymatic index (EI) was calculated from the ratio between the diameter of the halo obtained and of the fungus colony.
RCV% results revealed that PDA is the best medium for *A. oryzae* growth when compared to the other media, allowing to obtain the highest fungus viability for all the tested strains (LK 30.82, KK 69.57 and BK 103.72) (Figure 1(a)). Regarding amylolytic activity potential (Figure 1(b)), based on the EI values obtained, KK strain presented the highest value (1.34), revealing a greater potential for the production of extracellular amylases, capable of degrading carbohydrates and antinutrients[2].
After establishing inocula growth conditions, we started the fermentation, following the process over fermentation time. New miso will be periodically evaluated and compared with traditional miso for microbiota (cell viability and dominant species); physicochemical parameters (pH, soluble solids, sugars consumption and metabolites production, colour, texture and linear viscoelasticity). Nutritional and sensory characteristics, as well as bioaccessibility and bioactivity, will also be evaluated in the final products.
Finally, we believe that this novel and healthy miso will fit the *"back to roots"* movement – a tendency that promotes sustainable agriculture – being produced from traditional Portuguese crops, reducing carbon's footprint and supporting national and local food production.

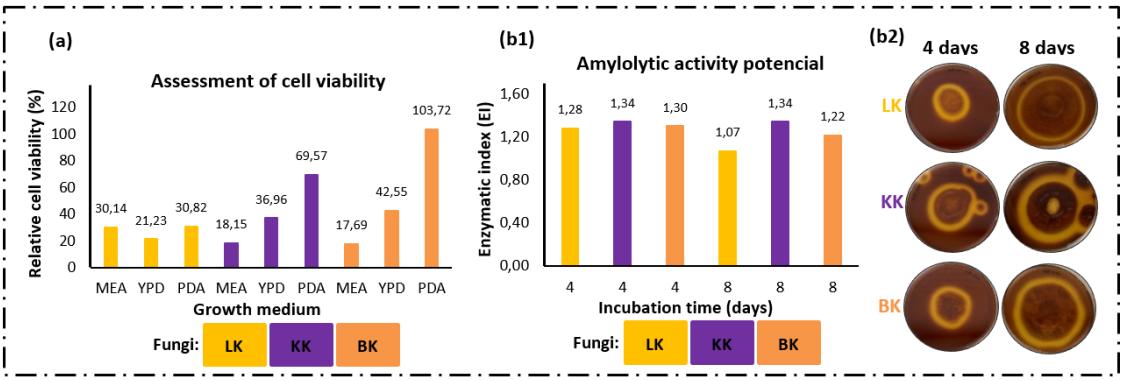

Figure 1: Assessment of cell viability and amylolytic activity potential. (a) determination of relative cell viability (%): ratio between cell viability and Total cell counts. (b1) calculation of enzymatic index: ratio between the clear zone diameter and the fungus colony diameter (b2) diameters of fungus colony and of clear zone formed around the fungus colony after iodine addition into the plates.

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Non-commonly used Edible Vegetable Substrates for Fermentation: An Alternative and Sustainable Source for Innovative and Healthy Food Products

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Today, market trends show that consumers value sustainable and healthy products. Minimizing food loss and waste while guaranteeing healthy products are some of the main challenges faced by the food industry to respond to consumers concerns and sustainability expectations. Aligned with these trends, food fermentation has become an important biological process for food technology, where microorganisms' metabolism offers added nutritional value and organoleptic features to an increasingly large number of foods, allowing the development of innovative, safe, and appealing food products. [1] Also, fermented foods have been found promising for the promotion of human health as a potential source of pro- and postbiotic elements and bioactive compounds.[2]

FermVegAlgae project, a partnership between the Instituto Superior de Agronomia and the companies Mendes Gonçalves, S.A. and MC Sonae, aims to develop new fermented products, thus offering alternatives of innovative, healthy and sustainable food products that can address consumers' concerns. With this goal, surplus and damaged vegetables, which are currently treated as agricultural waste or low value by-products, were selected, such as kale's stalks, carrots and beetroot's stalks and leaves. Additionally, common and underused atlantic seaweeds were also chosen and are being explored as raw materials, due to their promising nutritional profile and interesting characteristics.

In accordance with the goals of the project, beetroot's stalks and leaves and Galician kale stalks both bleached and non-bleached were used for fermentation by a consortium of two lactic acid bacteria (LAB) commonly found in vegetable fermentations, as starter culture. Fermentations were performed in the presence of 1% added salt. For seaweed fermentation, four LAB strains selected for their metabolism and probiotic properties were tested.[3] Screening trials were conducted with four different edible seaweed species - *Ulva rigida* (green seaweed), *Gracilaria gracilis* (red seaweed), *Saccharina latissima* (sugar kelp) and *Alaria esculenta* (brown seaweed) - with testing concentrations between 0,3% and 1,5% of salt. Fermentations were followed for 14 days by measuring pH, titratable acidity and °Brix until stabilization. CFU at the beginning and end of the fermentation process were also evaluated. The proximal composition, antioxidant capacity, texture and viscosity of the fermented products is currently being determined.

Regarding the vegetables, for beetroot's stalks (Figure 1), °Brix increased and pH decreased throughout the 14 days' fermentation process reaching a °Brix of 5,5 and a pH value below 4,0, typical of lactic acid fermentation. In accordance with pH, the titratable acidity revealed the increase of the lactic acid percentage throughout the trial period. The same did not happen with the Galician kale and beetroot's leaves.

Concerning seaweed fermentation (Figure 2), *U. rigida* and *A. esculenta* achieved the lowest pH results, between 4,0-5,0 and 3,0-4,0, respectively, and the highest °Brix values, between 4,2 (0,3% salt) and 3,8 (1,5% salt) for *U. rigida* and 2,8 (0,3% salt) and 5,0 (1,5% salt) for *A. esculenta*. Titratable acidity also increased during the trial period, both for *U. rigida* and for *A. esculenta* reaching to 3,0% lactic acid (initial values between 0,5 and 1,5%). CFU counts revealed that the number of viable cells has risen two- to three-fold (10^8-10^9) regarding the initial inoculum of starter cultures (10^6-10^7), indicating that the lactic acid bacteria had grown and the fermentation occurred properly. *G. gracilis* and *S. latissima* failed to obtain the desired results and characteristics. Further trials are underway for *U. rigida* and *A.* esculenta, to optimize the fermentation process and evaluate the antimicrobial potential.

The results obtained so far demonstrate that both beetroot stalks and seaweeds *U. rigida* and *A. esculenta* are quite promising for fermentation by LAB, with potential to be incorporated in food products such as vegetable pastes, sauces among others. It is expected that FermVegAlgae project will offer innovative and more sustainable fermented products, with additional nutritional and health benefits that satisfy the consumer's needs.

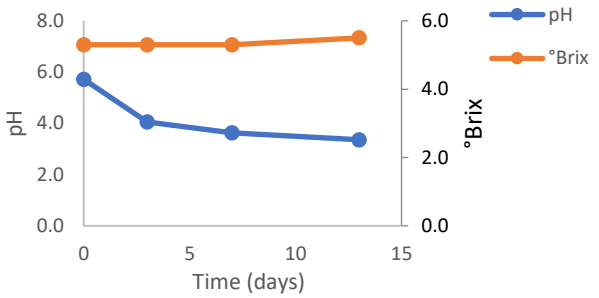

Figure 1 - Evolution of the pH and °Brix of non-blanched beetroot's stalks fermentation with LAB consortium.

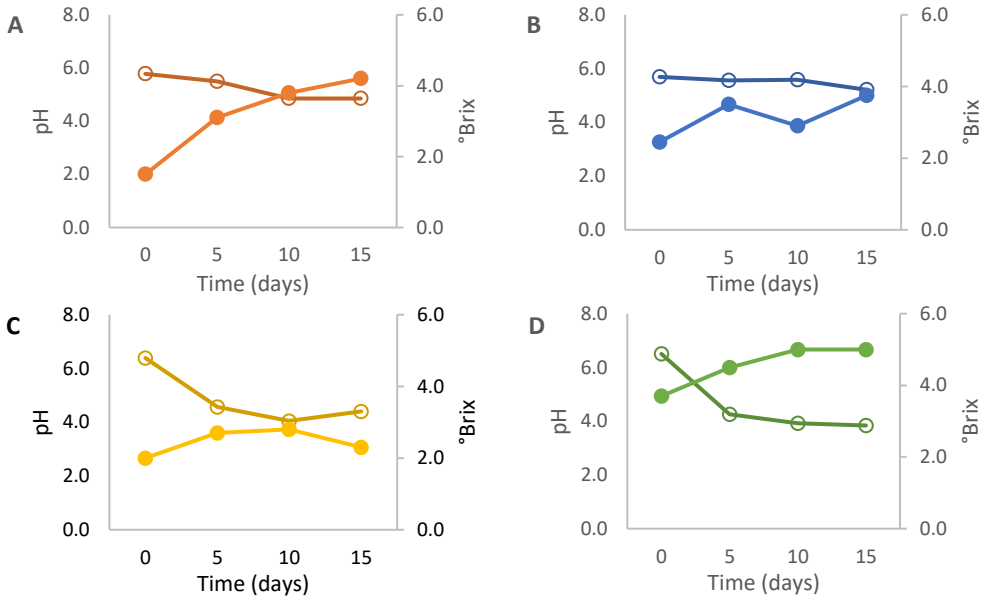

Figure 2 - Evolution of the pH, °Brix throughout seaweed fermentation with LAB consortium. A: *U. rigida*, 0,3% NaCl; B: *U. rigida*, 1,5% NaCl; C: *A. esculenta*, 0,3% NaCl; D: *A. esculenta*, 1,5% NaCl. pH open circles; °Brix closed circle.

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Funding: The FermVegAlgae Project is financially supported by Compete 2020, Lisboa 2020, Portugal 2020 and European Social Fund and Portuguese Foundation for Science and Technology (FCT), UIDB/AGR/04129/2020 LEAF Research Unit.

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Ultrasound extraction to improve the sensory profile of microalgal biomass

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With the world's population expected to reach nearly 10 billion by 2050, the challenges of feeding it are only increasing. In this context, new dietary strategies must be devised to provide the population with the necessary nutritional intake on a daily basis. Microalgae are emerging as a promising commodity to help address the food problem. Their inclusion in foods usually needs a pretreatment to ensure cell disruption and the consequent release of the bioactive molecules[1]. Furthermore, one of the main drawbacks for the industrial scale-up is related to the challenging colours, flavours and tastes present in microalgae-containing formulations[2]. In this work, ultrasound extraction is used as a valuable technique to address these two objectives: controlled cell disruption and improvement of the microalgal sensory profile, with a reduced loss of antioxidant components. It is intended to optimise the ultrasound extraction process of *Nannochloropsis oceanica* and *Dunaliella salina*, promoting the full recovery of the two fractions, in a circular economy logic - zero waste. Further, the solid protein-rich fraction obtained after the extraction, can be incorporated into bread, and the liquid bioactive-rich fraction can be incorporated into mayonnaise.

Extraction by traditional methods require more solvent and are time-consuming. Extraction by ultrasound (US) treatment appears to be an interesting alternative as it is efficient, easy to implement and reproducible. Several extraction conditions were tested with varying microalgal concentration (1% / 5% w/v), ethanol/water proportion (96% / 60%) and temperature (room temperature / 50°C). The other US extraction conditions were fixed, acoustic power density was 1.9 W/mL and extraction duration was 5 min. The extractions yielded up to 97%. The results showed a reduction in undesirable smells from the microalgae and a pigment removal when ultrasound treatment was applied, with different impact on total phenolics (*Folin-Ciocalteu* assay) and antioxidant capacity (DPPH and FRAP assays) (**Figure 1**), which are dependent on microalgal cell wall morphology. Microscopy was also used to evaluate the impact of US on cell disruption.

The extraction conditions of 1% microalgae, 60% ethanol and room temperature appear to be more interesting. Indeed, the antioxidants were presents in both solid and liquid phases in a balanced way. 60% ethanol solvent seem to be better than 96% ethanol, as solid phases retain well antioxidant activity, and it is also more sustainable to use less ethanol for extraction.

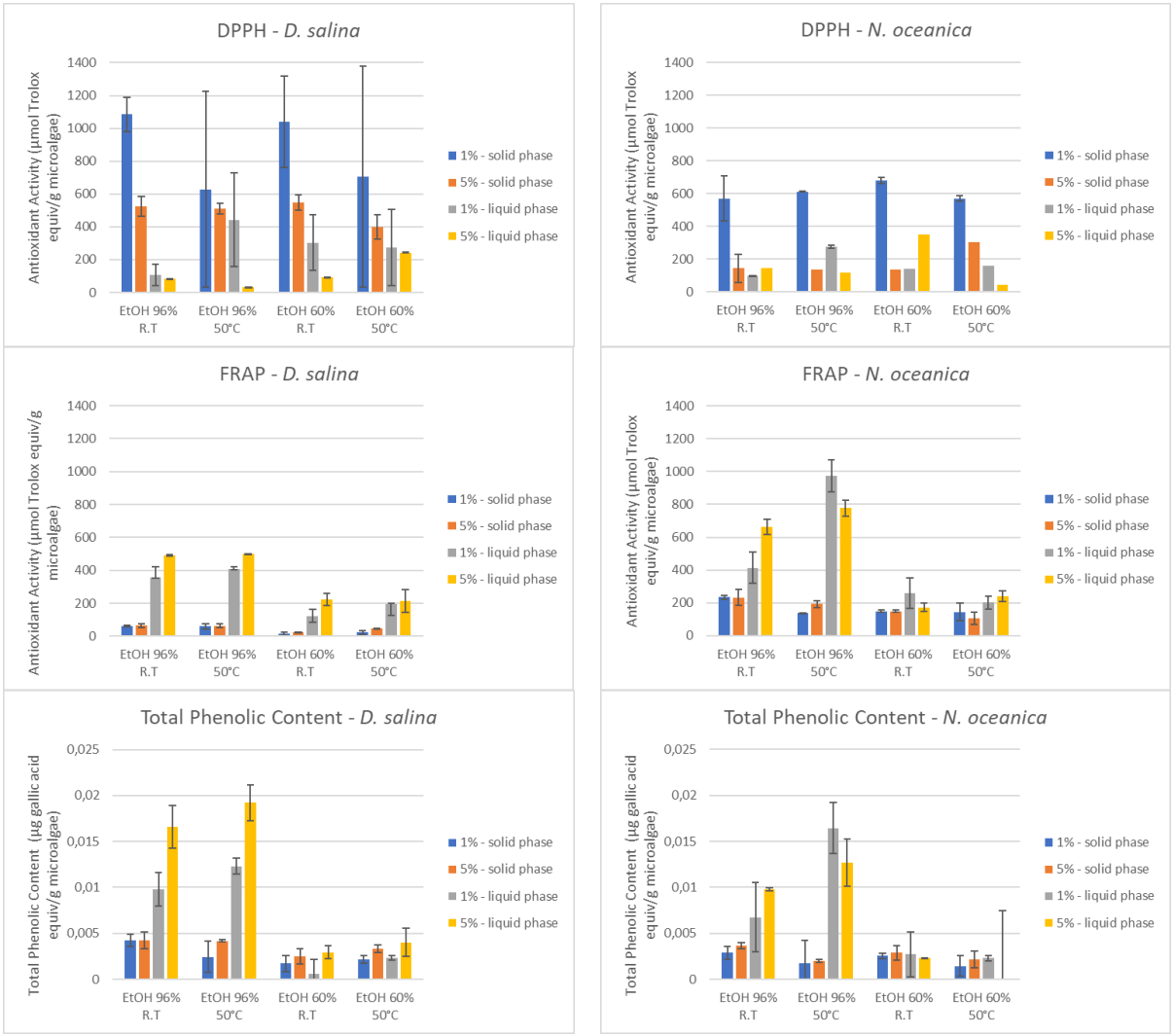

Figure 1. Antioxidant activity (µmol Trolox equivalent/g of microalgae) and total phenolic contents (µg gallic acid equivalent/g of microalgae) in *D. salina* and *N. oceanica*. Data shown mean *± SD, n=2.*

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Optimized microwave-assisted extraction of fish oil from fish industry by-products

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The seafood industry annually produces large amounts of fish by-products, of which a small part is used directly for animal feed.[1] However, the valorization of these by-products is still minimal and the industry is looking for more profitable ways to sustainably transform these by-products. As these contain high levels of fat-soluble constituents, fish oil production looks promising.[1] However, conventional oil extraction methods can be time consuming and unattractive for the industry. Although unconventional methods have been implemented for some high value-added compounds, their application to fish oil recovery has been little explored. Therefore, this work was carried out with the main objective of optimizing the microwave-assisted extraction (MAE) of oil from fish by-products and comparing the efficiency of this method with that of Soxhlet extraction (SE). Two batches of fish by-products supplied by the industry and composed of different types of fish were characterized and lyophilized. The MAE was made according to a central composite design combining five levels of processing time (1–30 min), microwave power (50–1000 W) and solid/liquid ratio (70–120 g/L), and the response surface methodology was used for process optimization.[2] For comparison and evaluation of MAE efficiency, a conventional SE was performed for 6 h, using solid/liquid ratios of 20 g/L.[2] Hexane was the solvent used in both methods. Then, the oil yield was determined gravimetrically and the fatty acid profile was characterized by gas chromatography with flame ionizing detection (GC-FID) after a derivatization process.[2] Afterwards, the experimental data were fitted to a quadratic equation using Design-Expert® software and the developed models were statistically validated based on a high a coefficient of determination and a non-significant (p > 0.05) lack-of-fit, among other statistical criteria. The oil yields were significantly affected by the three independent variables through linear, quadratic, and/or interactive effects. The extraction yields also varied as a function of the sample batch, which justified the determination of individual and global optimal extraction conditions. In general, MAE allowed fish oil yields similar to SE, but with extraction times shorter than 20 min. The model-predicted optimal MAE conditions were successfully experimentally validated. Regarding the fatty acid profile, unsaturated fatty acids predominated over saturated fatty acids, given the high levels of oleic, docosahexaenoic acid (DHA) and linoleic acids, and it was not affected by the process variables. Accelerated shelf-life studies are now being carried out to stabilize the obtained lipid fraction with plant-derived bioactive compounds.

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Mayonnaise with table olive flours: development and characterization of an innovative product

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The olive sector has been investing in innovation, exploring new products and presentations to respond to the market, not only to satisfy consumer tastes and needs but also to reduce losses in the food industry within a circular economy perspective. Our research group has recently characterized "table olive flours", a new product gaining increasing expression, prepared from fruits at different maturation stages - green, turning colour, and black olives, with interesting results[1], aiming to increase the value of table olives not prone for commercialization. Following these studies, this work intended to explore a new valorisation approach for those "flours", by incorporating them into mayonnaises.

Four mayonnaises produced without (control) and with three different "table olive flours" from cv. Cobrançosa were studied, with the support from rheology (texture and viscosity), lipid fraction characterization (fatty acids and tocopherols), antioxidant activity (Total Reducing Capacity, and Free Radical Blocking Effect of DPPH) and phenolic profile by High-Performance Liquid Chromatography - Diode-Array Detection (HPLC-DAD), together with quality parameters for lipid oxidation (peroxide indices and specific extinctions at 232 nm and 268 nm). The mayonnaises with "table olive flours" had different colours (**Figure 1**) and textures than the control mayonnaise, with few exceptions. The mayonnaise with the "green olive flour" stood out in terms of firmness (maximum positive force) compared to the control, being firmer than all the others approaches. When evaluating the viscosity of the mayonnaises, hysteresis was observed, presenting a behaviour closer to a thixotropic fluid.

Figure 1: Mayonnaises with "olive flours": control (without "olive flour"), with "table olive flours" from fruits at different maturation stages - green, turning colour, and black olives (from left to the right).

The main fatty acid found in mayonnaise was linoleic acid (C18:2c), with percentages between 53.6% (corresponding to mayonnaise with the addition of "black table olives flour") and 56.0% (value corresponding to the control). The mayonnaises with "olive flours" presented higher percentages of saturated (SFA) and monounsaturated fatty acids (MUFA) and lower percentages of polyunsaturated fatty acids (PUFA) than the control mayonnaise, influenced by the olive flour addition. Among the tocopherols, α-tocopherol was the major, with the control mayonnaise having the highest value. However, in this sample (control), β- and γ-tocopherols were not detected, suggesting that these compounds are only present in mayonnaise if "table olive flour" is added. Furthermore, it was found that the control had the highest total reducing capacity (2.60±0.42 mg equivalents of gallic acid/g mayonnaise). On the contrary, the inhibition percentages of the DPPH free radical varied between 16.0 and 18.4%, with no significant differences between the four analysed mayonnaises. Thus, adding "table olive flour" did not affect this parameter. Regarding the phenolic compounds, hydroxytyrosol, tyrosol and luteolin were detected. The mayonnaise with "green olive flour" had the highest levels of these compounds. The mayonnaise with "turning colour table olives flour" was the one that showed the lowest peroxide values. Still, all the mayonnaises showed similar levels of lipid oxidation, evaluated by

the specific extinctions at 232 nm and 268 nm in iso-octane. Therefore, mayonnaises with the incorporation of “table olive flours from cv. Cobrançosa” can be an innovative product, reaching new clients.

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Study of odorant compounds and sensory changes associated with wine spirit ageing using chestnut wood and Limousin oak under different technologies

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Wine spirit, resulting from wine distillation, usually undergoes ageing in wood before being marketed. Traditionally, the oak wood, especially from the French region of Limousin (mostly *Quercus robur L.*), is used for manufacturing the barrels for this purpose. However, the results from several studies pointed out the suitability of chestnut barrels for the ageing of wine spirits[1]. Usually, this ageing process is carried out by placing the distilled beverage in wooden barrels for a more or less extended time. However, recent works[2] showed that alternative systems, in which wood fragments are placed into the beverage kept in stainless steel tanks, are an interesting technology. Some of these works also highlighted the importance of oxygen, providing promising results through the combination of staves with micro-oxygenation in the ageing of these beverages[2,3].
This work aimed to study different levels of micro-oxygenation applied simultaneously with wood staves in the ageing of wine spirit in glass demijohns and their comparison with the traditional system using wooden barrels. A two way experimental design was established with two levels of the first factor (Chestnut wood *versus* oak wood from Limousin) and five levels of the second factor (different micro-oxygenation conditions) and two replicates of each assay modality, giving a total of 20 experimental units. The same wine spirit produced in the Lourinhã region (Portugal) was used to fill these units. The ageing process was followed for 12 months, and samples were collected over time. The volatile composition of the samples was evaluated by GC-FID and GC-MS. After 12 months, the wine spirits were bottled and analyzed regarding their volatile composition (GC-FID and GC-MS) and sensory profile, which was carried out by a trained sensory panel. Statistical analysis (ANOVA and principal components analysis-PCA) were applied to the chemical and sensory results.
The volatile determination was focused on the compounds (Fig. 1) previously assigned as odorant compounds.

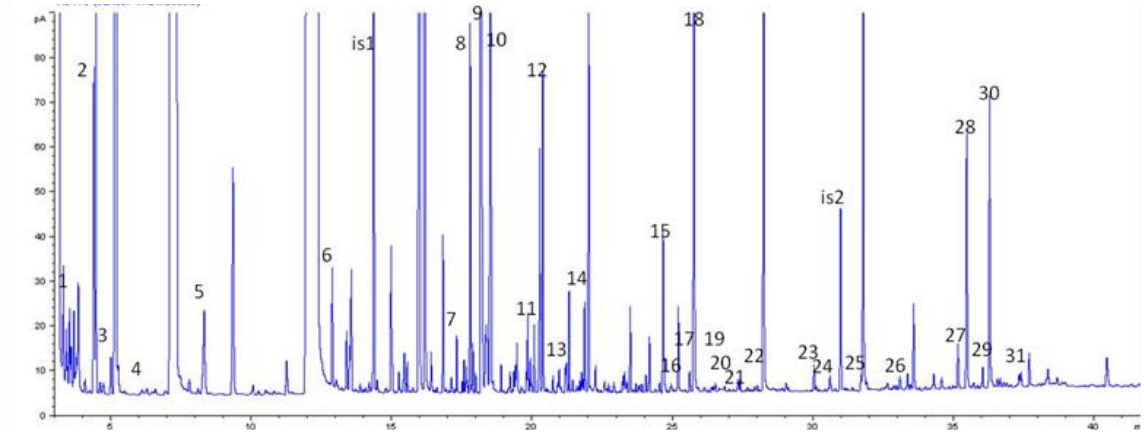

Figure 1. Chromatogram of an extract from an aged wine spirit in Chestnut wooden barrel

1: Ethyl isobutyrate; 2: isobutyl acetate; 3: ethyl butanoate; 4: ethyl isovalerate; 5: isoamyl acetate; 6: ethyl hexanoate; pi1: 5-methyl-2-hexanol (internal standard 1); 7: trans-2-hexenol; 8: ethyl octanoate 9: acetic acid; 10: furfural; 11: linalool; 12: 5-methylfurfural; 13: butanoic acid; 14: isovaleric acid; 15: hexanoic acid; 16: guaiacol; 17: trans β-methyl-γ-octalactone; 18: 2-phenylethanol; 19: *cis* β-metil-γ-octalactone; 20: 4-methylguaiacol; 21: 4-ethylguaiacol; 22: diethyl malate; 23: eugenol; 24: 4-ethylphenol; pi2: 3,4- dimethylphenol (internal standard 2); 25: syringol; 26: 4- methylsyringol; 27: dodecanoic acid; 28: HMF; 29: 4- allylsyringol; 30: vanillin; 31: acetovanillone.

The obtained results confirmed the significant influence of the wood's botanical species on the volatile composition and the sensory profile of the aged wine spirits. Those aged with chestnut wood had significantly higher overall quality than those obtained with Limousin oak wood. Regarding the ageing system, the wine spirits aged by alternative system seemed to present higher contents of some odorant compounds, namely some volatile phenols. Particularly, the modality of highest flow of micro-oxygenation (2 mL/L/months during 60 days followed by 0.6 mL/L/months during the remaining ageing time) resulted in the best sensory results.

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Inactivation of *Escherichia coli* and *Listeria monocytogenes* in raw goat milk by pulsed electric fields and mild heating

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Pulsed electric field (PEF) is a nonthermal processing technology that consists of exposing food to pulses of high-voltage electric fields during a short period of time (from nanoseconds to milliseconds) at moderate temperatures. PEF technology has been presented as advantageous in comparison to conventional heat treatments in microbial inactivation studies at low temperature without adversely affecting the color, flavor and nutritional value. In order to increase the lethality of PEF treatments and reduce the sublethal fractions of microorganisms and/or inhibit their recovery, the use of several hurdles in combination or in a successive manner may act additively or synergistically and difficult the survival of poisoning microorganisms. Combining PEF with mild heating allows softer individual treatments to be applied, being an effectively preserved, secure and minimally processed product. One of the most studied products has been milk, particularly bovine milk and because of that, studies with raw goat milk processed with PEF are very scarce. Raw goat milk is usually employed to produce goat cheeses because of its strong typical flavor and other organoleptic attributes. To ensure the microbial safety of such cheeses and maintain nutritive value close to raw cheeses, a low thermal load during treatment is a useful alternative to conventional heat treatments that destroys the heat-sensitive microflora and enzymes involved in the ripening of goat cheeses. This study was carried out to evaluate the effectiveness of low intensity PEF combined with mild heating in the inactivation of *Escherichia coli* and *Listeria monocytogenes* in raw goat milk and evaluate the possibility of reducing the pasteurization temperature/time, therefore minimizing any potential nutritional/organoleptic losses as well as keeping their food security.

The strains of *Escherichia coli* (ATCC 11775) and *Listeria monocytogenes* serotype 4b (ATCC 13932) used in this study were inoculated to get an initial microbial level of approximately 8-9 $\log_{10}$/mL in raw goat milk (from a traditional cheese dairy – Prados de Melgaço). The milk was pasteurized in a FT74XTA HTST/UHT system-Armfield at temperatures of 63, 66, 69, 72 and 75ºC and 2 s holder combined with and without a continuous flow system with a collinear treatment chamber design of PEF (EPULSUS®-LPM1A-10, EnergyPulse Systems, Lda) treatment with a flow rate of 2,92 L/h and an electric field strength, pulse width and frequency of 10 kV/cm, 50 µs and 3 Hz, respectively.

E. coli was more sensitive to the thermal treatments when compared with *L. monocytogenes*. Pasteurization at 75ºC with PEF as pre-treatment reduced the microbial load by a maximum of 7,49 log cycles for *E. coli* and 5,01 log cycles for *L. monocytogenes*. Applying only pasteurization at 75ºC lead to a reduction of 4,62 and 2,87 log cycles for *E. coli* and *L. monocytogenes*, respectively. The combination of PEF-pasteurization decreases the D value in all temperatures for both microorganisms, which is particularly noticeable at lower temperatures. A D_{75}=0,43s for *E. coli* without PEF pre-treatment was obtained, and after PEF pre-treatment a D_{72}=0,45 s was obtained. This reduction is more pronounced for *L. monocytogenes* with a D_{75}=0,69s without PEF pre-treatment and a D_{69}=0,69s after PEF pre-treatment.

The results of this study showed that through the combination of treatments it is possible to obtain a better microbial reduction, allowing to reduce the temperature and obtain the same inactivation level. Results indicate that the PEF has the potential to be used as a complementary method to mild thermal pasteurization as a hurdle processing technique to ensure the safety and maintain nutritive and sensorial qualities of goat milk at the industry level.

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Colorimetric labels based on pyranoflavylium-cellulose acetate films for food intelligent packaging

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Polymeric-based formulations enriched with anthocyanins as pH-sensitive natural dyes have been widely studied to produce films for food spoilage monitoring. However, anthocyanins are not promising because of their instability issues namely color fading with time, chemical and thermal degradation, and prone to oxidation reactions. On the other hand, some anthocyanin derivatives such as pyranoanthocyanin-like compounds are much more stable pigments because of their simplest chemistry and are poorly reported for those applications. Bearing this, a suitable and bio-inspired pyrano-3,7-deoxyanthocyanidin (PyF) dye previously developed was immobilized (0.2 % (w/w)) into cellulose acetate (CA)-based films with glycerol (30 % (w/w)) as a plasticizer, building up a colorimetric pH-freshness indicator for food packaging applications.[1,2] The films were obtained by the casting method and characterized by the thickness, morphology and barrier properties, thermogravimetric analysis, and color, among others. The pH-responsive properties of the films were tested in buffer solutions at different pH values (pH 4 to 8) and in amine-rich simulated environments. Releasing studies of film components to the medium and photostability assays are ongoing and validation tests on real samples are envisaged. The results showed that the incorporation of glycerol (up to 30 % (w/w)) in CA films tunes their chromatic variation suitable and efficient towards buffer solutions with different pH values (between 4 - 8), as well as in amine-rich headspace of model-like solutions (biogenic amines and ammonium hydroxide) at different concentrations (**Figure 1**). The films showed relevant and remarkable color change at the pH range of food spoilage indicating a great potential for application as a food freshness indicator in intelligent packaging.

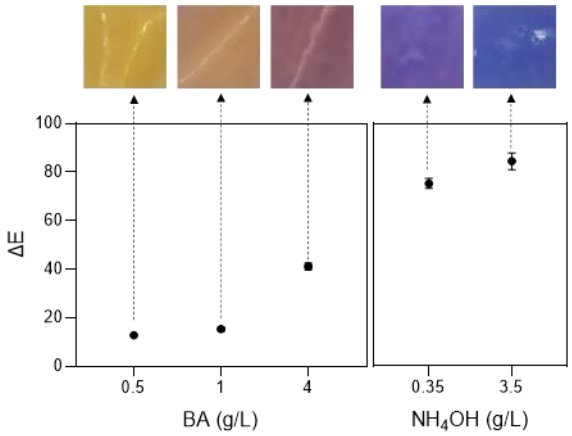

Figure 1. Color variation (ΔE) for the indicator developed, after overnight headspace exposition to different BA (g/L) and ammonia solutions (g/L), and photographs of the final color of the films. Adapted from (2).

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Vegetable extracts as alternatives to nitrite in cured meat sausages

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Processed meat is classified as carcinogenic to humans, since 2015, due to the association between colorectal cancer and dietary nitrites in processed meats[1]. Although meat products are not the only nitrite ingestion pathway, this classification was speculatively overvalued by the media and resulted in consumers' distrust of these products. Consumers are increasingly searching for "clean label" foods, meaning products without chemical additives. Therefore, several strategies to substitute nitrite in meat products' formulations are being studied, and vegetable extracts associated, or not, with starters, have shown promising results[2]. This work aims to develop a clean label meat product, using *Thymus citriodorus* and *Salvia elegans* as natural replacers of nitrate in a cured meat sausage (CMS), combined with a *S. equorum* starter. Three batches of each of the eight CMS formulations were produced: C1- Control without nitrate or starter; C2- Control with starter without nitrate; F1- 150 mg KNO3/kg; F2- 150 mg KNO3/kg with starter; F3- Sage 10.6%; F4- Sage 10.6% with starter; F5- Thyme 10.6%; F6- Thyme 10.6% with the starter. Analysis was performed on the finished CMS (day 0) and after 60 days of storage. A_w and pH were evaluated. The color using L*a*b* color space was measured with a Konica Minolta CR-400/410 (Konica Minolta, Japan) illuminant D65. Residual nitrate and nitrite, chlorides, and TBARS were determined according to Portuguese standards. Lactic Acid Bacteria (LAB), Coagulase Negative Staphylococci (CNS), and *Enterobacteriaceae* were counted according to ISO Standard. Considering technological flora, staphylococci counts in the product conditions with the starter were approximately 7 Log cfu/g for both days 0 and 60. LAB counts presented an increase in the course of time, from below 7 Log cfu/g on day 0 to 7.5 and 8.2 Log cfu/g on day 60. *Enterobacteriaceae* counts were inferior to 4 Log cfu/g on day 0, and inferior to 3 Log cfu/g on day 60, indicating that the product was satisfactory, according to Portuguese guidelines. Regarding color, on day 0, no significant differences in L* value were observed, but on day 60, values ranged from 46.86 (F6) to 51.16 (F2), being these two extremes statistically different. On day 0, except for the Sage formulation, all products inoculated presented higher a* values. F2 (a*=13.16) and F6 (a*=13.19) products were redder ($p<0.05$). These effects were maintained until the 60th day of storage. Control samples presented b* values significantly higher than F3 at day 0. No statistical differences were observed for the b* value at day 60. A_w ranged from 0.94 to 0.95 on day 0 and from 0.93 to 0.94 on day 60. CMS presented pH values ranging from 5.7 to 5.8 on day 0 and from 5.8 to 5.9 on day 60. Results from residual nitrite determination from days 0 and 60 were all inferior to 2 mg $NaNO_2$/kg. At day 0, residual nitrate levels were generally inferior to 5 mg $NaNO_3$/kg, except for formulations with added nitrate, without starter (F1= 102.1 mg $NaNO_3$/kg) and with starter (F2= 40.0 mg $NaNO_3$/kg). Undesirable color is one of the main disadvantages associated with the use of plants as alternatives for nitrites[3]. Concerning this study, color of CMS with thyme and starter was similar to those with nitrate and starter, with the advantage of having much less residual nitrate content in the final product. Therefore, results from the present work demonstrate that *Thymus citriodorus* use in CMS inoculated with *S. equorum* might be a viable alternative to the use of synthetic nitrite.

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Evaluation of corncob as carbon source in the production of xanthan gum

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Xanthan gum (XG) is a biopolymer used as rheology control agent in aqueous systems and in stabilizing emulsions and suspensions. The XG is normally produced from biosynthesis in fermentation processes by Gram negative obligate aerobic chemoorganotrophic bacteria of the genera *Xanthomonas*. It has applications in several different sectors of industry, including the food industry. Xanthan alone does not form gels but together with other polysaccharides can form soft, cohesive composite gels. The XG is an exopolysaccharide (EPS), and a biopolymer, chemically characterized by a structural chain with a molar mass ranging between 2 x 10^6 and 20 x 10^6 Da [1]. The monometric structure of the XG is formed by two units of glycose (cellobiose) bonded in the main chain (the backbone) with a branch formed by two units of mannose and one unit of glucuronic acid. The carbon source in the fermentative process is responsible for one-third of the production costs, and the search for less expensive and sustainable alternatives is ongoing. The use of agricultural residues such as the corncob is highly suggestive due to their abundance. Maize is the second most produced crop worldwide, with 1.1 billion metric tons produced in 2021, and representing 21% of the global crop production between 2000 and 2019 [2]. Based on data previously obtained [3], we have calculated the weighted of the corncob in 5.6% of the weight of the grain harvested, and therefore, we calculate the production of cob as a residual in 616 million metric tons, worldwide. The parameters influencing XG production apart from the carbon sources, are also the micronutrients available during fermentation (potassium, iron and salts) and nitrogen. The carbon sources are provided in concentrations ranging two and five percent, once bacterial growth inhibitions are observed with higher percentages [1]. This study aims to evaluate the use of derived hemicellulose fractions from alkaline extraction of corncob as carbon source in the production of xanthan gum in trials using four strains of *Xanthomonas sp.* (629, 1078, 254, and S6), and different medium (**Table 1**). The results indicate that strain 629 provided the higher yield (8.37 ± 5.75 g L^{-1}) (**Table 2**) while using a fermentation medium containing a carbon source of saccharose (1.25%), hemicellulose fractions (3.75%), and salts. In this same medium, the strain 629 produced, gum in 3% aqueous solution showing the higher apparent viscosity (9298 ± 31.13 mPa s^{-1}) at a shear rate of 10 s^{-1} at 25 °C (**Table 3**). The corncob proved to be a promising sustainable alternative carbon source in the obtention of xanthan gum, improving the economic viability of the process within a biorefinery context.

Table 1. Composition of the fermentative medium for production of XG used in this study: saccharose (%), hemicellulosic fraction (%), and addition or not of salts.

Fermentation Medium	Saccharose (%)	Hemicellulosic fraction (%)‡	Supplementation with salts§
Medium 1	5.0	0	yes
Medium 2	5.0	0	no
Medium 3	1.25	3.75	yes
Medium 4	1.25	3.75	no
Medium 5	0	5.0	yes
Medium 6	0	5.0	no

‡Compounds obtained after the alkali extraction of the corncob.

§Salt solution composition: $NH_4H_2PO_4$ (2.5 g L^{-1}), KH_2PO_4 (5.0 g L^{-1}), H_3BO_3 (0.006 g L^{-1}), $(NH_4)_2SO_4$ (2.0 g L^{-1}), $FeCl_3$ (0.0024 g L^{-1}), and $(CaCl_2)2.H_2O$ (0.002 g L^{-1}).

Table 2. Mean yields (g L^{-1}) of different Xanthan Gums produced in fermentation with different *X. campestris* strains and carbon sources at 25 °C.

X. campestris	Medium (carbon source)					
Strain	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5	Medium 6
S6	5.26 ± 1.14^{bcA}	2.88 ± 2.34^{cB}	5.08 ± 5.15^{bA}	1.88 ± 0.36^{cC}	2.34 ± 0.08^{aB}	$1.81 \pm 0{,}22^{aC}$
629	7.23 ± 0.61^{bB}	5.02 ± 3.10^{cC}	8.37 ± 5.75^{aA}	6.56 ± 4.12^{bBC}	0.84 ± 0.11^{bC}	$0.42 \pm 0{,}067^{cD}$
254	11.58 ± 0.16^{aA}	8.56 ± 1.18^{bB}	5.35 ± 0.93^{bC}	1.69 ± 0.67^{cD}	1.57 ± 1.23^{bD}	$0.63 \pm 1{,}23^{cD}$
1078	6.84 ± 1.01^{bA}	4.42 ± 0.39^{cBC}	6.09 ± 0.76^{bAB}	2.93 ± 0.87^{cCD}	1.50 ± 0.05^{bD}	1.27 ± 0.421^{bD}

Means with different lowercase letters in columns and uppercase letters in rows are significantly different ($p < 0.05$).

Table 3 Means of the apparent viscosity (mPa s^{-1}) of aqueous solution of the different Xanthan Gums produced in fermentation with different *X. campestris* strains and carbon sources at 25 °C.

X. campestris	Medium (carbon source)					
Strain	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5	Medium 6
S6	4869 ± 9.19^{aC}	2543 ± 11.31^{bE}	6599 ± 7.77^{cB}	4779 ± 5.65^{bC}	8790 ± 10.60^{aA}	3899 ± 12.02^{cD}
629	3292 ± 13.43^{dD}	1106 ± 4.24^{cE}	9298 ± 31.13^{aA}	7687 ± 12.72^{aC}	8710 ± 24.74^{abB}	8488 ± 8.48^{aB}
254	3711 ± 13.43^{cD}	3468 ± 15.55^{aE}	9278 ± 12.72^{aA}	2870 ± 22.62^{cF}	8209 ± 20.50^{bB}	6572 ± 21.21^{bC}
1078	3711 ± 15.48^{bC}	3468 ± 12.71^{bE}	9278 ± 11.51^{bA}	2870 ± 15.55^{dF}	8209 ± 8.48^{cB}	3750 ± 14.04^{cD}

Means with different lowercase letters in columns and uppercase letters in rows are significantly different ($p < 0.05$).

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Funding: This work did not receive any specific funding

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The dynamics of sustainability claims and certifications in new food products

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The aim of this work is to present the latest market trends in food and beverage sector related with sustainability. Across the globe consumers are concerned about the state of the environment. Consumers' perception affects their decision-making process— almost 60% of European Union (EU) consumers considered the environmental impact more important than the product's brand when buying. [1] Sustainability can be perceived in new food products when they contain, either as logos and/or labels with certification, sustainable claims such as environmentally friendly packaging, recycling (e.g., recycled plastic), and sustainable habitat/resources as can be seen by the growth in the number of product launches (**Figure 1**). According to Mintel, there is a yearly increase of 8% in the number of new products launched with sustainability claims between 2019 and 2021. In the same time-line, ethical and environmental claims correspond to 34% of the total new launches in food and beverage products.

Brands focusing on animal welfare standards and in reducing packaging footprint will likely resonate with consumers seeking more sustainable and ethical products. Vegan claims do not translate into lower environmental impact. Sustainable claims specifying environmental impact aid the consumer with numerical figures (e.g., carbon/water footprint; % of recycled plastic) in comparing products and selecting the most environmentally friendly one.

Food companies must be transparent to gain the consumers' trust and buying intention. One strategy is through third-party certification and labelling such as eco-score, enviroscore, planet score, eaternity, etc. Consumers believe their behaviour can have a positive impact on the environment and will leverage third-party certification to achieve it. [2] In the future, the impact of an individual food product would be communicated to consumers via front-of-pack score. The communication of transparency can also be done without claims, but through a QR code allowing the consumer to meet the farmer. [2] The European Commission is preparing a proposal that will force companies to substantiate their environmental claims, using a single EU methodology called Product Environmental Footprint (PEF). This method measures the environmental performance of a product or organization throughout the value chain, from the extraction of raw materials to the end of life, using 16 environmental impact categories. That could be an instrument to tackle 'greenwashing', or companies making false claims about the environmental footprint of their products. In turn, this could help consumers to make better-informed choices about the products they buy. In the future, it will be easier for consumers to be 'in control', that is, able to buy food, drink, and foodservice made with ingredients from traceable sources with lower environmental impact.

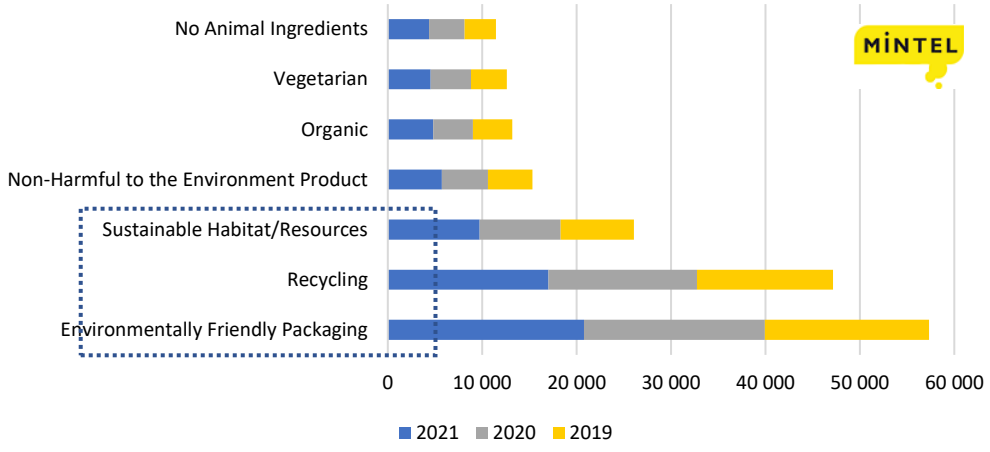

Figure 2: Number of product launches in Europe, containing sustainable claims, between 2019-2021 (Mintel GNPD database).

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Liquefaction Optimization of Peel of Potato *Solanum tuberosum* L. var Monalisa

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The potato (*Solanum tuberosum* L.) is native to South America, in the Andes Mountains where it was consumed by native populations [1]. According to the Food and Agriculture Organization of the United Nations (FAO), there has been a large increase in potato production in Latin America and Asia, especially China, that in 2018 was the largest producer followed by India. These two countries represented almost a third of the potatoes consumed in the world [1]. In Portugal, the most widely planted potato is *Solanum tuberosum* L. var Monalisa, that is used by potatoes' processing industry, generating tons of potato peel waste annually. The waste from the potato industry accounts for approximately 27% of total production. The objective of this work was to evaluate the potentiality of potato industrial residues to be liquefied by polyhydric alcohols and the chemical transformations observed in this process with subsequent use to produce polyurethane foams.

Potato peel waste (PPW) was dried in an oven, crushed in the Retsch SMI mill and sifted in a vibratory sieve model Retsh 5657 HAAN 1 for 30 minutes. The fractions obtained were > 35 mesh, 35-40 mesh (0.500-0.425 mm); 40-60 mesh (0.425-0.250 mm); 60-80 mesh (0.250-0.180 mm) and 80 mesh (< 0.180 mm). The liquefactions were made in an oil-heated double-shirt reactor with a mixture of glycerol and ethylene glycol 1:1, catalyzed by 3% sulfuric acid. The effect of particle size (<80 mesh at >35 mesh) temperatures (140 °C - 180 °C), ratio material/solvent (1:5, 1:7, 1:10, 1:12) and times (15-60 min) were studied. The Fourier Transform Infrared Spectroscopy by Attenuated total reflection (FTIR-ATR) was used to evaluate the functional groups present in the original sample of PPW, in the liquefied sample and in the solid residue obtained.

Liquefaction percentage with increased temperature, time, material/solvent ratio and granulometry is presented (**Figure 1**). Results show that liquefaction performed at 180 °C with a 1:10 material/solvent ratio, increases along time, reaching a maximum at 60 min. Similarly, liquefactions made during 60 min with a 1:10 material/solvent ratio show that there is an increase in liquefaction yield with the increase in temperature until 180 °C.

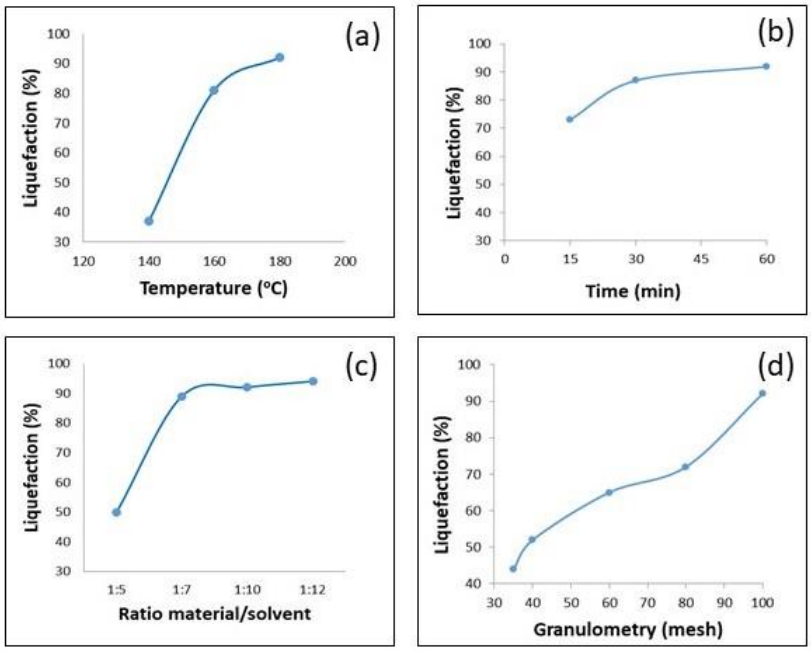

Figure 1: Liquefaction percentage with increased temperature (a), time (b), material/solvent ratio (c) and granulometry (d).

A higher temperature could increase the liquefaction yield but would lead to a higher energy consumption in the process. There seems to be no significative advantage in increasing material/solvent ratio above 1:7, although the liquefaction yield increases for higher ratios. Granulometry testing shows that the smaller the particle the best is the liquefaction percentage. It was concluded that the best liquefaction yield, of approximately 90%, was obtained with a temperature of 180 ºC, for 60 min and particle size <80 mesh for PPW. This material has good properties to be converted in a liquid mixture that can be used later, on the production of polyurethane foams (**Figure 1**).

The PPW spectrum exhibits the common bands for agricultural materials (**Figure 2**). The main differences between the solid material and the liquefied material is the high OH band with a peak at around 3300 cm^{-1} for both the original material and the liquefied, while the peak for the solid residue is at higher wavenumbers. The liquefied sample has a considerable higher OH peak than the solid samples, which is probably due to the polyalcohols used for the liquefaction. The band at 1740 cm^{-1} (non-conjugated C=O bonds) is higher in the solid residue spectrum and smaller in the liquefied material. Similarly Jin et al. [2] observed the absence of C=O groups after the liquefaction of enzymatic hydrolysis lignin. The highest peak in the original and in the liquefied material spectra is the peak at 1100 cm^{-1} which has been attributed to C–O stretching vibrations in carbohydrates. This is in accordance with several chemical compositions reported for PPW [1]. In the liquefied material a new peak appears at around 860 cm^{-1}, which can be due to stretching in the pyranose ring as stated before [3].

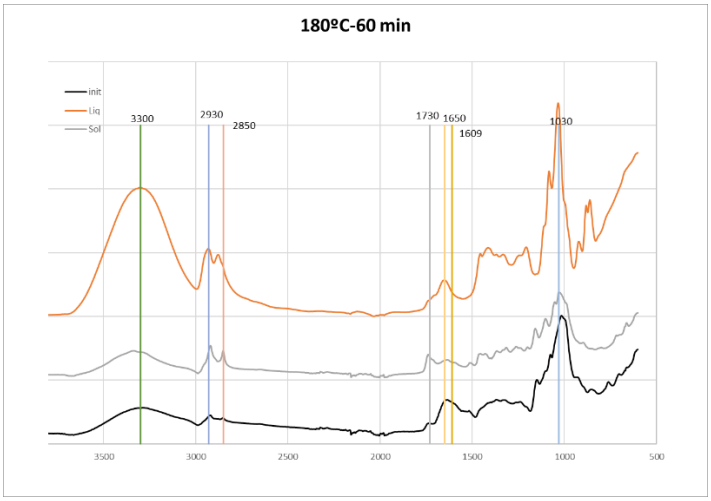

Figure 2: FTIR_ATR spectra of initial PPW, liquefied material and solid residue.

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Compostos Bioativos

Exploring the effects of *Cynara cardunculus* L. besides milk clotting: antioxidant properties

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Cardoon flower, also known as thistle flower, is one of the main ingredients in the production of several kinds of cheese in the Mediterranean area. It belongs to the perennial plant *Cynara cardunculus* L. (Asteraceae), which comprises three botanical varieties such as the globe artichoke (var. *scolymus* (L.) Fiori), the cultivated cardoon (var. *altilis* DC.), and the wild cardoon (var. *sylvestris* (Lamk) Fiori) [1,2]. *C. cardunculus*, commonly known as cardoon, has several uses as the fleshy stems and the immature heads are used in Mediterranean cuisine. The flowers are used as vegetal rennet in the production of some cheeses. In addition, cardoon is rich in cynarin and silymarin, being of great interest in traditional medicine in several areas such as diabetes, high blood cholesterol levels, and cancer [1–3]. Cardoon's leaves, stems and seeds can also produce biomass for energy, biodiesel, seed oil, animal feed and paper pulp [1,2].

Cardoon flowers are commonly used as milk clotting in some cheeses produced in countries like Portugal, Spain and Italy. Some of these cheeses can be classified as Protected Designation of Origin (PDO), where cardoon flower is responsible for its quality and authenticity. The "Queijo Serra da Estrela", "Queijo Serpa", "Queso de La Serena", and "Queso de Flor de Guía" are examples of PDO cheeses produced with cardoon flower as the coagulant agent [1,3]. Cardoon flowers are rich in cardosin, responsible for the milk clotting in cheese making. Cardosins A and B are present in higher concentrations in the cardoons flower (stigma and style). Furthermore, cardoon flowers have presented antimicrobial activity against *Listeria monocytogenes*, methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), *Morganella morganii* and *Pseudomonas aeruginosa*. Besides, cardoon flowers have also been reported as a source of antioxidant compounds [1–3]. Cardoon leaves are an excellent source of bioactive compounds, such as chlorogenic acid, cynarine and luteolin and these compounds are responsible for its antioxidant and antimicrobial activities [1,2].

This study aims to determine the antioxidant activity and the total content of phenolic and flavonoids of cardoons flower methanolic and ethanolic extracts and globe artichoke (var. *scolymus* (L.) Fiori) leaves, both methanolic and ethanolic extracts. Hence, four different assays were performed: the free radical DPPH scavenging and the β-carotene bleaching assays for antioxidant activity and Total Phenolic Compounds (TPC) and the Total Flavonoids Content (TFC) were determined. For the free radical DPPH scavenging assay, globe artichoke leaves' extract presented lower value of EC50 than the cardoons' flower extract in both extracts (1.6 mg/ml vs 3.2 mg/ml, respectively for methanolic extract and 3.9 mg/ml vs 5.2 mg/ml, respectively for ethanolic extract), whereas a lower value means a higher antioxidant capacity. For the β-carotene bleaching assay, the methanolic flower extract presented a higher antioxidant activity coefficient (AAC) than the methanolic leaves' extract (AAC= 161.74 ± 11.51 vs 109.05 ± 9.25). Likewise, the ethanolic cardoon flower extract presented a higher antioxidant activity coefficient than the ethanolic globe artichoke leaves extract (176.34 ± 19.57 vs 90.98 ± 11.98). Finally, cardoon flower methanolic extract presented a higher TPC (44.03 ± 2.23 mg GAE/g vs. 29.79 ± 1.30 mg GAE/g) and TFC (71.51 ± 4.42 mg ECE/g vs. 21.24 ± 1.70 mg ECE/g) than the globe artichoke leaves' methanolic extract. On the other hand, the leave ethanolic extract presented higher values of TPC (51.79 ± 3.74 mg GAE/g vs 45.47 ± 5.49 mg GAE/g) and TFC (81.33 ± 9.87 mg ECE/g vs 9.90 ± 1.46 mg ECE/g) than the flower ethanolic extract, in agreement with the DPPH results. These results suggest that the cardoon flower and globe artichoke leaves are natural sources of antioxidant compounds that can be explored by food industry taking in account this effect.

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Virtual screening of medicinal compounds present in mushrooms as potential tyrosinase inhibitors

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Tyrosinase (EC 1.14.18.1) is a multicopper enzyme, involved in melanogenesis (formation of dark macromolecular pigments), its inhibitors are very attractively used in the cosmetic and medicinal industry as a form of purification and prevention of severe skin diseases. However, the continued use of many of these inhibitors is considered unsafe [1,2]. In the present study, as an alternative to conventional inhibitors, we looked for compounds present in mushrooms as potential tyrosinase inhibitors. The present paper provides an investigation with the enzyme tyrosinase (PDB: 5m8m), in which a virtual screening was performed with a library of 211 mushroom compounds of different families (steroids, terpenes, flavonoids, amines, amides, amino acids, among others...) with medicinal activity for the discovery of new potential inhibitors using *in silico* investigation (molecular docking method). The top 3 compounds as potential tyrosinase inhibitors were (A) acetyl eburicoic acid, (B) naringin, and (C) 3,11-dioxolanosta-8,24(Z)-diene-26-oic acid with binding free energy (ΔG) values of -9.1 kmol/kcal, -9 kmol/kcal and -8.9 kmol/kcal. The predicted Ki values compare well with the result obtained for (D) kojic acid (-5.9 kmol/kcal), a known inhibitor that is used as a skin whitening agent and insights were observed such as the aminoacid His381 had interaction presence with all mushroom compounds and Ser394 that showed interaction with compounds (B), (C), (D). These three compounds (A), (B), and (C) can be considered as promising tyrosinase inhibitors, however experimental evaluation is required.

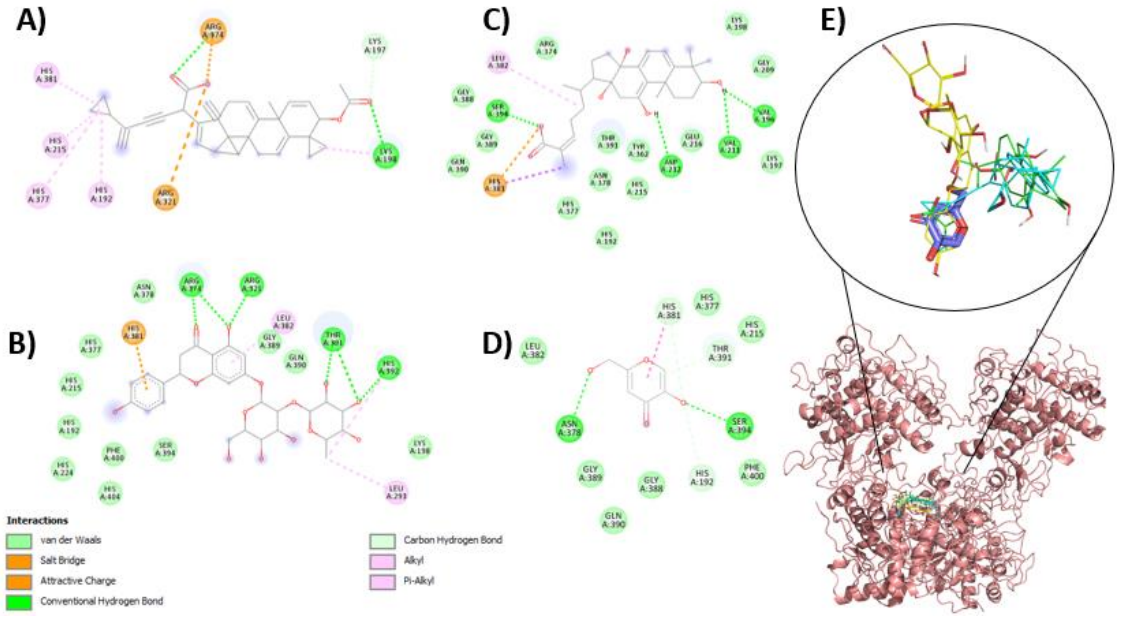

Figure 1: The five top-ranked compounds as potential Tyrosinase inhibitors were (A) acetyl eburicoic acid (*Laetiporus sulphureus*), (B) naringin (*Lentinus lepideus*), (C) 3,11-dioxolanosta-8,24(Z)-diene-26-oic acid (*Jahnoporus hirtus)*, (D) kojic Acid (Control), (E) overlapping molecules in line format (top 3) and control in sticks format in purple observed in the Pymol software, with predicted constant of inhibition values (Ki) of (A) 213.7 nM, (B) 252 nM, (C) 300 nM. The predicted Ki values compare well with the result obtained for (D) Kojic acid (78552 nM), a known inhibitor that is used as a skin whitening agent.

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Prunus lusitanica L. fruits as a source of bioactive compounds

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Reactive oxygen species (ROS) generated in living organisms, trigger, under certain conditions, oxidative stress that contributes to the development of several diseases.[1]
In recent years, the incidence of stress-mediated diseases has increased, leading to the need to complement the cell molecular tools to tackle the negative effects of ROS. This challenge has been addressed by using powerful naturally occurring antioxidants, as is the case of (poly)phenols.[2]
Prunus lusitanica L., also known as cherry bay or Portuguese-laurel has an important role in maintaining ecological balance and ecosystem sustainability. To date, there are only two studies regarding the chemical composition and bioactivity of its leaf's extracts.[3]
The aim of the present study is to analyze the phenolic profile of *Prunus lusitanica* L. fruits (grown in northern Portugal) and correlate it with the antioxidant capacity, focusing on its potential future application in the food and/or phytopharmaceutical industry, considering the uses and applications of other related species belonging to the same genus. To the best of our knowledge, this is the first study that addresses both the phenolic composition of the fruits of this species as well as their antioxidant capacity.
The antioxidant capacity was evaluated through three different spectrophotometric methodologies, the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and FRAP (ferric reducing antioxidant power) methods. Identification and quantification of the phenolic compounds was performed by high-performance liquid chromatography with pulsed amperometric detection coupled to electrospray ionization tandem mass spectrometry (HPLC–PAD–ESI-MS/MS).
Twenty-eight phenolic compounds belonging to different classes were identified and quantified in *Prunus lusitanica* L. fruits extracts, which also showed promising antioxidant capacity.

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Funding: This work was supported by National Funds by FCT - Portuguese Foundation for Science and Technology and by the Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro

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Antineurodegenerative and antioxidant properties of bioactive compounds extracted from olive seeds of three cultivars by ultrasound-assisted extraction

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Despite the beneficial effects from olive oil and its phenolic compounds, which have been extensively explored on neurological disorders,[1,2] to the best of our knowledge, olive seeds have never been investigated in this subject. Although few reports have been developed, concerning phytoconstituents of olive seeds, the ones carried out, have shown being a source of phenolic compounds. Additionally, the use of phenolic compounds from olive seed, could be a cost-effective alternative to synthetic antineurodegenerative compounds. Thus, contributing simultaneously to the sustainability of olive oil industry, and to improve co-products management. On the other hand, since life expectancy is increasing, is also predicted an increase of neurodegenerative diseases, which will raise the search for natural antineurodegenerative compounds. Therefore, and taking into account that the use of Ultrasound Assisted Extraction (UAE) is gaining a wide acceptance due to several advantages over other conventional and non-conventional one,[3] this work aimed to evaluate the phytochemical composition of olive seeds extracts from different cultivars (Cobrançosa, Galega and Picual), as well as their antioxidant capacity. In addition, it also intended to appraise the ability to inhibit enzymes associated with neurodegenerative diseases: acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and tyrosinase (TYR).

The results have shown that seed extracts present a high content of phenolic compounds, and a great ability of scavenging ABTS•+ and DPPH. The HPLC-DAD with Mass Spectrometry indicated the presence of one phenyl alcohol (tyrosol), two flavonoids (rutin and luteolin-7-glucoside), and three secoiridoids (nüzhenide, oleuropein, and ligstroside). Galega was the most promising cultivar, not only due to its high concentration in phenolic compounds, but also because of its high antioxidant and strong inhibition of AChE, BChE, and TYR activities.

It can be concluded that olive seeds extracts may provide a new and alternative source of agents for medical and industrial applications.

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Evaluation of antioxidant capacity and phenolic composition of muffins fortified with grape pomace from the Douro region

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Nowadays, the use of the bioactive compounds present in the agro-food by-products as a source of functional and antioxidant ingredients for the development of new products has been increasing.
In fact, the agro-industries activity produces massive quantities of by-products, which represent serious environmental and disposal problems, such as for wineries. On the other hand, these residues have been described as a natural source of polyphenols which are mainly responsible for several biological properties. One of them is grape pomace which is a waste resulting from the vinification process and is considered an economically attractive source for the exploitation of bioactive compounds (Pintać et al., 2018). This by-product is considered a major solid waste from the wine sector, and is composed by skins, seeds, and residual stems after the grapes are pressed and the fermentation processes (Troilo et al., 2021). In this way, these residues can be used as colorants, preservatives, and food supplements.
Since grape pomace has high levels of polyphenols such as flavanols, catechins, anthocyanins, stilbenes, and phenolic acids, there are studies that incorporate this by-product into foods. In all studied foods (for instance, bread, biscuits, brownie, muffins, yogurt, cheese, sausages, seafood, puree), the fortification was successfully achieved (Antonić et al., 2020). The addition of grape pomace to foods increased their nutritional value, and improved their sensory properties, which can provide some health beneficial properties, such as the prevention of chronic diseases and cancer, and the maintenance of intestinal health.
In this context, the aim of this study was to evaluate the effects of the incorporation of grape pomace from the Douro region in two distinct forms (as whole pomace flour and as pomace hydro-ethanolic extracts) in the formulation of muffins on their taste, appearance, and functional properties, since muffin is one of the most consumed bakery products. A control muffin was also performed (without pomace). Afterwards, the content of total phenols, flavonoids, ortho-diphenols and the antioxidant capacity by three different methodologies (DPPH, FRAP, and ABTS) were determined by spectrophotometric methods. The color L∗ (lightness), a∗ (redness), and b∗(yellowness) of the muffins were also determined using a colorimeter. A sensory analysis of the muffins formulations was carried out to evaluate the consumers' acceptance.
With this study it was possible to evaluate the differences between the muffins prepared with the whole grape pomace and the ones prepared with bioactive extracts obtained from this by-product.
Overall and based on other studies, it is expected that the levels of phenolic compounds and antioxidant capacity of muffins will increase with the incorporation of pomace in the muffin's formulations.
We can conclude that grape pomace can be an excellent raw material for the enrichment of muffins.

Acknowledgements: We would like to thank Rozés company for providing the pomace samples. Rui Dias Costa received a Ph.D. scholarship from Fundação para a Ciência e Tecnologia (FCT)— PhD grant 2021.07571.BD.

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Subcritical Water Extraction of chestnut shells: A promising source of bioactive compounds

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Subcritical Water extraction (SWE) is a green promising technology for the large-scale recovery of bioactive compounds from by-products due to its low-cost equipment, minimal solvent employment, fast processing time, high yield and selectivity, and low probability of compounds deterioration[1]. *Castanea sativa* fruits (chestnut) have significant ecological and economical interest and its industrial processing generates huge amounts of by-products, mostly burs and shells[2]. The aim of this study was to optimize the extraction temperature (110°C- 180°C) of *C. sativa* shells through SWE, aiming to obtain a bioactive extract with high antioxidant and antiradicals scavenging capacity as well as low toxicity effect on buccal epithelial cell lines (HSC3 and TR146). The extract obtained at 110°C showed the highest phenolic and flavonoid contents (239.53 mg of gallic acid equivalents (GAE)/g dry weight (dw) and IC_{50}=148.68 µg/ml, respectively) as well as the highest antioxidant activity (4240.38 µmol of ferrous sulphate (FeS)/g.), as shown in **Table 1**. Also, the extract prepared at 110°C exhibited the best antiradical activity (IC_{50}=426.88 µg/ml for DPPH assay) and a good ability to scavenge the reactive oxygen species HOCl and $ROO^{\bullet}$ (IC_{50}=4.47 µg/ml and 0.73 µmol of Trolox equivalents/mg dw, respectively). However, the extract prepared at 140°C presented the best IC_{50} (31.14 µg/ml) for the scavenging activity of $O_2^{\bullet-}$. In the case of reactive nitrogen species, the extracts obtained at 120°C and 140°C had the best performance in scavenging $ONOO^-$ in presence and absence of HCO_3^-, respectively (IC_{50}= 1.41 µg/ml and 2.09 µg/ml, respectively). The MTT assays demonstrated that the SWE *C. sativa* extracts did not lead to a decrease of the viability until 2000 µg/ml. This study demonstrates the potentialities of SWE to valorize *C. sativa* shells as a valuable source of compounds for nutraceutical and pharmaceutical applications. Further studies should be performed to evaluate other biological properties, such as the antimicrobial activity, also testing the encapsulation of the optimal extract to develop a potential application for oral disorders.

Table 1 – Total phenolic content (TPC), and antioxidant and antiradical activities evaluated by FRAP, DPPH and ABTS assays of *C. sativa* extracts, prepared by SWE. Values are expressed as means ± standard deviation (n=3). GAE, gallic acid equivalents. FeS, ferrous sulphate equivalents.

C. sativa extracts	TPC (mg GAE/g dw)	FRAP (µmol FeS/g)	DPPH IC_{50} (µg/ml)	ABTS IC_{50} (µg/ml)
110°C	239.53 ± 23.17 [a]	4240.38 ± 10.04 [a]	426.88 ± 13.42 [a]	148.68 ± 13.20 [a]
120°C	201.75 ± 13.02	4092.98 ± 92.82 [b]	489.49 ± 5.96 [a]	228.32 ± 16.48
140°C	162.52 ± 7.14	3398.98 ± 57.23	460.21 ± 14.81	232.97 ± 4.23
160°C	122.31 ± 5.89	2728.06 ± 35.04	496.15 ± 8.75	220.40 ± 7.83
180°C	126.19 ± 6.33	2464.32 ± 68.44	583.47 ± 14.68	256.59 ± 5.55

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Fruit and vegetable pomaces from Juice Industry as a source of bioactive compounds

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Fruit pomaces, as the main byproducts from the fruit juice industry, are rich in bioactive and can have a strong potential for healthier diets. Our goal was to evaluate the antibacterial and anti-inflammatory potential apple and carrot pomaces in different granulometry fractions to understand the influence of the particle size on the pomace's biological potential. Pomaces were milled and antibacterial activity was evaluated using two reference bacteria (Gram+ and Gram-): *S. aureus* (MRSA) and *E. coli* (O157:H7) by a standard microdilution broth assay, using 96-well plates, as a function of optical density (OD600)[1]. The inhibitory activity of apple and carrot pomaces against matrix metalloproteinase MMP-9 was assessed using the DQ-gelatin assay[2] as a way to determine its anti-inflammatory and anticancer potential.

Results showed that at concentrations higher than 0.75 mg/ml, both apple and carrot pomaces significantly reduced the growth of both Gram- and Gram+ bacteria (**Figure 1**). Also, as is illustrated in **Figure 2**, apple pomace showed MMP-9 inhibitory activity reducing its activity to less than 50% when compared to controls. High activity of MMP-9 is related to cancer development and inflammation. Thus, our results indicate that apple and carrot pomaces have a promising potential to be used as cheap and valuable ingredients to develop novel functional foods with health-promoting effects on humans.

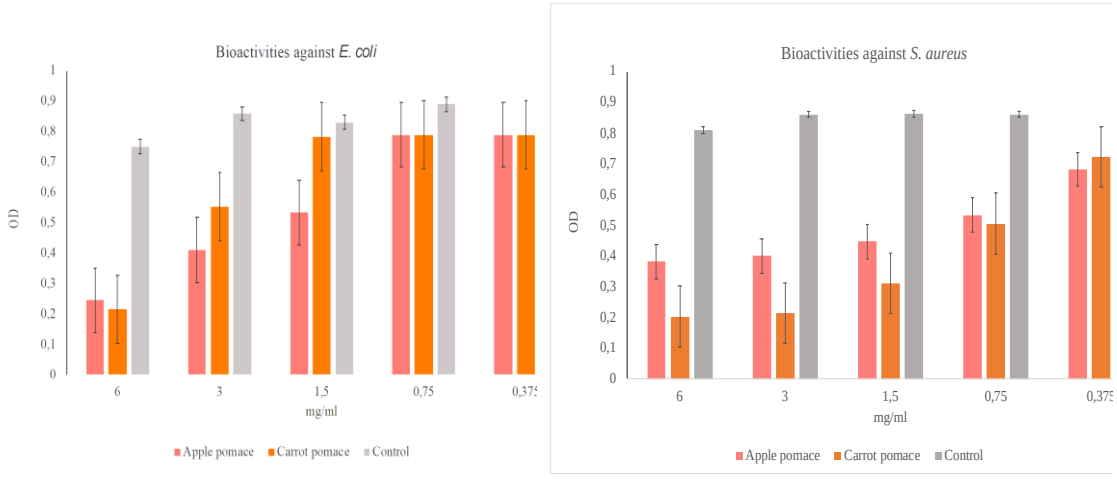

Figure 1: Antibacterial activities of apple and carrot pomaces against *Escherichia coli* and *Staphylococcus aureus*.

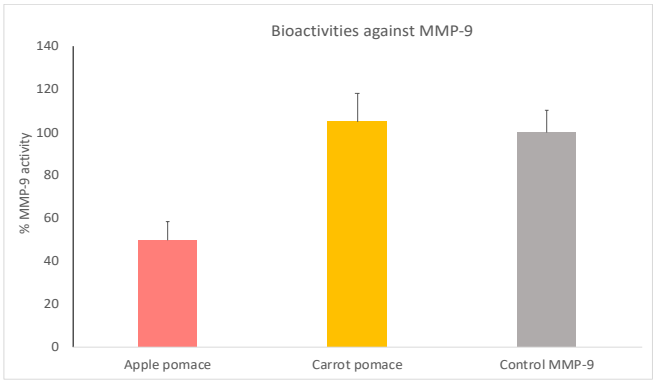

Figure 2: Anti-MMP-9 activities of apple and carrot pomace.

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New insights into phenolic compounds-proteins complexes as natural emulsifiers in mayonnaise models

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Consumers are increasingly concerned with healthy and natural foods. Phenolic compounds (PCs) have been widely attributed to antioxidant, anticancer, antiaging and anti-inflammatory properties[1]. Besides the health-promoting effects, PCs are bioactive compounds which can be described as promising tools to reduce the use of synthetic additives[2]. In fact, they have been described as able to modulate the main organoleptic characteristics of plant-derived foods[3]. Moreover, their natural ability to bind to proteins can bring new insights in the use of PC as emulsifier agents.

In this study, the molecular perspective of the use of PCs as emulsifiers has been studied in a yeast protein extract (YPE)-based mayonnaise in comparison with the traditional egg derived mayonnaise Thus, the molecular mechanisms of the interaction between egg or alternative protein (YPE) models and PCs (gallic acid-GA, tannic acid-TA, and grape seed extract-GSE) were unravelled by fluorescence quenching. The molecular binding models were studied at pH 7.4 (biological conditions) and at pH 3.5 (mayonnaise conditions) and at different temperatures (4 °C and RT) simulating the storage conditions.

Overall, different mechanisms of molecular interaction were found for the different PCs. Molecular affinity constants were calculated by using the Stern-Volmer equation.

In general, the higher affinity constant was observed in YPE model when compared to egg proteins. At the concentration tested the GA show no interaction with both protein models. On the other hand, the GSE and TA show interactions with both (egg and alternative) protein models. Overall, two main binding mechanisms were found in this study depending on the PC tested. The PCs were found to be the main factor affecting the affinities, which also depended on the temperature and the pH. The results obtained within this study clearly showed the potential of PC to be used as natural emulsifiers, which can conquer the food industry in response to the consumer demand for clean labelling and potentially health-beneficial foods. However, future studies are required to understand the structure/activity relationships and main dose/response behaviours.

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WPI active edible coatings to prevent cheese color defects

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Cheese is a type of food that has been used over time as a way of preserving milk. It is a popular food because of its excellent versability and palatability. All around the world a huge variety of cheeses, flavors, and shapes can be found. This food product depends on the culture and available resources of each region. In 2021, global cheese production amounted to about 21.86 million metric tons [1]. The European Union was by far the top producer of cheese worldwide, with a production volume of around 10.35 million metric tons of cheese that year [1].

Color is one of the most important attributes that will be first seen by consumers before eating the cheese and is a primary consideration of consumers when making purchasing decisions. The color is also an indicator of the cheese quality with attributes such as flavor sanity, and ripeness.

Cheese is a living food, considered as a bio complex ecosystem colonized by a diverse group of microorganisms, known as cheese microbiota, by raw milk, starter, and adjunct cultures. These microorganisms are the main contributors to the perceived sensory attributes of different cheeses due to their complex interaction with milk proteins, carbohydrates, and fats that occur mainly in an important technological process in cheese making, known as ripening. Cheese ripening is a complex phenomenon involving several biochemical reactions. Proteolysis is the most important multi-step, biochemical event in cheese ripening. It accounts for the development of several organoleptic features, encompassing both flavor and texture. High proteolysis resulted in a high free amino acid content, which has been described as a substrate for cheese color defects (blue, pink, and brown).

For some years now, cases of color defects in cheeses surfaces have been reported in the literature. Sporadic inconsistencies in cheese appearance may result in a downgrading of cheese and a consequential economic loss to producers [2].

To control the appearance of fungi and other bacteria of the genus *Pseudomonas* spp, edible films/coatings have been developed as active packaging that can contain various compounds to extend the shelf life of cheese without compromising the organoleptic characteristics of the cheese.

Thus, the objective of this work was to study several formulations based on whey protein isolate [3]. We used organic acids (lactic and citric) as antibacterial agents, nisin as anti-listeria, and natamycin as an antifungal agent in various formulations. We also used melanin produced by *Pseudomonas putida* ESACB 191[2], as a colorant and antioxidant to develop a new line of edible coatings. Physic-chemical characterization of the edible coatings was performed FTIR-ATR analysis and various rheological parameters such as tensile stress, extensibility, and water vapor permeation. The main objective of this study is to select the best formulation to be applied to the cheese and prevent color defects during cheese maturation and shelf life.

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Effects of gastrointestinal digestion on the anti-inflammatory properties of phlorotannins from *Himanthalia elongata*

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Phlorotannins are phenolic compounds made of several units of phloroglucinol that are exclusive from brown macroalgae and have been recognized for their promising bioactive properties[1]. However, the bioactive properties described for these compounds have usually been described on pure compounds and/or phlorotannin-rich extracts without considering the possible alterations that they may incur during their passage of the gastrointestinal tract. In this study, a phlorotannin extract was obtained from *Himanthalia elongata*, an edible brown seaweed species that grows profusely along the shores of the north-east Atlantic. Since the phlorotannin profile of this species remains poorly studied, an UHPLC-ESI-MS/MS analysis was performed, revealing that fucophlorethol-type followed by carmalol-type compounds were the most common phlorotannins present in this species. Afterwards, the phlorotannin extract was submitted to a simulated gastrointestinal digestion which caused a reduction of their concentration and consequent loss of antioxidant activity measured in vitro via $NO^{\bullet}$ and $O_2^{\bullet-}$ scavenging assays, thus suggesting that these compounds' integrity and bioactivity are negatively affected by the digestive process. Nevertheless, when non-digested vs digested extracts were used on LPS-stimulated Raw 264.7 macrophages, both showed strong inhibitory effect on the cellular $NO^{\bullet}$ production. In fact, although not statistically significant, the digested extract revealed a tendentially stronger effect than its non-digested counter, suggesting that even though there is a decrease of the phlorotannins' concentration after digestion with consequent loss their scavenging properties, the possible degradation products that are being formed may exert their effects through the modulation of the intracellular signaling mechanisms. Overall, this study not only contributed to a better understanding of the phlorotannin composition of the species *H. elongata* but also allowed to understand that, although the digestive process may affect the integrity and concentration of these compounds, it does not necessarily translate into loss of bioactivity, in particular the anti-inflammatory activity, most likely owing to the possible bioactive effects that the degradation products of these phenolics may have on intracellular level.

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Pumpkin by-products as a source of preservative compounds for food application: valorization of industrial bioresidues towards a sustainable system

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Considering the interest in replacing synthetic additives with healthier natural alternatives, bioresidues from industrial processes can be an alternative source of rich and cheap compounds to be explored for this. Parts of fruits and vegetables, such as peels, seeds, and leaves, which are often discarded, have been investigated due to their important amounts of high value-added compounds, as well as their potential to be recovered and incorporated into food products. Pumpkin industrialization fits into this scenario: in the pulp processing, large amounts of bioresidues are generated and still undervalued despite being rich in nutritional and bioactive compounds.[1] In this work, the by-products of three varieties of Portuguese pumpkins were evaluated as source of preservative compounds, fostering the circular economy and the valorization of local products. For this purpose, the peel, seeds, and fibers of butternut squash, common pumpkin, and kabocha squash, grown in Bragança – Portugal, were evaluated in terms of their bioactive potential and their composition in tocopherols. For the antioxidant capacity assessment, the hydroethanolic extracts were evaluated through five methods, three chemical (DPPH scavenging activity, reducing power, and β-carotene bleaching inhibition) and two biological (TBARS and OxHLIA) methods. The antibacterial and antifungal capacity of the extracts was tested against five strains of gram-negative bacteria, three gram-positive bacteria, and two strains of fungi with relevance in food, in the maximum concentration of 10 mg/mL. The cytotoxicity was tested in a primary culture of non-tumor porcine liver cells, PLP2, using the sulphorrodamine B (SRB) assay. The samples composition in terms of tocopherols was determined by HPLC coupled to a fluorescence detector.

The evaluated pumpkin by-products presented great bioactivity. Regarding the antioxidant activity, the seeds stood out in both biological methods, being the butternut and kabocha varieties the best ones. In the chemical assays, the results were more heterogeneous, but it is possible to highlight the fibers as the samples presenting the best results, followed by the seeds and, then, the peel. Moreover, in terms of pumpkin varieties, the kabocha squash presented the best result in three of the five assays (DPPH, β-carotene, and TBARS). In the antimicrobial and antifungal activity, the fibers of Butternut squash stood out inhibiting all the tested strains, followed by the fibers of common, and the seeds and the peel of kabocha, which revealed inhibiting capacity against seven bacteria and one fungal strain, in a lower concentration than the other samples. In fact, all samples have inhibited just one of the two fungal strains. None of the samples presented bactericidal nor fungicidal capacity at the tested concentrations and all samples inhibited at least three strains of bacteria. In addition, none of the tested samples showed hepatotoxic activity in a primary culture of non-tumor porcine liver cells (PLP2), at the maximum concentration tested of 400 µg/mL, demonstrating their safety for food application. Furthermore, all samples presented α-tocopherol, which is the most biologically active isoform of vitamin E. None of the samples presented β-tocopherol and three samples presented the δ isoform. In the samples where γ-tocopherol was found, it was the major one. The peel of common pumpkin presented the highest total tocopherol content. The fibers of kabocha were not assessed due the insufficient quantity of sample, once the content of fibers in this variety was very low.

These results demonstrate the potential of pumpkin by-products to be exploited as a source of high value-added compounds with preservative capacity. As a next step, studies can be carried out on the recovery and application of these compounds in food products, in order to promote a sustainable system focused on a circular economy and the development of healthier food products.

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Chritmum maritimum L. as natural preservative: in vitro antioxidant activity assessment, phytochemical characterization and nutritional profile

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Several plants have in their composition macro and micronutrients as well as phenolic compounds, phytoconstituents responsible for a few biological properties including antioxidant and antimicrobial. Furthermore, the addition of plant extracts in food matrices can contribute for the food conservation leading to the partial or total substitution of artificial preservatives, and enhance the nutritional properties. [1] In this work, an infusion and decoction of aerial parts of *Chritmum maritimum* L. (*C. maritimum*) were prepared with extraction yields of 36.27 % and 46.08 %, respectively. Phytochemical profiles of infusion (**Figure 1**) and decoction (**Figure 2**) were performed by HPLC-PDA. The antioxidant activity of both extracts was assessed by DPPH and ABTS methods, and nutritional profile of dry plant evaluated. For comparison, BHA antioxidant activity in DPPH method was determined. HPLC-PDA analysis of both extracts showed identical phytochemical profiles, being chlorogenic acid and derivatives the major phytoconstituents. [2] Relatively to antioxidant activity, IC_{50} values of infusion, decoction and BHA in DPPH assay were 38.3±4.55, 44.9±5.73 and 4.79±1.74 μg/mL, respectively. In ABTS assay, IC_{50} values of infusion and decoction were 36.6±4.18 and 38.4±2.47 μg/mL, respectively. The nutritional profile of *C. maritimum* aerial parts exhibited a considerable protein (8.04±0.01 %), fiber (45.7±19.7 %), ashes (23.6±4.78 %) and phosphorous (26.2±0.32 μg/mL) contents. According to the results obtained in DPPH and ABTS assays, both extracts exhibited identical antioxidant activity. The possibility of natural resources uses in foods contributes to healthier products free from synthetic preservatives and other chemicals harmful for health and for the environment. The incorporation of plants in food matrices is an important strategy for the sustainable development of new food products. Taking into account the nutritional profile and antioxidant activity of *C. maritimum*, this plant has potential as a natural preservative in food matrices such as pre-prepared sauces (mayonnaise, ketchup or mustard, *e.g.*), also adding nutritional value to the food. The observed antioxidant activity is due to the phenolic composition of *C. maritimum* extracts, namely chlorogenic acid and its derivatives. According to the literature, chlorogenic acid, in addition to exhibiting antioxidant activity, has antimicrobial activity. [3] Thus, *C. maritimum* extracts have potential as substitutes for synthetic antioxidants and antimicrobials (such as EDTA or potassium sorbate) in pre-prepared sauces.

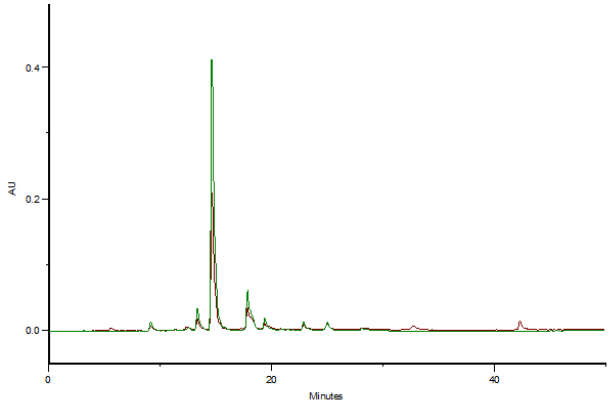

Figure 1: HPLC-PDA profile of *C. maritimum* infusion, recorded at 280 and 366 nm.

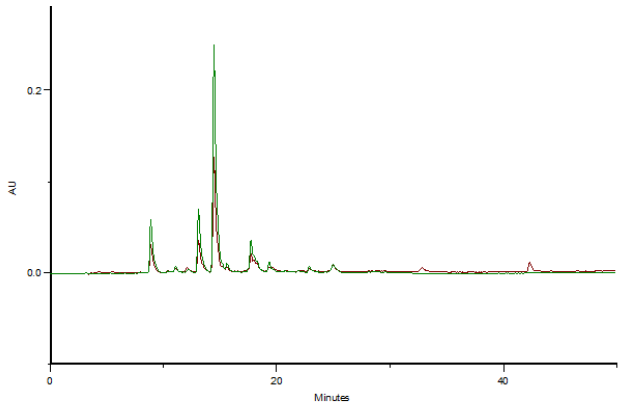

Figure 2: HPLC-PDA profile of *C. maritimum* decoction, recorded at 280 and 320 nm.

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Chemical characterization and antioxidant activity of wild mushroom extracts

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Mushrooms are foods that have always been used throughout history due to their great medicinal and nutritional properties. Due to this fact, they are often consumed by people for many centuries. In recent decades, with advances in biology, technology and medicine, mushroom extracts and their secondary metabolites began to be used to evaluate various biological effects, such as antioxidant, antimicrobial, anticancer, anti-inflammatory and immunomodulatory activities.[1] Today, it is known that mushrooms are a source of phenolic compounds with antioxidant and antimicrobial activities; however, more studies must be carried out to evaluate a possible correlation between phenolic compounds and the bioactivities of the mushrooms.[2] It was found that phenolic compounds have a inhibitory effect on bacterial growth, mainly because they cause structural or functional damage to the bacterial cell membrane that affects their survival and multiplication, so they can be a viable alternative to antibiotics.[3] Therefore, the aim of the this study was to extract the phenolic acids from several species of mushrooms (*Sarcadonon imbricatus*, *Tricholoma colossus*, *Gymnopilus junonius*, *Russula sardonia*, *Macrolepiota procera*, *Russula parazurea*, *Hydrophobic aurantiaca*, *Tricholoma ustale*, *Boletus aereus*, *Tricholoma equestre*, *Lactarius deliciosus*, *Lactarius aurantiacus*, *Suillus* mediterraneensis, *Amanita virosa, Amanita ponderosa*), edible and inedible properly known and catalogued from the region of Trás-os-Montes and Alto Douro, to evaluate their antioxidant potential and to determine the phenolic content present in the methanolic extracts by HPLC-DAD-Electrospray (ESI)/MS. Antioxidant properties were determined by FRAP, β-carotene and lipid peroxidation assays. The main phenolic compounds identified in the extracts were gallic acid, p-hydroxybenzoic acid, cinnamic acid, protocatechuic acid and p-coumaric acid. Gallic acid was present in all mushrooms extracts except for *Tricholoma ustale*. It should be noted that *Hygrophopis aurantiaca*, *Boletus aureus* and *Lactarius deliciosus* contained the greatest variety of phenolic compounds after reading the chromatograms. *Tricholoma equestrian* (edible) contained the highest amount of total phenolic acids, whereas *Russula sardónia* (inedible) contained the least amount of total phenolic acids. Furthermore, our results showed that inedible mushrooms contained lower values of phenolic acids when compared to edible ones. In this study, the mushroom species that obtained the best antioxidant activities were *Sarcadonon imbricatus*, *Gymnopilus junonius* and *Suillus mediterraneensi.* There was not a direct relationship between the amount of total phenolic acids and the antioxidant activity. However, there was a positive relationship with the different types of phenolic acids that each mushroom extract contained. Mushrooms are a great source of phenolic compounds with good antioxidant activity. Further studies will be carried out to investigate the antimicrobial activity of these mushrooms' extracts.

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Influence of the particle size and extraction process of pear pomace in their health-promoting properties

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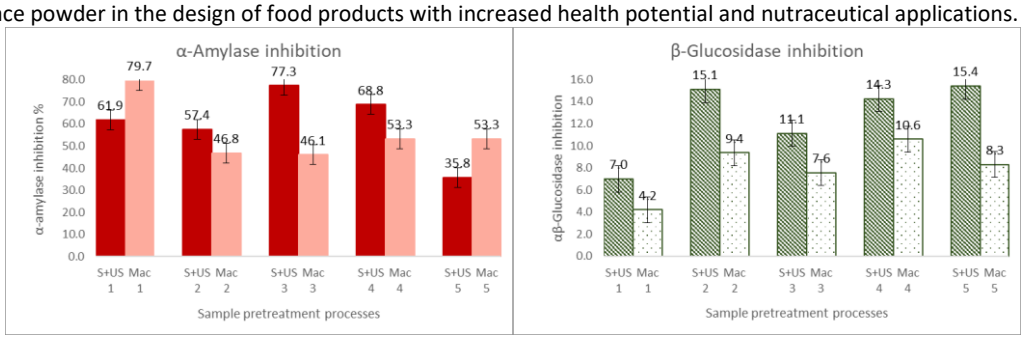
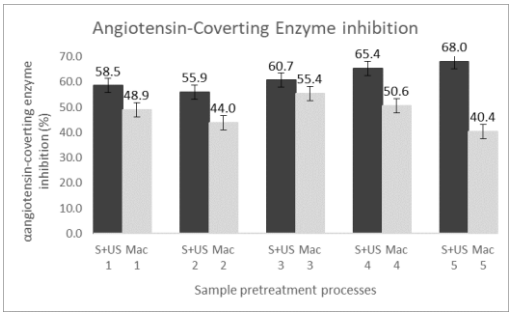
In recent years, more and more attention has been paid to the impact of food on the human health.
The development of chronic, metabolic, autoimmune and neurodegenerative diseases and cancer is positively correlated with oxidative stress and epidemiological studies provide evidence that consuming food rich in antioxidants reduces the risk of developing the above mentioned chronic diseases[1]. Pear fruits (*Pyrus communis* L.) are an excellent source of substances with antioxidant and pro-health properties that include carotenoids, triterpenoids and polyphenols, which possess strong antioxidant, anti-inflammatory, antiviral, and anticarcinogenic properties[2]. Proposals for alternative raw materials with a desirable effect on the body seem to be justified due to the growing interest of consumers and processors for new materials with health-promoting properties. Such raw materials, characterized by a high content of proteins, fibres, polysaccharides, polyphenolic compounds and high antioxidant capacity include the pear juice industry sub-product, the pomace, which is produced in significant amounts (up to 35% of mass of raw material). These bioactive compounds can be valorised while developing innovative food and non-food products with health-promoting benefits and at the same time contributing to an efficient waste reduction management[2]. And although the fresh raw material has been been associated with antioxidant, anti-obesity, anti-aging, anti-inflammatory and antidiabetic activities[2], these raw materials have not been described to date in the literature in terms of detailed bioactive properties.
The main goals of this study were determine the effect of the degree of granulation of the pear pomace powder and ii) evaluate the effect of the extraction process on the phytochemical profile and bioactivities including antioxidant, antidiabetic and antihypertensive. Concerning both extraction processes (S+US) soxhlet extraction with dichloromethane prior to ultrasound with methanol, and (Mac) maceration with methanol, there is a strong relationship between the ferric reducing power of a sample and its total phenolic and total flavonoid contents (both related as well), as well as with α-amylase and angiotensin-converting enzyme inhibitory activities. When analyzing the results using the pretreatment (S+US), it is still visible a clear relationship between the ferric reducing power of a sample and its total flavonoid content (also correlated with the phenolic contents) and with the angiotensin-converting enzyme inhibitory activity. The pretreatment (Mac) allowed a positive correlation between the α-amylase and angiotensin-converting enzyme inhibitory activities and between the ferric reducing power of a sample and the β-glucosidase inhibition (**Figure 1)**.
The particle size and the extraction processes influenced the bioactivities analysed: α-amylase inhibition was higher in particles of 75-180 µm using sequential solvent fractionation (S+US) and in particles of higher size when using maceration with methanol. ACE and β-glucosidase inhibition was higher in fractions with small particle sizes (lower than 75 µm) especially when samples treated with a sequential solvent fractionation (S+US).
Therefore, the method of pretreatment and the particle size of the pear pomace flour are able to modulate the biological activity of the samples and, therefore, the results can direct the pear powder production process in terms of the selection of the degree of grinding, selection of fractions with a specific granulation in order to maximize their potential while supporting waste-free production. The report will shed new light on the possibility of using pear pomace powder in the design of food products with increased health potential and nutraceutical applications.



a) b)



c)

Figure 1: Determination of antidiabetic and antihypertensive activities by α-amylase inhibition (a), β-glucosidase inhibition (b) and angiotensin-converting enzyme inhibition (c) assays in fractions 1 (1 mm), 2 (710 µm), 3 (180 µm), 4 (75 µm) and 5 (53 µm), in both treatments (S+US) soxhlet extraction with dichloromethane prior to ultrasound with methanol, and (Mac) maceration with methanol.

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Chia (*Salvia hispanica* L.*)* whole flour: phenolic profile and authenticity

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Seed and grain whole flours have been widely used in the development of functional foods, making them a potential target of adulteration around the world. Knowing that food fraud poses risks to public health, it is necessary to develop strategies to assess the quality of food products, such as the use of chromatographic fingerprinting and chemometric methods.[1] In this sense, this study aimed to evaluate the authenticity of commercial chia flours. Therefore, the phenolic profile of chia flours was evaluated by HPLC-DAD and the chromatographic data were analyzed by PCA.

The samples were acquired in commercial establishments in Salvador-Bahia-Brazil: 5 commercial samples of chia flour and, to guarantee authenticity, 15 samples of chia seeds used for the production of reference samples of chia whole flour. The seeds of one of the chia samples were manually separated by color for the independent production of flours and separately evaluation of the phenolic profile of each variety (black and white). The samples were homogenized in a granulometric sieve (32 mesh) and subjected to extraction. The samples were homogenized (32 mesh) and subjected to extraction.2 The methanolic extracts were analyzed by HPLC-DAD in order to identify 17 bioactive compounds (catechin, rutin, naringenin, quercetin, kaempferol, chrysin, vanillin, and gallic, protocatechuic, chlorogenic, caffeic, syringic, p-coumaric, ferulic, sinapic, ellagic and trans-cinnamic acids).

Only vanillin was not detected in the analyzed samples. Among the bioactive compounds identified, high levels of phenylpropanoid trans-cinnamic acid (19.64 to 238.56 µg g-1) were obtained, the most abundant flavonoid was catechin (30.95 to 263.71 µg g-1) and the phenolic acid present in higher concentrations was ferulic acid (1.16 to 39.12 µg g-1). Comparing the chromatograms of chia flour samples (Figure 1) it is evident that the chromatographic profile of black chia flour (pink), white chia flour (blue) and chia seed mix flour (black) is similar, so that there was no great variation between the quantified bioactive compounds. In view of this, it is suggested that the proportion of black and white chia seeds in the mixture for the production of whole flour should not influence its phenolic composition.

PCA was applied to verify the authenticity of commercial chia flours based on their phenolic profile. The unrotated matrix was used. Five principal components presented eigenvalues above 1.0, of which three are able to explain more than 10% of the total variance of the experimental data. As PC1 (29.62%) and PC2 (19.80%) explain almost 50% of the variation, it was considered sufficient to extract information and the interpretations were based on the graphs in the PC1 × PC2 plane (Figure 2). The 95% confidence ellipse (highlighted in blue) in the case projection plot indicates that there was no significant difference between the composition of authentic produced chia flour samples (red triangles) and commercial chia flour samples (green squares). It is also evident that the flours produced with chia seeds separated by color (marked in the graph with pink circles) do not differ significantly
from the others.

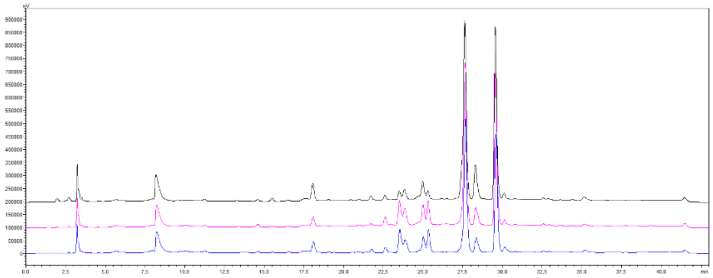

Figure 1: Chromatograms of chia flour samples (max plot 260-360 nm).

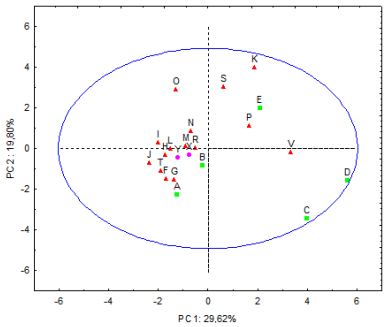

Figure 2: Projection of cases in the PC1 × PC2 plane

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Olive phenols stability and selective extraction steps from olive leaves

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Olive leaves are an alternative source of bioactive compounds, such as hydroxytyrosol (HT) and oleuropein (OLE), which do not compete with the food chain. Olive phenolic compounds have gained much attention due to their bioactive properties, and their use as a food ingredient to improve food quality has been widely exploited [1]. Indeed, HT is recognised as safe and the consumption of 5 mg of HT with other derivatives, including OLE, is recommended to prevent cardiovascular diseases [2]. OLE is composed of an elenolic acid linked to an o-diphenol (catechol moiety), i.e., hydroxytyrosol, and a glucose residue. This is mainly abundant in olive leaves and drupes. HT can result from OLE degradation, being most abundant in olive oil and cured olives, although this is also detected at lower levels in olive leaves and other products such as grapes, wine and olive by-products [1]. In general, phenolic compounds from olive leaves obtained by maceration with organic solvents such ethanol and methanol result in the preferential extraction of OLE, whereas the use of water foments the HT recovery [3]. Subsequent acid hydrolysis and purification treatments have also been applied to improve the HT recuperation [3]. This study aimed to assess the value of the selective extraction steps applied to water extraction of olive leaves regarding the recovery yields of HT and OLE, total phenolic compounds and antioxidant activity, as well as evaluate the HT and OLE stability in the resulted fractions along four weeks.

The extraction conditions were as follow: i) solid-liquid extraction using olive freeze-dried leaves powder, performed with acidified water (distilled water with 5% HCl, 8.4 M), 1:14 m/v for 4 h at room temperature, allowing the obtaining of a crude water extract (CRD); ii) same conditions as in i) with an additional step of purification with ethyl acetate, resulting in the obtaining of a purified (CP) fraction; iii) same conditions as in ii), with the additional step of acid hydrolysis (CHP fraction). The purification step was done through a liquid-liquid extraction using three times ethyl acetate volume relative to the extract volume, followed by cotton filtration the evaporation of ethyl acetate phases in a rotatory evaporator and resuspension in water. The hydrolysis step was performed with HCl 8.4 M in a proportion of 1:20 of acid: sample at a 100°C sand batch for 1 h. HT and OLE were identified by ultra-high-performance liquid chromatography and HT levels were quantified by internal standard quantitation using cinnamic acid. Moreover, the total amount of phenolic compounds in the samples was estimated by the Folin-Ciocalteu method and their antioxidant activity was accessed through ABTS scavenging assay. The stability of the extracts regarding HT content during four weeks was analysed along the storage conditions from 5 to -18°C.

OLE and HT were the most abundant compounds extracted. Still, the treatments applied after the acidified water extraction had a great influence on the concentration ratios of these compounds. Extraction procedure i) allowed the recovery of HT at 1.66 ± 0.03 mg/g olive leaves powder (OLP) and OLE, but their recovery decreased with the addition of the purification step in the CP fraction (1.17 ± 0 .15 mg HT/g OLP). This suggests that a significant part of HT and OLE was kept in the aqueous phase. In turn, CHP fraction, which was hydrolysed, allowed a maximum recover of HT (11.4 ± 0.9 mg/g OLP) in the absence of OLE that was probably degraded under these conditions.

The HT content in CRD increased over four weeks of storage (6.09 ± 0.29 mg/g OLP after 29 days), accompanied by a significant rise in the antioxidant activity (from 2.3 to 3.72 mg ascorbic acid equivalents/mL) and visible loss of OLE. Even without significance, the antioxidant activity of fraction CHP also increased over time (from 0.65 to 1.3 mg ascorbic acid equivalents/mL at 0 and 29 days) but was still lower than that of CRD. This could be related with the existence of other compounds visible by the chromatogram peaks of CRD. In the CP fraction, HT levels were stable, while variations in antioxidant activity and total phenolic compounds were similar to CHP. These results showed that OLE, HT and other non-identified compounds, even in lower amount, contribute with higher antioxidant activity than high levels of isolated HT. Moreover, HT-rich extract seems more stable under laboratory conditions than OLE, that showed some susceptibility to degrade over time.

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Effect of pasteurisation on bioactive compounds of human milk

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The human milk is the optimal food for an infant to keep its nutritional and health status. Because it contains a large group of bioactive compounds such as proteins, vitamins, nucleotides, oligosaccharides, immunoglobulins, extracellular vesicles (EVs) and bioavailable minerals. These bioactive components of human milk play an essential role in preventing the gastrointestinal and respiratory infections, anemia, and bone-related problems as well as it enhances the immune function and helps in the maturation of the digestive system (1).
For these reasons, professionals and the World Health Organization (WHO) recommend exclusive breastfeeding for feeding newborns and especially premature newborns.
The purpose of human milk banks is to provide safe donor milk to premature infants who cannot be breastfed by their mothers. Donor breast milk undergoes a number of different processes including freezing and pasteurisation. These processes alter the nutritional composition of the milk and loss of bioactive and immunological properties. Therefore, it would be of great interest to evaluate the impact of pasteurization on bioactive compounds of human milk like lactoferrin and extracellular vesicles
Lactoferrin is one of the most abundant bioactive glycoproteins in human milk that can be found as its iron-free form (apo-lactoferrin), or as iron-bound form (hololactoferrin)(2). In this investigation, an isotopically labelled iron-lactoferrin complex was used to fortify donor milk samples, which were submitted to pasteurisation. The effect of pasteurization on the levels of lactoferrin and iron-lactoferrin isotopically labelled complex was evaluated.
Another milk bioactive compound of great interest is the so-called exosomes: “extracellular vesicles (around 10–200 nm) that contain genetic material (DNA, RNA), proteins and lipids” and that act as cellular messengers transporting material and information to other cells, where they intervene in multiple processes with important health benefit (Figure 1). This study a methodology for separation and characterization milk exosomes was developed. The effect of pasteurization on the number and integrity of isolated milk EVs and their bioactive components was investigated.

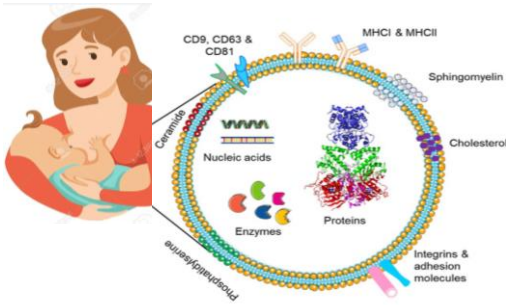

Figure 1. Exosomes structure with their cargos including nucleic acid (mRNA and DNA), protein, and lipids.

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Chemical composition and biological activity of different residues obtained from the wine industry

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In recent years, the bioactive compounds present in agri-food sub-products have attracted increased attention due to their health benefits and advantages within a circular economy context. Annually, wine production is responsible for the production of large amounts of phytotoxic waste, which elimination is considered challenging since these residues can be hazardous to the environment when used as fertilizers or just discarded. Phenolic compounds are secondary metabolites processed by plants that have shown several health benefits, acting as antioxidants, antimicrobials, anticarcinogenic, or antidiabetics, among others. Bioactive phenolic molecules have attracted considerable attention from the pharmaceutical, cosmetic, and food industries. So far, several studies have been developed on the characterization of grape pomace and its components (seeds, skins, and stems), especially directed to the residues of red varieties.[1] However, less attention has been paid to other by-products generated during winemaking, such as waste from the production of white wine, wine lees and diatomaceous earth. The latter is used in the filtration of wine and constitutes about 250 tons/year of waste from the wine sector in Portugal alone, making it a very pertinent residue with still scarce information being found in the literature. In this context, within the framework of the BacchusTech project that seeks to develop new innovative processes, comprising the extraction, purification, and concentration of bioactive compounds present in winemaking residues, different residues including pomace, lees and diatomaceous earth were evaluated for their chemical composition and bioactivities. Residues were extracted using an hydroalcoholic solvent (80%, *v/v*), total phenolic compounds were estimated using the Folin-Ciocalteu reagent and individual phenolic compounds were identified and quantified by liquid chromatography coupled to mass spectrophotometry (HPLC-DAD-ESI-MSn). Additionally, the biological activity was assessed through TBARS, DPPH, and reducing power assays to determine the antioxidant activity, and the antimicrobial activity was evaluated by broth microdilution against eight bacteria and two fungi.

The phenolic composition was in accordance with the previously reported in red wines.[2,3] Fifteen non-anthocyanin phenolic compounds were found, five phenolic acids (gallic acid and derivatives, *p*-hydroxybenzoic and *p*-coumaric acid), four flavan-3-ols (procyanidin dimers), two *O*-glycosylated flavanols (isorhamnetin and quercetin derivatives), three flavanol aglycones (quercetin, kaempferol, and myricetin), and one unknown compound. Regarding anthocyanins, five compounds were found, namely malvidin derivatives linked to acyl groups. Wine lees and white grape pomace before distillation presented the highest contents of phenolic compounds; however, only diatomaceous earth sample reveal the presence of *O*-glycosylated flavonoids. All samples showed antibacterial and antifungal activity against most of the tested microorganisms. The best bacteriostatic activity was evidenced by the red and white grape pomace before distillation and diatomaceous earth, while the wine lees stood out for their fungistatic activity. In general, all samples showed promising antioxidant capacity, with very good results being obtained on TBARS assay, particularly for the white pomace after distillation (EC_{50} = 0.016±0.002 mg/mL), diatomaceous earth (EC_{50} = 0.063±0.001 mg/mL) and red pomace before distillation (EC_{50} = 0.08±0.04 mg/mL).

Overall, the results obtained showed that the residues analyzed are good sources of bioactive compounds, namely anthocyanins and other phenolic compounds, which can be used as raw materials for the steps of concentration, purification and/or isolation of compounds of added value.

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Autenticidade e rastreabilidade dos Alimentos

Detection and quantitation of added water in octopus using a rapid and non-destructive method based in Time Domain Reflectometry (TDR)

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Seafood is often the target of practices that may affect product integrity, especially in those species with high added value[1]. One example is the abusive and non-reported water addition to allegedly compensate for moisture losses[1]. Consumers complain for an enormous weight loss of octopus after cooking, and market studies regarding the addition of water have confirmed abusive practices. A study with octopus from the Portuguese market showed that 85 % of commercial products had moisture contents higher than 86.0 g /100 g[2], while reported baseline moisture contents for *Octopus vulgaris* were 80.7 ± 1.6 g/100 g[3]. Rapid and non-destructive methods can be used for a prompt evaluation of abusive practices before first sale, as early as the primary processing stage for product qualification in the industry, or in fresh products in markets.

A rapid and non-destructive method of measurement of the product's dielectric properties based in time domain reflectometry (TDR) analysis was developed for the control of abusive water addition to octopus, not only for detection, but also for quantitation of water content in water-added octopus. Common octopus (*Octopus vulgaris*) and curled octopus (*Eledone cirrhosa*) were captured in Portugal (west coast), and a total of 77 specimens were used in the case of *O. vulgaris* and 44 for *E. cirrhosa*. Octopus samples were immersed in freshwater for different periods of time (0.5 – 36 h) to give a wide range of moisture contents, and thus represent different commercial conditions. Control and water-added octopus were analyzed with a TDR sensor, and data correlated with moisture content were used for calibration and method validation.

TDR results were different between the two octopus species. Control samples of *E. cirrhosa* showed higher TDR values than *O. vulgaris*, and the average moisture contents were 78.9 g/100 g and 82.3 g/100 g, respectively. Regarding the immersion treatments, in the region between 1.2 and 1.5 ns, the reflected TDR signal increased with the increase in immersion time, in a similar trend between species (**Figure 1**).

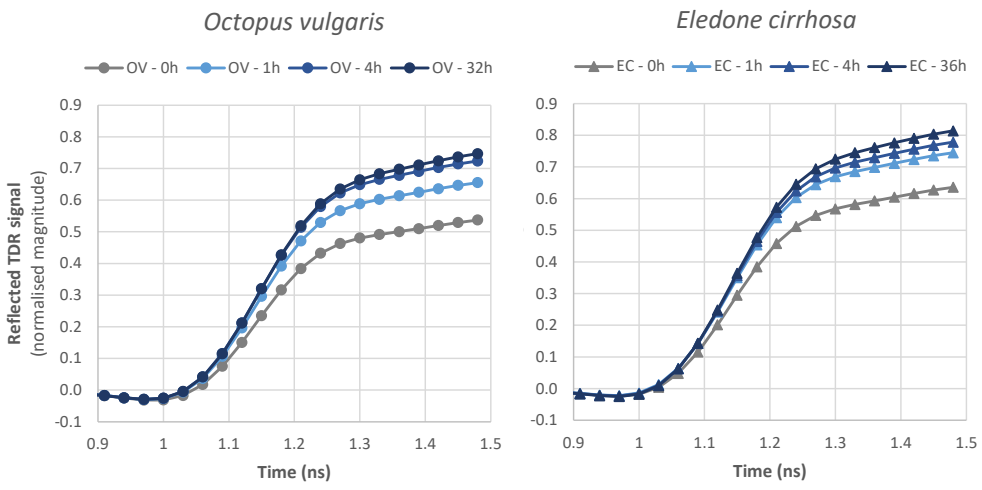

Figure 1: Time domain reflectometry data of *Octopus vulgaris* and *Eledone cirrhosa* samples. Data represent the average of all control samples (0 h) and for each immersion time (up to 36 h).

Rapid and non-destructive method able to discriminate between control and water-added octopus, independently of the species under test, would be advantageous as it would allow the analysis inclusively by non-trained personnel. In this sense, principal components analysis and discriminant analysis were performed in TDR data. Results showed that

TDR analysis can be used for the detection of water addition in octopus samples, independently if it is an *O. vulgaris* or an *E. cirrhosa* sample that is analyzed. In terms of discriminative analysis classification, 98.8 % of the samples were correctly classified. Control samples were never classified as water-added samples, although some were misclassified in terms of species. In an alternative model, using one third of the samples for testing, although a discriminative analysis misclassification was also observed in terms of species, a good classification was obtained regarding the discrimination between control and water-added octopus.

For development of a model allowing the quantitation of the moisture content in octopus samples a principal components analysis and multiple linear regression were applied to the TDR data. The TDR equipment was firstly calibrated with the experimental data and then validated for the quantitation of moisture content in *O. vulgaris* (between 80 and 90 g/100 g; RMSE = 1.1 %). Calibration and validation results showed similar root mean squared errors (RMSE). As moisture baseline levels of *O. vulgaris* and *E. cirrhosa* species are different, species specific calibration models need to be developed for quantitation of moisture content.

TDR data and correlation with moisture content show that this non-destructive methodology can be used by the industry and quality control inspections for assessment of octopus quality and to verify compliance with legislation, promoting fair trade practices, and further contributing to a sustainable use of resources.

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Multielement analysis to trace authenticity using potential markers of PDO pears and PGI apples cake fillings

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The authenticity of food products with Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) is important for the food industry, giving an added value and allow them to be distinguished in the European economy. The authenticity of PDO "Pera Rocha do Oeste" and PGI "Maçã de Alcobaça", some of the most relevant Portuguese fruits in terms of area production, economic and export, is easily recognizable when commercialized in retail markets but not when processed in fruit fillings used in pastry. Apple and pear fruits have their consumption in fresh, as well as industrially processed, as constituents of cake fillings. Processed products require the identification of unique characteristics of the target products, which may serve as markers of authenticity. It is possible that the fruits of a region possess characteristics associated to the environment, conferring them these required unique characteristics for their identification, even when processed. As there a growing interest in certified products by the pastry industries, the identification of authenticity markers is very important to be able to sell the fruit fillings with PDO and PGI certification due to economic value. Aiming to contribute to find markers of PDO pear and of PGI apple in fruit fillings that enable establishing the basis for geographical origin, multielement analysis by Inductively Coupled Plasma (ICP) together with chemometric tools can provide discrimination of processed fruits. In this study, the mineral profiles of PDO "Pera Rocha do Oeste" and PGI Alcobaça Apple var. Golden Delicious (fresh fruit and fruit fillings) were analyzed by ICP and identified to evaluate the applicability of multielement data on the determination of geographical origin and authenticity markers. The results show that 4 elements (Mn, Ce, B, and Rb) are significatively different between PDO pear fillings and pears fillings from Alentejo. In case of apple, PGI fresh apples and fruit fillings have lower caesium (Cs) and rubidium (Rb) concentrations than apples and fruit fillings from other Portuguese geographical areas. These differences can be explained by the soil characteristics.
The present study shows that multielement analysis by Inductively Coupled Plasma (ICP) combined with the chemometric tools can provide discrimination of processed fruits and can be a valuable contribution for the identification of the geographical origin authenticity markers of PDO pear and PGI apple in fruit fillings.

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DNA-based techniques for the entomological authentication of honey: comparison of high-resolution melting (HRM) analysis and next generation sequencing (NGS) approaches

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Honey is a food widely consumed worldwide and highly appreciated for its aroma, taste, and nutritional properties, as well as for its beneficial health effects. However, honey is also ranked as one of the products most prone to adulteration, either by admixing with lower quality honey, by sugars addition, or by mislabeling botanical and geographical origin, among other possible frauds.[1] Up until now, different methodologies have been proposed for honey authentication, mainly focusing on these types of frauds, yet the authentication of geographical origin is still difficult to be achieved. Recently, more attention has been paid to the entomological origin of honey since it is also related to its geographical origin based on the different natural distribution of honeybee species and subspecies around the globe. In Europe, different *Apis mellifera* subspecies carrying mitochondrial DNA (mtDNA) of distinct ancestries can be naturally found.[2,3] Besides being an additional parameter contributing to a holistic approach towards honey's authentication and avoiding unfair competition among producers through the identification of frauds, establishing the entomological origin of honey can also contribute to valorizing autochthonous honeybee subspecies and promoting biodiversity.

Therefore, as part of the Autent+ project, new approaches were explored and tools developed for the authentication of honey produced by different honeybee subspecies and mitochondrial lineages, namely *A. m. iberiensis* (lineages A and M), *A. m. mellifera* (strain M), *A. m. carnica* (lineage C) and *A. mellifera ligustica* (lineage C). In the present work, two developed methodologies were developed and applied to a set of commercial honeys from different countries and the obtained results were compared. The first methodology was based on the amplification of an informative short fragment of the cytochrome c oxidase I (COI) gene by real-time PCR coupled with high resolution melting analysis (HRM). This methodology allows the differentiation of the three mtDNA lineages (A, M and C) in a single step. The second developed methodology consisted of using next-generation sequencing (NGS) with newly designed primers targeting a 406 bp fragment of the COI gene followed by the bioinformatic analysis of the obtained data. The two methodologies were validated by using a set of honeys of known entomological origin provided by beekeepers from apiaries previously studied, and further applied to a set of 44 commercial honeys labeled as being from different geographical origins.

In general, the results obtained by the two methods were in good agreement and corroborated the declared geographical origin of the commercial samples. Particularly in the case of four French honeys labelled as Corsican PDO honeys, which specification indicates that should be produced by *A. m. mellifera* (lineage M) both methods confirmed their entomological origin. Overall, the two methodologies were suitable for verifying the labelling compliance of PDO honeys that should be produced with specific autochthonous honeybee subspecies. The first method has the advantages of being a simple, fast, and cost-effective approach. However, honey may contain DNA of different maternal lineages when it is harvested from different hives headed by queens from different lineages and then combined. Using real-time PCR coupled with HRM, some commercial honey samples did not cluster with any honeybee lineage, suggesting the presence of mixtures of honeys produced by honeybees from different ancestries, which cannot be identified with this technique. In turn, DNA-metabarcoding is emerging as a promising alternative for identification, since high-throughput sequencing platforms are capable of yielding millions of reads due to massively parallel sequencing. NGS confirmed the presence of mixtures in those samples and allowed the identification of the mtDNA ancestries, offering a reliable and high-throughput alternative to establish the entomological origin of honey. However, this approach is more laborious and costly.

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Multiple correspondence analysis of microbiological profiles as markers of authenticity of PDO cheeses

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Due to an ever-increasing demand of the market for quality products, production of Protected Denomination of Origin (PDO) cheeses has grown constantly over the years, becoming these products more susceptible to adulteration
Serra da Estrela is the oldest and most recognizable traditional Portuguese Protected Designation of Origin (PDO) cheese produced following artisanal procedures using ovine raw milk, *Cynara cardunculus L.* flower and salt. Despite it has been extensively studied over the years, a hiatus of 20 years emerged between the present manufactures and the most recent survey, focused on microbial characterization. The present work aimed to perform an updated characterization of Serra da Estrela PDO cheeses and raw materials focused on lactic acid bacteria and evaluate the potential of microbiological profiles when used in conjunction with multiple correspondence analysis, as markers of authenticity of PDO cheeses.
Microbial characterizations were performed on cheese, curd, ovine milk and cardoon samples obtained in November, January; February, March and May – June periods within the 2018/2019 production campaign. This approach allowed an assessment of autumn, winter and spring manufactures. The Serra da Estrela cheeses were manufactured in a certified PDO cheese producer from Gouveia, Portugal. The biochemically confirmed LAB isolates obtained from anaerobically incubated MRSA plates were subjected to molecular genetic identification throughout the amplification and sequencing of the 16S rRNA gene.
The correspondence analysis performed on LAB isolates revealed that *L. paracasei, L. lactis, E. durans, E. faecium and L. mesenteroides* species are most common species found in larger amounts n Serra da Estrela cheese production, being present in ovine raw milk, curd and cheese matrices. Furthermore, lactobacilli were more closely associated to winter and spring manufactures, while enterococci were preferably found in autumn and winter manufactures.
Overall, this study, contributing to an updated microbiological characterisation and complemented with multiple correspondence analysis, revealed a good potential regarding the attribution of microbiome profiles as a marker of authenticity of the technological process applied to PDO cheeses. The results obtained revealed that this statistical tool could probably be useful in the future and possibly used in conjunction with the characterisation of the microbiome as a marker of authenticity of the process involved in cheese production. However, large-scale studies should be carried out to ensure the good reliability of this approach.

Segurança Alimentar

The mycoestrogen zearalenone and rice: occurrence and risk assessment

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Rice is the second most important cereal crop and an essential component of the diets and livelihoods of billions of people [1], including infants and celiac patients. Global rice production has been steadily rising over the last decade. The Portuguese adult population annual average consumed 25.1 kg of rice per capita. As for children and adolescents, the annual consumption was, 19.1 and 28.3 kg/year, respectively. [2] This high consumption, above the European Union (EU) mean, can enhance human exposure to chemical hazards, namely mycotoxins, such as zearalenone (ZEN). ZEN, a metabolite primarily associated with several *Fusarium* species, is a phytoestrogenic compound, already reported in rice.[3]

The goal of this study was firstly to evaluate the occurrence of ZEN in rice and the Portuguese population exposure. Rice intended for human consumption was commercially acquired and provided by Portuguese rice producers. The determination of ZEN was accomplished by a competitive enzyme immunoassay (ELISA). From the 36 samples analysed, 88.89% of the total samples showed levels above the LOQ, up to 14.25 µg/kg, with an average of 2.75±2.26 µg/kg. None exceeded the maximum limit (ML) established by the EU.

Regarding ZEN exposure and risk assessment (Figure 1), for every population studied, the maximum estimated daily intake (EDI) value was of 77.81 ng/kg bw/day, considering the worst case scenario approach and the 95th consumption. Children presented the higher risk values, followed by adolescents and adults, considering the Tolerable daily intake (TDI) value of 250 ng/kg bw/day.

Current results can be justified by the application of good agricultural practices and rice production, storage and distribution. However, in a time of concern about world hunger, impact of climate change, population growth and future food security, rice production might be threatened, as well as both, the quantity and the quality of rice that is available for consumption to continue to monitor the population's exposure, namely those with higher susceptibility or consumption, to these contaminants in order to protect public health.

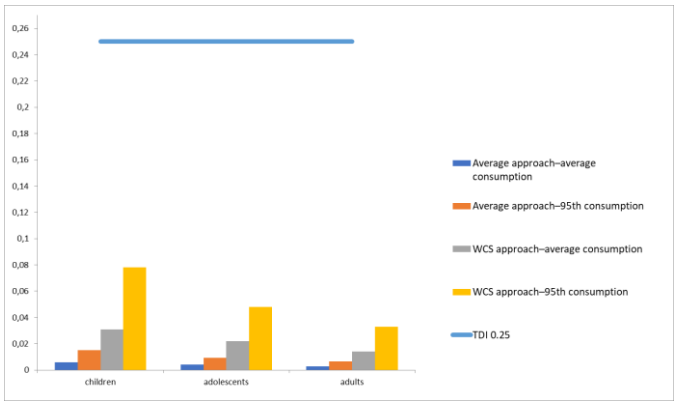

Figure 1: ZEN estimated daily intake (EDI) and risk assessment.

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Arsenic in Portuguese rice. Is there any risk?

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Arsenic is a metalloid with natural and anthropogenic sources and its inorganic form is toxic to humans. It is considered carcinogenic belonging to Group 1 of the International Agency for Research on Cancer.[1] Organic and inorganic arsenic is mainly present in water, originating from different sources, with foods that are irrigated with large amounts of water, such as rice, accounting for a greater exposure when compared to other cereals, additionally, rice is highly consumed worldwide.[2] Therefore, this study evaluated the inorganic arsenic content of 70 Portuguese rice samples; 48 were sampled from a rice factory located in Coimbra District (Portugal) during the months of September and October 2019 and 22 samples were acquired from Portuguese supermarkets in January 2020.
The inorganic arsenic was extracted with nitric acid and the extracts analysed through inductively coupled plasma mass spectrometry (ICP-MS) with a detection limit of 3.3 µg kg^{-1}. The total frequency of detection for inorganic arsenic in rice was 81% (57 samples) with an average of 29.3 µg kg^{-1}, with brown and short rice presenting higher values, with statistical difference, than white and long rice. The highest concentration, 100 µg kg^{-1}, equalled the maximum residue limit (MRL) for rice destined to infant's consumption. The results obtained in the present study were clearly lower when compared with other scientific published works, even when comparing with data from similar regions. The estimated daily intake (EDI) surpassed the benchmark dose (lower confidence limit 10%) ($BMDL_{10}$) of 0.3 µg kg^{-1} of bw/day considering children in the 95th percentile of rice consumption and the worst-case scenario concentration (**Figure 1**). It should be noted that rice is not the only source of inorganic arsenic. Therefore, other sources that also contribute to the daily intake should also be considered for a correct risk assessment. Additionally, there are population groups that present a higher risk to the exposure of inorganic arsenic like children, celiac people, some ethnic groups and high consumers of algae-based products that can highly exceed the BMDL10.[3]

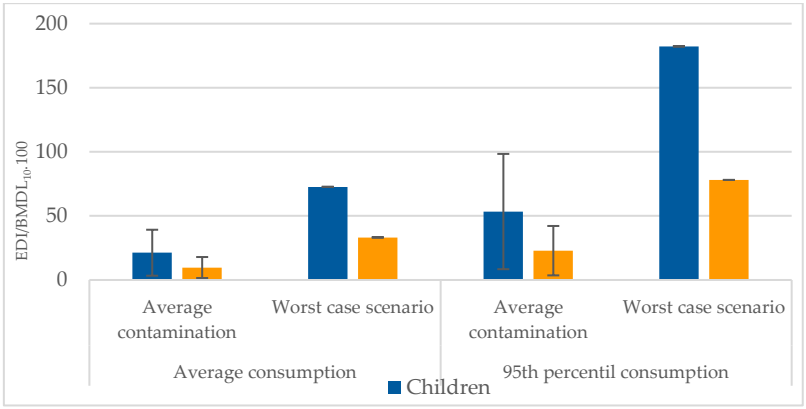

Figure 1: Percentage of the EDI (µg kg^{-1} bw/day) versus the $BMDL_{10}$ (0.3 µg kg^{-1} bw/day) with standard deviation.

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Funding: This work was financially supported by PT national funds (FCT/MCTES, Fundação para a Ciência e a Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020.

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Heavy metals and metalloids in shrimps from northwest Portuguese coast

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Seafood is highly consumed and suggested as part of a balanced human diet, the seafood market has grown in the last years, making seafood a valuable source of nutrients for the human population. Shrimps are one of the most consumed seafood in the world and are highly consumed by the Portuguese population. Although shrimp can be a good source of nutrients, they can also accumulate heavy metals and metalloids from the surrounding environment. Heavy metals and metalloids are a group of trace pollutants that can accumulate in marine organisms and be bioconcentrated along the food chain. This way heavy metals and metalloids could reach humans through their diet and pose health risks[1]. The European Union has set maximum levels for some heavy metals in food. For crustaceans, the maximum level for lead (Pb) and cadmium (Cd) is 0.5 mg/kg wet weight (ww)[2].

This study aimed to evaluate the concentrations of heavy metals and metalloids, such as Pb, Cd, arsenic (As) and aluminum (Al) in two shrimp species (*Palaemon serratus* and *Palaemon varians*) from different sites along Portuguese coast (namely, Vila do Conde, Matosinhos, Aveiro, Figueira da Foz and Sado estuary).

For the sample preparation and analysis, microwave-assisted wet digestion of shrimp samples was performed using approximately 0.5 g of each shrimp sample and 10 mL of HNO_3 in a teflon microwave vessel, all samples were analyzed in duplicate. Heavy metals and metalloids were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

The results for the evaluation of Pb, Cd, As and Al showed significant differences between the diverse sampling sites. As was higher in samples from Figueira da Foz (11.2 mg/kg), while Pb content was higher in wild samples from Sado estuary (0.087 mg/kg) and higher contents of Al and Cd were observed in the aquaculture samples from Sado estuary (90 mg/kg and 0.006 mg/kg respectively). Regarding the specie, As and Cd were found in significantly higher levels in *P. serratus*, while *P. varians* showed significantly higher levels of Al. For *P. serratus*, As mean content was higher in spring compared to autumn samples. For *P. varians,* As mean content was significantly higher in spring, while Cd was higher in autumn. The maximum limit for Pb and Cd in legislation is 0.5 mg/kg ww in crustaceans[2]. All samples analyzed in this study presented values below the legislated maximum.

Risk assessment was performed using Target Hazard Quotient (THQ). The THQ values were all below 1 with exception of Al in *P. varians* where the value was above 1. Although, the value for Al was above 1, this element is practically not absorbed by the gastrointestinal tract, not presenting a particular risk.

The results from this study highlight the need for continuous monitoring of coastal areas, ensuring environmental and food safety[3].

Acknowledgements: We would like to thank to FCT and the European Union's H2020 Research and Innovation Programme for funding through the project SYSTEMIC - An integrated approach to the challenge of sustainable food systems: adaptive and mitigatory strategies to address climate change and malnutrition. Maria Luz Maia is grateful to FCT and ESF (European Social Fund) through POCH (Programa Operacional Capital Humano) for the PhD grant SFRH/BD/128817/2017 and COVID/BD/151852/2022.

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Luminescence Sensors based on nano-MOFs to detect biogenic amines

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Food safety is one of the major concerns in the world. Moreover, detection and control of hazardous substances is always necessary. In this reagard, it is a major concern to establish the shelf life of a given food product during storage and commercialization, because depending on the product nature, its degradation in terms of microbiological and the physicochemical properties can be different. Some compounds which appear because of the metabolic activities of microorganisms in food are Biogenic amines (BA). This BA can be present in all types of foods with high protein or free amino acid content, such as fish, meat or wines, their presence in non-fermented foods is usually undesirable and is related to microbial spoilage. Putrescine and cadaverine are two of the most common BA, their presence in high concentrations can produce severe toxicological effects in humans [1]. The best method for the detection of BA in food would be a fast, simple and non-invasive, that does not destroy the sample, allowing the analysis in the production line or even incorporating the sensors in the packaging. [1].

NanoMOFs (metal organic frameworks) are crystalline nanoparticles composed of ions of a metal or a cluster held in a three-dimensional structure connected via organic ligands. They have aroused great interest in designing sensors due to their porous structure, large surface area, modifiability and luminescent properties [2]. This behaviour and the possibility of being used as sensing platforms have increased the interest of MOF based luminescent sensors.

This research works proposes the use of nanoMOFS as luminiscent indicators of BA presence in food samples. A Cu-nano MOF (CuDOBCD or "green MOF") has been characterized and evaluated in liquid (using Milli-Q as solvent) and gas phase as alternative to detect amines. The effect of amines on the luminiscent spectra and/or visual colour changes have been studied (Figure 1.)

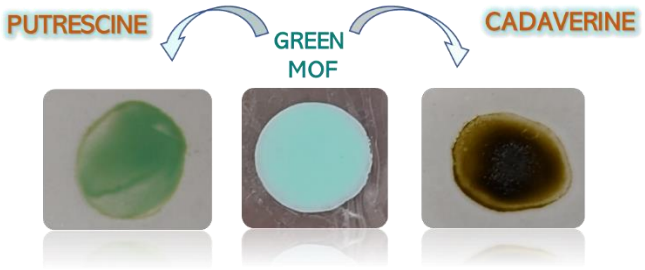

Figure 1: **Visual test on isolated environment with biogenic amines.**

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Determination and human health risk assessment of mercury in fish samples

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Mercury is a chemical element whose toxicity is a worldwide concern. This metal causes damage to human health, sometimes irreversible, mainly affecting the kidneys, liver, digestive system, heart, lungs, and central nervous system. Also, problems in the cardiovascular system such as hypertension, coronary heart disease, and myocardial infarction are caused by mercury.[1]

The leading indices employed for human health risk assessment due to the consumption of foods are estimated Weekly Intake (EWI), Provisional Tolerable Weekly Intake (PTWI), Target Hazard Quotient (THQ), Maximum Safe Consuming Quantity (MSCQ), Carcinogenic Risk (CR), and Hazard Index (HI).[2]

Developing works determining human health risks from food contamination requires a meticulous sample collection process. In this way, sampling projects must have a statistical basis, considering the following points: availability of food for the community, availability of other foods of the exact nature, seasonal effects, population age, cultural issues, etc.

This work presents the determination of mercury in fish purchased at a public market. The mercury quantification was performed using the DMA method, which allows limits of detection and quantification of 0.004 and 0.012 ng, respectively. Method accuracy was confirmed using a certified reference material of fish protein from (NRCC) National Research Council, Canada. The analyzed species were: *Dourada* (Brachyplatystoma rousseauxii), Filhote (*Brachyplatystoma filamentosum*), Pescada Branca *(Cynoscion leiarchus*), Piramutaba (*Brachyplatystoma vaillanti).* The mercury contents expressed as wet sample weight varied from 0.078 to 0.150 µg g^{-1}. Afterward, the health risk assessment indices Estimated Weekly Intake (EWI), Target Hazard Quotient (THQ), and Maximum Safe Consuming Quantity (MSCQ) were applied to the analytical data, and the results obtained were exhaustively interpreted and discussed. All the indices demonstrated that the daily consumption of 25 g of these fishes does not risk the local population's human health. No systematic sampling was performed. Risk indices were applied and exhaustively interpreted. However, the conclusions, including public policies, cannot be used in formal matters.

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Regulated, non-regulated and emerging multi-mycotoxins in raw milk: UHPLC-QTrap-MS/MS method validation for control of biosafety measures

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Milk plays an important role in the human diet, due to its rich composition in micro and macro-nutrients, especially in vulnerable groups such as children and the elderly. Milk quality is directly related to the type and quality of the animal feed, being the main contamination route of raw milk by consumption of naturally contaminated feed. The presence of mycotoxins in these food matrices are mainly due to the carry-over process of such toxins from animal feeding, or due to biotransformation and secretion into milk from these contaminated sources, consequently becoming a risk to human health. The most studied mycotoxin in milk is AFM1, being the only regulated mycotoxin in the EU legislation[1], though other mycotoxins that exert negative health effects, either individually or synergistically, can be carried-over into this food product. Therefore, it is important to have improved analytical tools for rapid and robust analysis, that can be applied for specific requirements on a biosafety level.

This study was focused on the validation of an analytical methodology by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-QTrap-MS/MS) for the determination of regulated, non-regulated and emerging mycotoxins in raw milk samples. Blank raw milk samples fortified with mycotoxin standards were subjected to an optimized extraction procedure based on a modified QuEChERS protocols. Validation parameters, including linearity, limits of detection (LoD) and quantification (LoQ), repeatability, reproducibility, and recovery were assessed, and compared among the different published analytical methods. Performance criteria for aflatoxins, ochratoxin A, zearalenone, fumonisins, T-2 and HT-2 toxins, and citrinin were evaluated according to the specific requirements for confirmatory methods stated in Commission Regulation nº 401/2006[2]; and for non-regulated mycotoxins general method validation guidelines were followed. For application purposes, a qualitative characterization of the contamination profiles in raw milk samples from Portuguese dairy farms was performed.

The present methodology successfully permitted the extraction and determination of regulated, non-regulated and emerging mycotoxins (n=23) in raw milk samples, proving to be specific and selective, with low matrix interferents or peaks that could co-elute with the target compounds, and, therefore, low ionic suppression effects. Good performance criteria were also obtained in compliance with Commission Regulation (EC) Nº 401/2006[2]. LoDs and LoQs demonstrated the method's capacity to determine concentrations below the maximum residue levels for such samples, as established in Commission Regulation (EC) Nº 1881/2006[3]. From the real samples analysed, at least one mycotoxin was present in 97% of the samples, with emphasis on the high occurrence of emerging mycotoxins, namely beauvericin (90%) and enniatin B (77%). The only regulated mycotoxin for milk, aflatoxin M1 (AFM1), was not found in any of the analysed samples.

Data on mycotoxin (co-)occurrence in milk is very scarce, and continuous monitoring on multi-mycotoxin presence in such samples are essential to perform accurate risk assessments and implement adequate biosafety measures, thus allowing to protect consumer's health, especially in a food highly consumed by age vulnerable population groups. The high occurrence of emerging mycotoxins also points out the need to perform further occurrence surveys in this matrix.

Acknowledgements: Marta Leite wishes to acknowledge the Portuguese funding institution FCT - Fundação para a Ciência e a Tecnologia for supporting the present research, through the Ph.D. grant SFRH/BD/141778/2018.

Funding: This work was financially supported by the FCT – Portuguese Foundation for Science and Technology [grant nº UIDB/50006/2020].

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Monitoring Contaminants in Food: from Food Production to "One Health" approach

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The impact that animal production has on the environment and the resulting effects on "One Health" (human, animal and environmental health) are an increasing concern for all stakeholders along the food chain. One of the factors responsible for the negative consequences is related to the continued use of pharmaceuticals in food producing animals, which may result in the presence of undesirable residues in food and in the environment. Since the increase of antimicrobial resistance is one of the current topics of concern, the possibility that the continued use of antibiotics may contribute to the further development of such resistant strains in animals, as well as in humans and in the environment, is a hypothesis to be highlighted. Consequently, it is also to be considered a possible transfer of resistant bacteria between animals and humans through the food chain and even into the environment by manure and wastewater. For the efficient protection of consumers, the control of veterinary drug residues is essential to ensure that the entry of animal products into the food chain does not threaten the quality of the final food products. Likewise, the evaluation of environmental matrices for the control of pharmaceuticals that may be considered persistent contaminants, as for example in aquaculture, is also an issue of great importance in the "One Health" concept. In other words, dealing with health-related issues in a global and truly holistic way, bringing together, at least, human health, animal health and environmental health. In the same sense, it is also important to evaluate contaminants of natural origin, such as the occurrence of mycotoxins, which must be evaluated along the entire food production chain, given their recognized link to cancer diseases.[3]

Advances in chemical analytical technologies have allowed detecting more and more substances at residual concentrations, allowing a deeper knowledge of the occurrence of persistent contaminants. Methods for multi-detection of pharmacologically active compounds and other natural contaminants, such as mycotoxins, have been developed and improved, using liquid chromatography coupled to mass spectrometry systems, namely UHPLC-QTRAP-MS/MS and UHPLC-TOF-MS. Safeguarding food safety, consumer health, industrial and commercial interests, as well as obtaining data and tools for the evaluation of the effects caused on the environment and animal health, is the main objective of the work developed. The analytical strategies recently developed as Food Safety tools, and here presented, are focused on the following topics:

1. Determination of antimicrobials in food samples intended for human consumption,[1]
2. Evaluation of persistent drugs in representative samples of the environment,[2]
3. Occurrence of mycotoxins in the food chain: from corn seed to milk consumption.[3]

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Byssochlamys nivea ascospore germination and inactivation by hyperbaric storage – dependence of thermal and nonthermal pre-activation steps

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Food storage under pressure (hyperbaric storage, HS) is being increasingly studied as a replacement/complement of the conventional refrigeration processes, considering the energetic savings attained by HS (as energy is only mobilized during the short compression/decompression phases of the pressure vessel) and no additional energy is needed to keep the product under pressure. This methodology makes use of hydrostatic pressures (up to 150 MPa) to hurdle microbial development and inhibit several deteriorative reactions in foods and can be performed at room temperatures (RT)[1]. Considering that generally pasteurized acidic foods are to be kept under refrigeration, due to the possible presence of specific spores (as fungi spores), such as those from *Byssochlamys nivea*, that are quite resistant to thermal and nonthermal pasteurization procedures, and can produce mycotoxins, such as patulin, novel methodologies to destroy spores are of upmost interest, particularly causing milder impact in food [2].
So, the present work aimed to evaluate the effectiveness of HS/RT to control the germination of *B. nivea* ascospores in commercial apple juice (pH 3.70) and the impact of pre-ascospore activation procedures by thermal (70 and 80 ºC for 30 seconds) and HPP (600 MPa, 3 minutes, RT). To do so, samples were processed in the aforementioned conditions and placed under HS conditions (25-150 MPa) for 30 days at uncontrolled RT (20-25 ºC), and also at atmospheric pressure at RT and under refrigeration (unprocessed samples were also placed at the same aforesaid conditions). The microbiological analyses were made in potato dextrose agar, and an aliquot of each sample was previously thermally treated at 70 ºC/10 minutes to inactivate vegetative forms.
The results showed that there was a clear dependence on the activation methodology on the ability of HS to control the germination of B. nivea ascospores. Unprocessed samples and samples processed at 70 ºC for 30 sec. evolved quite similarly under HS conditions, wherein neither ascospore germination nor inactivation was observed. A different scenario was observed for samples processed at 80 ºC for 30 sec and 600 MPa/3 min, wherein not only ascospore inhibition was observed (25-50 MPa) but also ascospore inactivation (75-150 MPa) of more than 3 log units of for both cases (initial load of 5.17 log CFU/mL). Differently, conventional refrigeration only inhibited ascospore development, being a possible threat for food safety due to possible germination after the 30 days of the study and mycotoxins production.
Concluding, a fully nonthermal process, HS with a previous 600 MPa/3 min HPP treatment for germination activation, resulted in at least more than 3 log units of *B. nivea* ascospores inactivation along storage, while this ascospore cannot be inactivated by thermal pasteurization or HPP (is only inactivated by sterilization), resulting in enhanced food safety, with the added advantage of being performed at uncontrolled RT, thus allowing considerable energetic savings and milder effects on foods.

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Characterization of coagulase-negative *Staphylococci* as potential starter cultures to substitute the addition of nitrate in the production of meat products

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One of the essential quality characteristics of meat products is their color, and the most common way to reach the ideal color is by adding nitrate and/or nitrites. During fermentation, nitrites are reduced to nitric oxide (NO) by coagulase-negative staphylococci (CNS) present in the meat or added as a starter culture. Then, this molecule binds to myoglobin to form the nitrosomyoglobin. NO-myoglobin is responsible for the meat's dark red colour.[1] However, adding nitrites has been linked to the production of hazardous N-nitrosamines associated with carcinogenic diseases. One of the alternatives currently explored is the nitric oxide synthase (NOS) found in CNS.[2] NOS is an enzyme encoded by the nos gene, first described in 1989. This enzyme is a possible alternative to nitrites since it produces NO from arginine without the addition of nitrate or nitrites.[3] The work aimed to characterise CNS for their potential to be used as substitutes for the addition of nitrate by studying the presence and expression of the nos gene. To accomplish this objective, we studied ten Staphylococcus equorum and ten Staphylococcus xylosus for the presence of the nos gene by PCR, with fifteen strains being further selected to be tested for their nitrate reductase activity. In total, six of the fifteen staphylococci were chosen to test for gene expression. The six strains included two S. equorum positive for both parameters evaluated (S2M7, F7Ca_2), two reference strains (S. equorum S03-0154 and S. xylosus S00413), one negative control for the nos gene (F2Z1_3) and one negative control for the nitrate reductase activity (K78). To test the expression of the nos gene, we prepared two Tryptic Soy Broth media, one supplemented with metmyoglobin and the other supplemented with metmyoglobin and potassium nitrate. After overnight incubation, the production of total pigments was analyzed based on the absorbance curves. It was possible to detect that the S. equorum strains F2Z1_3 and S2M7 presented the highest absorbance values at the characteristic wavelengths for the nitrosomyoglobin (547 and 582 nm). However, since another pigment derived from metmyoglobin has the same characteristic wavelength can also be present, extraction with acetone was necessary to confirm the production of NO-myoglobin. After extraction, it was observed that the S. equorum strain F2Z1_3 presented one of the highest absorbance values in the medium without adding potassium nitrate. This was of particular interest since, as previously mentioned, this strain was the negative control for the nos gene. To confirm the absence of nos gene in the genome of F2Z1_3 strain and the other mechanisms that could be behind the production of the pigment, whole-genome sequencing was performed. Sequencing of the S. equorum strain F2Z1_3 resulted in an assembly length of 4 493 653 bp with 3 322 contigs. The assembled genome was annotated using Prokka and 3 731 genes, 3 705 CDS, and 26 tRNA were identified. Several genes involved in the color development were identified in the genome, including genes coding for enzymes with nitrate reductase activity (nar) and nitrite reductase activity (nas, nir). The nos gene was also found, indicating a possible error in the initial PCR. Other genes involved in other important characteristics such as flavor, adaptation to the meat substrate and adaptation to the manufacturing conditions were also identified. The presence of these genes and the phenotypical expression of nos gene detected previously indicate that this strain is an excellent candidate to be used as a starter culture in the production of meat products without nitrate addition which may improve the safety of food products. However, when investigating the presence of undesirable characteristics, the F2Z1_3 strain carried the lin(A) gene in the genome conferring resistance to lincomycin and the possible existence of a plasmid. Further investigation should be performed to evaluate the potential expression of the antibiotic resistance gene and characterize the likely plasmid to ensure food safety. In conclusion, it was possible to detect the nos gene in several staphylococci strains. The strains that were able to have their expression studied demonstrated the capacity to express the nos gene through the formation of the NO-myoglobin pigment. The strain F2Z1_3 showed high promise in its capacity to be a good starter culture. However, the possible presence of plasmids and resistant genes needs to be further investigated before applying this S. equorum in the production of meat products.

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Alimentos Funcionais

Novel type of *Camellia sinensis* green tea rich in polyphenols and L-theanine, a promotor of cognitive functions

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Originated from China, the tea plant (*Camellia sinensis* (L) O. Kuntze) gradually expanded into many tropical countries and since the last decade of the 19th century, tea is also produced in one unique place in Europe: S. Miguel Island, Azores.[1] *Camellia sinensis* tea has received considerable attention due to its scientifically proven beneficial effects on health.[2] Some of these beneficial effects have been attributed to the non-protein amino acid (AA) L-theanine, the predominant AA in *C. sinensis* that can improve memory function, and has influential effects on lifestyle associated diseases, such as: stress relief, diabetes, hypertension, cardiovascular disorder, tumor suppression and liver injury. L-theanine, is also responsible for quality of tea, the tea's *umami* taste and has a strong influence on the neurotransmitters brain levels such as: dopamine, serotonin, acetyl choline, norepinephrine and gama-aminobutiric acid (GABA) and also stimulates the generation of alpha-waves that cause a relaxation sense.[3] Polyphenols, the major antioxidant constituents of *C. sinensis* tea, are also considered responsible not only for their flavor characteristics, but also for their wide variety of health benefits.[2]

The determination of L-theanine was achieved by HPLC following the Baptista *et al*.[1] methodology, and the total phenolic and flavonoid contents were determined by colorimetric methods. The aim of this study was to show the variation of polyphenols and L-theanine in Azorean *C. sinensis* green tea with different drying conditions of leaves and in different collecting zones of Gorreana Tea Plantation. The results presented in **Table 1** revealed that drying temperature strongly affect the level of polyphenols and L-theanine content and revealed differences between zones. The highest levels of L-theanine were observed when tea leaves were dried at low temperature and the same pattern were shown for the flavonoids contents. For phenolic compounds, the results revealed that the contents were higher at high temperature. The difference in the L-theanine content in different tea plantation zones may be explained by different soil composition and altitude, that interfere with the L-theanine synthesis in the root of plant and/or different sunlight exposure during the growing process.

Table 1. L-theanine, total phenolic and flavonoid contents (TPC and TFC) in *C. sinensis* on different collecting zones and under different drying temperatures[a]

	Leaves dried at high temperature			Leaves dried at low temperature		
	Zone A	Zone B	Zone C	Zone A	Zone B	Zone C
L-theanine (mg/g)	3.92 ± 0.04	4.45 ± 0.03	6.41 ± 0.03	7.57 ± 0.11	9.55 ± 0.07	14.60 ± 0.16
TPC (mg GAE/g DE)	302.47 ± 1.76	313.86 ± 1.29	303.08 ± 1.27	297.26 ± 0.63	300.26 ± 0.79	287.87 ± 2.02
TFC (mg RE/g DE)	60.26 ± 1.34	47.07 ± 0.86	40.82 ± 1.05	75.48 ± 2.30	67.56 ± 2.37	73.95 ± 1.74

[a]Values are mean ± SD (*n = 3*). GAE, Gallic acid equivalents. RE, Rutin equivalents. DE, dry extract.

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Obtention of lipid enriched extracts from microalgae for food applications

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Microalgae are a trend in agri-food, animal nutrition, cosmetic and pharmaceutic industries, due to their high nutritional value and bioactive properties.[1] To this end, microalgae cultivation is a promising way to supply biomass in a sustainable fashion.[2] The main aim of this work was to obtain lipid-enriched extracts from food grade microalgae for application in food products, namely in the substitution of fat in bakery and pastry products. Pastry products are, in general, rich in saturated fats, which are known to increase the risk of cardiovascular diseases. Thus, the total or partial substitution of the saturated fats with lipidic extracts richer in mono and polysaturated fats could be a strategy to develop healthier pastry products.

Three different commercial strains of *Chlorella vulgaris*[3] (organic, smooth and white) and *Tetrasellmis chuii*[3] were used, and the extracts were produced using solvent extraction (Ethanol), solvent extraction assisted by ultrasounds (Ethanol/UAE), and solvent extraction with a pre-treatment with a deep eutectic solvent (Ethanol/DES).

Extraction with ethanol after a pre-treatment with the eutectic solvent choline chloride:urea (1:2) resulted in higher extraction yields and in extracts with higher fatty acid contents (**Figure 1**). The analysis of the fatty acid profile of the extracts showed that *C. vulgaris* smooth and *T. chuii* extracts have lower contents of saturated fatty acids, which makes them good candidates for food applications. The extracts were further used as partial substitutes of fat in brioche (sweet bread), which was nutritionally characterized. A reduction of the saturated fatty acid content was observed, without affecting the sensory features of the product.

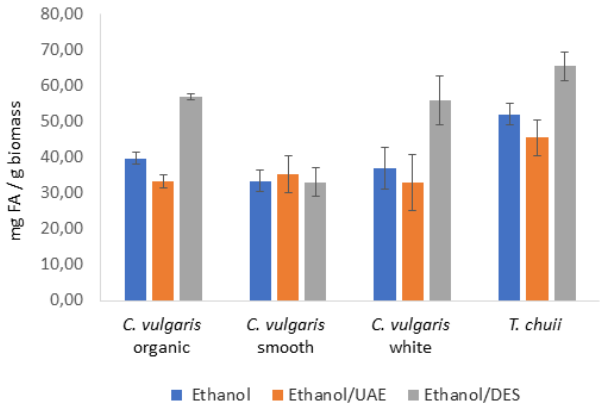

Figure 1: Fatty acid contents of the microalgae extracts obtained by the different extraction methods.

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Cheese prototypes enriched with microalgae: impact on structure, chemical composition, and sensory acceptance

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Over the years the health food market has been growing due to the increasing demand of nutrient-rich food products **(1)**. The production of foods fortified with bioactive ingredients has been recognized by food companies to position their products in healthy food markets. Microalgae biomass is a natural food ingredient with a great nutritional profile **(2)**. The development of microalgae-enriched products is a challenge for the food industry, as appearance, safety, preparation, storage stability and sensory properties should not be compromised by incorporating bioactive ingredients. Algae Green Cheese is a project developed in partnership with Queijos Santiago Portuguese cheese company. The main goal of this work is to evaluate the impact of *Chlorella vulgaris* (from 0.5 to 4% w/w) on industrial prototype cheeses **(Figure 3)** structure, chemical composition, bioactivity, and sensory profile.
Smooth *C. vulgaris* by *AllMicroAlgae*, is a variant that was obtained through heterotrophic production. Heterotrophic production consists in the exclusive use of organic compounds as a carbon source for the development and biomass production of the microalga. This type of production when compared to the autotrophic one has a faster growth rate, better productivity, and takes less space to produce the same amount of biomass. The microalga is grown in a fermenter, in the dark, resulting in a lower production of chlorophyl. Thus, Smooth *C. vulgaris* has a good nutritional profile and milder organoleptic features (color and taste) when compared to the autotrophic *C. vulgaris* which facilitates consumer acceptance **(3)**.
In this work, cheeses were prepared in an industrial scale based on the processing diagram used at Queijos Santiago. To evaluate the impact of *C. vulgaris* on the cheeses´ mechanical properties, empirical rheology methods (Texture Profile Analysis and Cut Test) and fundamental rheology measurements (oscillatory tests) were performed. The effect of *C. vulgaris* on pH, color, nutritional composition, and bioactivity were evaluated using AOAC methods - protein, lipids, carbohydrates, minerals, caloric value - and bioactivity through the determination of antioxidant capacity (FRAP and DPPH assays), total phenolic compounds, and total flavonoids compounds. A sensory analysis was conducted in individual booths at the Sensory Analysis Laboratory of *Instituto Superior de Agronomia* using untrained panellists. The evaluation was carried out by comparing the green prototype with the control.
The results obtained in this research work concluded that the addition of *C. vulgaris* affected some of the physicochemical properties of the cheeses, namely texture, color, antioxidant activity and mineral content. Such results suggest that the green microalga is a good bioactive ingredient that brings a potential positive health impact to consumers, when compared to traditional dairy products, obtaining innovative and more nutritive products, with a greater bioactivity, although some technological processes might need adjustment due to texture variations in the final product.

A B C D

Figure 3 - Cheese prototypes (A) Fresh Cheese; (B) Cured Cheese; (C) Whey Cheese; (D) Cream Cheese.

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Fruit pomace macromolecular antioxidants: from wastes towards innovative food applications

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Agro-food by-products, such as fruit pomaces are relevant sources of macromolecular antioxidants. These are polymeric structures of phenolic compounds or polyphenols associated to plant food macromolecules, such as polysaccharides or proteins, corresponding to a non-extractable fraction. In the last years, there has been a considerable interest in understanding and exploiting the functions and health benefits of the macromolecular antioxidants, as they yield bioavailable metabolites by the action of the microbiota, with significant effects either local and/or systemic after absorption, thus presenting an increasing interest in nutrition and health. However, due to the complexity of macromolecular antioxidants' structures and the lack of methodologies for their characterization, their application in functional foods remains a challenge.[1]

In this work, it is intended to use fruit pomaces, a waste generated from the fruit juices production, to recover a macromolecular antioxidant fraction, and to assess their potential gut health benefits, through in *vitro studies*. Fruit pomaces will be subjected to an *in vitro* simulated gastrointestinal digestion to investigate the release of polyphenols, carbohydrates' hydrolysis and consequential glucose bioaccessibility during digestion. The undigested fraction retained in the colon will be analyzed regarding its potential antioxidant effects and phenolic compounds metabolites. The health effect of macromolecular antioxidants opens new opportunities for the exploitation of these agri-food wastes in food nutrition, the next step towards reaching a circular economy.

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Study on the effect of the concentration and drying of microalgae on *Chlorella vulgaris* and *Arthrospira platensis* enriched pasta

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Mainly due to their abundance in phytochemicals, which provide several health benefits beyond the basic human nutritional necessities, microalgae are considered a functional food. The WHO even recognizes *Arthrospira platensis* (commonly known as *Spirulina*) as a "superfood", due to having nearly 70% of protein in its composition and being a great source of numerous vitamins, fatty acids (including ω3 and ω6), natural pigments, antioxidants, neuroprotectors and immunological fortifiers. It has been consumed for centuries by ancient populations, mainly in Mexico and Africa. *Chlorella vulgaris*, traditionally used as food and alternative medicine in the Far East, has shown to be helpful in cases of gastric ulcers, wounds, constipation, anemia, hypertension, diabetes, and infant malnutrition, while having a similar nutritional and bioactive composition to *Spirulina*, with vast quantities of natural pigments, namely carotenoids [1].

From the environmental perspective, microalgae have shown appreciable potential, between some other reasons, because of their bigger protein yield when compared to soy and livestock, and because, unlike terrestrial agricultures, microalgae don't require arable soil. Although previous studies have resulted in a product with an attractive and innovative appearance and considerably enhanced the nutritional quality of pasta, without affecting its cooking and texture quality properties, with a favorable sensory evaluation [2], the main factor that is setting back the popularization and widespread consumption of microalgae is its inherent taste and smell, that in larger concentrations can present a strong "fishy" odor.

A pre-treatment of the biomass is needed to promote a controlled release of active compounds through a partial cell wall disruption, but different methods of drying will result in distinct levels of bioavailability of such compounds, nutritional composition, sensory profile, and mechanical quality. Another aspect to consider is the cost and efficiency of such methods, owing to the overall energy consumption that these processes require, which in turn may affect the level of sustainability of these products. While solar drying is cheaper and is done in open environments, it takes a longer time, a larger area and is dependent on meteorological conditions. On the other hand, spray drying has a more mainstream use, for it is faster, flexible and can be used in a continuous system while keeping costs relatively low. Its disadvantages stem from high installation costs, the loss of volatile compounds due to higher temperatures, being then unrecommended for ingredients sensitive to heat [3].

This study sets out to investigate the effect of adding *Chlorella vulgaris* and *Arthrospira platensis* obtained by spray-drying and solar drying methods on the technological, nutritional, and bioactive properties of fresh pasta.

After pasta preparation, the samples were characterized in terms of rheology (frequency sweep), texture (TPA, AACC 66-50.01, stickiness and tensile strength), and cooking quality (water absorption, total solid loss, and swelling index) tests to get better understanding of the impact of microalgae addition in the pasta's quality and technological properties. Furthermore, bioactivity tests that aim to obtain the total phenolic content and antioxidant activity (DDPH and FRAP), nutritional analysis (including minerals), and sensory analysis tests were performed.

The results pointed to an optimal 4% level of microalgae in the total composition of the dough, meaning that, at this percentage the technological qualities of the control product are maintained, the reason being that the sizable quantities of protein that both microalgae possess will bridge the gap that's left after the replacement of wheat semolina by microalgae, having impact on the dough and pasta quality properties. This is a positive result, opening the possibility for a widespread consumption of microalgae-based pasta since products as similar as possible to the ones the public is already used to should be an adequate way to convince it to opt for such. Differences in results in respect to the methods of drying have been demonstrated, namely in what concerns the pasta's technological and bioactive properties. The ones resulting from biomass dried using solar drying (**Figure 4**) have a larger bioactivity and different technological properties, i.e., swelling index, water absorption, total solid loss, firmness, and stickiness are lower in solar dried samples when compared to spray dried ones.

Figure 4 *- Pasta with 4% of Arthrospira platensis dried through Solar Drying*

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Microalgae biomass as a relevant source of vitamin B12, in vegetarian and vegan diets

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Vitamins are a vast group of organic compounds, usually accessible through a well balanced diet. With the exception of vitamin D, mammals do not possess the necessary enzyme pool to biosynthesize these compounds. Vitamin B12, generally known as cobalamin (CBL), is an indispensable, hydrophilic, organometallic complex with an extremely convoluted molecular structure. This compound is a cofactor in key enzymatic reactions, it is important in the metabolism of amino acids and fatty acids, the preservation of nerve cells, general cellular production, differentiation, metabolism and proliferation, and most noteworthy, the treatment of pernicious anemia.[1]
B12 undergoes a highly elaborated and modulated absorption and transport mechanism, throughout the body. This promotes the storage of the vitamin and helps to prevent any deficit, even after years of no ingestion.[1] Technically, if the diet includes animal-based food, then CBL will inherently be included and absorbed by the organism. However, in uncompromising vegetarian and/or vegan diets, CBL is commonly absent and may, therefore, lead to vitamin deficiency. This deficit is typically manifested in problems with cell division, in particular bone-marrow and intestinal mucosa cells, but also progressive neuropathy, with symptoms such as numbness, stiffness and general weakness. Some of the vegan and/or vegetarian diets are unable to comply with the daily requirements of CBL for physiological needs.[2] However, the transition from animal to plant-based foods, is necessary, for sustainability reasons, as well as other strong arguments such as ethical and health concerns.
It is becoming increasingly relevant to produce functional, vegan and/or vegetarian-based foods, that may allow strict vegans and/or vegetarians to safely meet the daily requirements of CBL, without having to turn to ordinary vitamin supplementation. The search for these new ingredients, rich in CBL, to be incorporated into functional foods or products, is the main objective of the present work. It started with the determination of CBL in different microalgae products, all from Chlorella vulgaris species, as presented in **Figure 1**: the commercially available Chlorella vulgaris, and the organic, smooth, white and honey variations, all provided by AllMicroalgae – Natural Products, S.A. (https://www.allmicroalgae.com/pt-pt/).

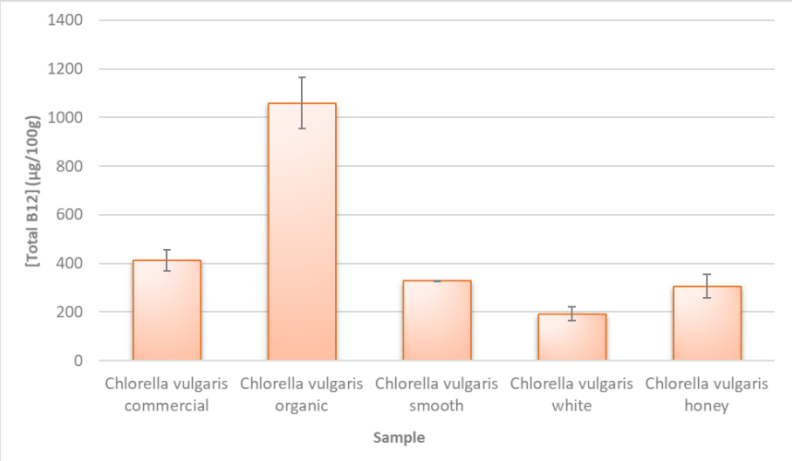

Figure 1: Total B12 concentration in variation of Chlorella vulgaris.

Extraction and analysis of vitamin B12 were carried out according to what is generally established and described in the Association of Official Analytical Chemists' (AOAC) official final action method 2014.02.[3] Purification of the samples, was
accomplished in a procedure using immunoaffinity collumns (Easi-Extract® Vitamin B12 (LGE), R-Biopharm Rhône Ltd., Glasgow), in a manner also described in the AOAC method 2014.02.[3] Analysis of the extracts, was carried out in a Dionex UltiMate 3000 HPLC system, with a solvent rack with a 4-channel degasser SRD-3400, a quaternary gradient pump LPG-3400SD, an analytical auto-sampler WPS-3000SL, a column compartment TCC-3000SD and a UV

spectrophotometric detector DAD-3000, with isocratic elution using acetonitrile and trifluoroacetic acid 0.025% (15:85) as mobile phase and at 0.25 mL/min flow rate.

In the present study, the organic Chlorella vulgaris, produced by an autotrophic process, that is, a photosynthetic organism, displayed the highest levels of CNCBL, slightly above 1000 μg/100g of sample. One must underline the fact that these values

represent total B12 concentration, including the readily bioavailable forms by origin and the forms that were bound to proteins in the matrix, and therefore, not bioavailable. The remaining Chlorella variations, namely Smooth and Honey, obtained by a heterotrophic process, namely in one organic medium, produced by a controlled chemical mutagenesis, in order to inhibit chlorophyll production, displayed values of total B12 similar to the commercially available Chlorella, that is, around 300-400 μg/100g of sample. The White variant displayed the lowest total B12 levels, around 200 μg/100g of sample. A possible explanation for this discrepancy, is the fact that the former doesn't undergo chemical mutagenesis, whilst the latter variants do.

Some microalgae species have been present in the diet of the average adult, ever since 1960, when they began being cultivated, marketed and globally commercialized, despite the fact that, in Asian countries, they had been part of the diet from ancient times. Subsequently, as is the case of Chlorella vulgaris, for example, they are no longer restricted by any regulations as to their application in food products. According to the European Food Safety Authority (EFSA), the recommended daily allowance (RDA) is about 4 μg per day, for the average adult. The AOAC method 2014.02 determines the total amount of B12, including the biologically active and inactive forms, by converting all into CNCBL. Some portion of the total B12 of microalgae, represent biologically inactive species, and therefore, not bioavailable or accessible for physiological requirements. In the case of Chlorella, research points towards most of the existing CBL being biologically active, which means that around 400 mg of organic Chlorella vulgaris would be enough to comply with the total RDA.

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Revalorization of *Prunus avium* L.: Determination of bioactive compounds

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Throughout history, human beings fought for their survival and wellbeing, where wild fruits played an important role, since they allowed mankind to have a varied diet with an important contribution of vitamins, mineral and bioactive compounds. Nevertheless, the development of industrialization and commercial agriculture led to a progressive decrease in their consumption and finally to the underestimation of their value[1]. On the other hand, nowadays the human beings are more aware of the importance and influence of diet on their health, this has led to an increase in the demand of healthier foods, therefore, the customer prefers products made with natural ingredients[2].
In the present work, anthocyanins content, as well as other phenolic compounds was quantified in the fruit of *Prunus avium* L., in order to determine its possible use in the context of a healthy diet. Specifically, individual anthocyanin profile (HPLC-DAD-MS) and total anthocyanin content (TAC; pH differential method), as well as total phenolic compound (TPC; Fast blue BB method), hydroxybenzoic acids (HBA), hydroxycinnamic acids (HCA) and flavonoids content (FC)[3] were determined. All methods were carried out through QUENCHER methodology, in which the homogenized sample is summited to direct contact with the respective reagents of each determination, this process allows avoiding the extraction steps, therefore, it is possible to measure the soluble and insoluble compounds, obtaining more reliable results. *Prunus avium* L. wild fruit were collected in Horcajuelo de la Sierra, Madrid, according to PN-NC_032021 National and Ref. ABSCH-IRCC-ES-257749-1 permission (Spanish Ministry of Agriculture, Fisheries and Food).
The fruit of *P. avium* presented a total of 361,9 mg GAE/100g of phenolic compounds, this value was higher than those previously reported by other authors (35,6 – 65,85 mg GAE/100g). In this work was obtained a total phenolic acids of 206,73 mg GAE/100g, where the value of hydroxybenzoic acids was almost double the value of hydroxycinnamic acids. Regarding to the flavonol content, an amount of 21,95 mg QE/100g was obtained. The total anthocyanin content achieved (2,96 mg cyanidin-3-glucoside/100g) agreed with the values previously reported (1.15 – 16.2 mg cyanidin-3-glucoside/100g). Regarding the individual anthocyanins profile, cyanidin-O-hexosyl-pentoside was the main anthocyanin compound found in this wild fruit.
It is known that the concentration of phenolic compounds may vary depending on different factor, such as soil, cultivar method, altitude, water, among others. However, the higher values obtained in this work with respect to those previously reported, may be due to the use of QUENCHER methodology.
Through this work it can be evidence that *P. avium* contains interesting amounts of these compounds, therefore, it can be a great option as a functional ingredient which will allow to achieve and maintain a healthier diet.

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Evaluation of microalgae enriched gluten-free bread as functional food

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Microalgae are innovative and sustainable ingredients, nutritionally rich in vitamins, proteins, carbohydrates, minerals (such as iron, potassium, magnesium, and zinc), lipids, polyunsaturated fatty acids (PUFA), antioxidants, fibers, pigments and antimicrobial agents. Additionally, they have already been shown to produce a wide spectrum of biologically active secondary metabolites, making them excellent elements to be incorporated in inherently low nutritional value products, such as pasta, biscuits, and gluten-free (GF) breads.

The present study is part of the Algae To Future (A2F) project, funded by the Research Council of Norway (https://www.algae2future.no), and the exploratory project AlgaeHealthyBread from LEAF, aiming to explore the potential of microalgae as new ingredients in both human and animal food.

The addition of *Tetraselmis chuii* (Tc), *Chlorella vulgaris* (Cv), and *Nannochloropsis gaditana* (Ng) algae biomass was tested to produce GF bread, with a formulationpreviously optimized [1–3]. These newly prepared gluten-free doughs led to a significant increase in proteins, lipids, minerals (Ca, Mg, K, P, S, Fe, Cu, Zn, Mn) and antioxidant properties, although compromising rheological and sensory properties of the GF breads[5]. Subsequent bleaching of microalgae biomass with ethanol was performed to address de sensory issues and both raw (and the corresponding treated (T) biomasses.

These highly nutritious breads with improved technological and sensory characteristics were evaluated concerning their bioactive properties in terms of developing health benefits for the consumers (Figure 1): antioxidant properties, total phenolic (a) and total flavonoid contents (b) and α-amylase inhibition ability (c).

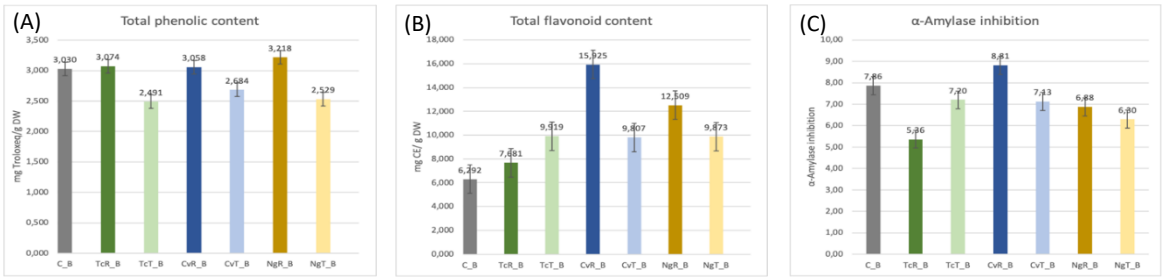

Figure 5 - Bioactive properties [A - Total penolic content; B – Total flavonoid content; C- α-Amylase inhibition] of different breads enriched with microalgae [*Tetraselmis chuii* raw (TcR_B), *Tetraselmis chuii* ethanol-treated (TcT_B), *Chlorella vulgaris* raw (CvR_B), *Chlorella vulgaris* elthanol-treated (CvT_B), *Nannochloropsis gaditana* raw (NgR_B) and *Nannochloropsis gaditana* elthanol-treated (NgT_B). One control (plain GF bread) was set up, called Control (C_B).]

Phenolic content was higher in bread enriched with microalgae, especially with *N. gaditana.* A considerable enhancement of flavonoid content was accomplished when introducing the microalgae in GF bread formulations, namely with *C. vulgaris* (Cv), and *N. gaditana* (Ng). The ethanolic treatment promoted a slight decrease of both phenolic and flavonoid contents. GF bread enriched with *C. vulgaris* showed α-amylase inhibition ability (almost 90%, when compared to the control bread).

The nutritional value in terms of protein of the prepared GF bread was evaluated before and after an *in vitro* static digestion method the protein content was around 85% higher in bread where microalgae were incorporated, but it deceased almost 50% in all GF bread after digestion. Also, a considerable loss of minerals after digestion was observed.

Overall, the microalgae bread incorporation represented a relevant strategy for adding nutritional and healthy benefits for the consumers.

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Crop rotation and irrigation regime affects the nutritional and chemical profile of *Cichorium spinosum*.

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The sustainable management of agricultural systems offers synergistic opportunities for the co-production of agricultural and natural capital outcomes.[1] A properly sized agricultural system is essential for the sustainable and ecological maintenance of crop productivity. Irrigation management is an important adaptation strategy to improve crop resilience to global climate change while crop rotation brings benefits such as increased crop yields through high soil fertility and reduced fertilizer inputs.[2] *Cichorium spinosum* L. (spiny chicory) is a wild edible plant that has received very recent attention as a potential alternative/complementary crop. It is a plant traditionally consumed in the so-called Mediterranean diet due to its high nutritional value and various beneficial health effects.[3] The study aims to improve and integrate the cultivation of this species in farming systems of the Mediterranean region. Thus, a combination of full or deficit irrigation with or without crop rotation with maize was established in an attempt to establish the commercial cultivation of spiny chicory. Two control samples were cultivated: C0 (rain-feed with crop rotation with maize) and C00 (rain-feed without crop rotation). The nutritional profile was evaluated using AOAC methods. Energy was calculated according to the equation: energy (kcal per 100 g) = 4 x (g protein + g carbohydrate) + 2 x (g total dietary fiber) + 9 x (g fat). The profile of organic acids, minerals, fatty acids and sugars were performed using UFLC-PDA, atomic absorption spectrophotometry, GC-FID and HPLC-RI, respectively. Although the impacts that a sustainable farming system generates on the crop involved is a long-term assessment and after the system has been repeated for several growing periods, however some changes are already noticeable in the first growing period. In the nutritional profile, there were no differences between the six experimental treatments, with the exception of the total dietary fiber content which samples C0 (control) and CFIC (full irrigation with crop rotation with maize) showed the highest levels. The samples presented low values of total fat, being the sample C0 the one that presented the highest value (3.5 g/100g dry weight). Promising levels of crude protein were indicated by all samples, however once again the control sample had the highest content (C00). The CFIC and CFIN samples (full irrigation with and without crop rotation, respectively) showed the lowest values of carbohydrates. The sample CDIC (deficit irrigation with crop rotation with maize) showed the highest energy (276.3 kcal/100g dry weight) probably due to the low fiber content and consequently the high carbohydrate content. Five organic acids were identified in the spiny chicory samples, mostly quinic acid, except in the CFIN sample in which oxalic acid had the highest concentration. In terms of minerals, the samples with full irrigation showed higher concentrations of iron, manganese and copper and lower calcium, while the samples without crop rotation showed lower concentrations of potassium. The predominant fatty acids identified and quantified were linolenic, linoleic, and palmitic acids, the sum of which represented 82 to 86% in the studied samples, while the sample with deficit irrigation and without crop rotation (CDIN) presented the lowest percentage. Finally, the sugars identified in higher concentrations were sucrose, glucose, fructose, and trehalose, respectively, however, it is suggested that crop rotation with maize altered the profile of sugars by increasing their concentrations. Considering that these are preliminary results, it was possible to point out positive impacts of the tested agronomic practices on nutritional parameters of the species that could be commercially applied aiming to integrate wild edible species in sustainable and low inputs farming systems.

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Influence of phytochemical composition and biological activity of Portuguese honeys from different botanical sources and geographical origins

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Honey is a saturated sugar solution that has been used as a only available natural sweetener produced by *Apis mellifera* bees from secretions of living parts of plants or excretions of plant-nectar.[1,2] This product has been recognized not only for a food or sweetener, it was also associated for its therapeutic purposes and health-promoting effects. These effects are dependent from its composition, being mainly justified by the presence of phenolic compounds that are associated to biological activity, including antioxidant and antimicrobial capacities.[3] The content and the profile of phenolic compounds are directly influenced, and may vary according to the botanical and geographical sources of the nectar collected by the honeybee.[2]

Oxidative stress in a given organism can occur when there is an imbalance between the production of free radicals and the antioxidant activity. Considering this stress and the different diseases associated with it, the study of antioxidant capacity has increasingly aroused the interest of the scientific community.[1] Antioxidant potential is affected by many constituents of honey, mainly phenolic compounds.[2]

Antimicrobial activity is a physiological effect associated to honey, turning this food matrix a promising solution as a natural antimicrobial agent against antimicrobial resistance.[2,3]

This study was proposed to determine, quantify, and identify the content in total phenols, *ortho*-diphenols, and flavonoids, as well as antioxidant capacity and antimicrobial activity in honey originated from different floral sources of different regions of Portugal and find a correlation between these parameters.

The content in phenolic compounds were evaluated according to several colorimetric assays, as well as antioxidant property including radical scavenging ability (ABTS, DPPH) and ferric reducing power (FRAP), and antimicrobial capacity by disc diffusion method in these honey samples. Furthermore, the Reverse Phase - High Performance Liquid Chromatography - Diode Array Detector (RP-HPLC-DAD) was performed to characterize the polyphenolic profile of honey samples to reveal the relationship between the phenolics and the biological activities previously mentioned.

The content in phenolic compounds in this study revealed extremely favourable findings, ranging from 20.82 to 112.13 mg of Gallic Acid/100 g of sample for the total phenol content, from 10.25 to 103.26 mg of Gallic Acid/ 100 g of sample for *ortho*-diphenols, and from 2.94 to 40.96 mg of Catechin/ 100 g of sample for flavonoids content. It was also verified significant results for antioxidant capacities (0.06 to 2.27 mmol Trolox/ 100 g, 0.04 to 0.45 mmol Trolox/100 g, and 0.05 to 0.69 mmol Trolox/ 100 g, for ABTS, DPPH, and FRAP, respectively), as well as significant differences between honey samples concerning the antimicrobial activity.

This study contributes to the valorization of honey in economy and/or industrial interests regarding the possible applications of these components in the food, cosmetic, and pharmaceutical industries, being this natural sweetener a product with several salient therapeutic properties.

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Exploring the technological potential of *Salicornia ramosissima* as a mineral accumulator

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Salicornia ramosissima is an extremophile species that integrates the Portuguese native flora and stands out for its remarkable ability to withstand extreme salinity levels (above 500 mM). It is an annual herbaceous plant that reaches about 30 cm height, and is characterised by its articulated and fleshy stems with peculiarly scale-shaped leaves, that exhibit an intense green colour during spring and summer and a reddish tone in autumn. As an obligatory halophyte, it thrives abundantly in saline environments, namely in salt marshes, in sandy-muddy soils, integrating low marsh halophytic shrub communities. To survive and multiply in this environment, *S. ramosissima* resorts to a set of mechanisms to tolerate osmotic, ionic and oxidative stress. As to saline stress resistance, one of its most important defence strategies involves the accumulation and compartmentalisation of ions in cell vacuoles in its aerial parts. Hence, knowing the mineral accumulation pattern of *S. ramosissima* can be especially interesting for its technological valorisation. [1] Thus, the present research aims to characterise the nutritional and mineral profile of the aerial parts of the wild *S. ramosissima* from the Mondego estuary (Portugal). For the analysis of the proximate composition, the AOAC procedures were followed, and a swift and a for the first time proposed "green" method was adopted for the analysis of mineral constituents. The said “green” method is based on water sonication extraction followed by potentiometric or photometric analysis, depending on the mineral, and displays excellent linearity (r^2> 0.999), good recoveries (80.46 – 108.29%) and repeatability (0.71 – 4.81%). Furthermore, the calculated values of the quantification limits (LOQs) attest to its high sensitivity (Na: 13.12 mmol/L; K: 0.64 mmol/L; Cl: 17.48 mmol/L; Fe: 15.50 µg/dL; Ca: 0.15 mmol/L; P: 0.13 mmol/L; and Mg: 0.75 mg/dL). On the whole, the combination of the ultrasound-assisted extraction followed by potentiometric/photometric determination proved to be valid and highly advantageous, mainly owing to its swiftness and consistency with the principles of “sustainable chemistry”.

Regarding the nutritional value of *S. ramosissima*, it presented low levels of lipids (0.45%) and protein (4.16%), important levels of fibre (10.36%) and especially minerals (47.38%). In view of these results, *S. ramosissima* shows great potential as a functional ingredient, in both food products and feed. Particularly, a possible field of application of high interest in terms of public health should be dietary reformulation strategies aiming at a reduction in salt consumption, an ingredient associated with an increased risk of cardiovascular diseases development. In fact, owing to its important content of minerals contributing to the salty taste, such as Mg, K and Ca, the addition of *Salicornia* should bring about a significant reduction in Na employment. Furthermore, it should be noted that the obtained results refer to wild *Salicornia* and that, by adopting a cultivation strategy, its nutritional and mineral profile can be improved in accordance with the intended application by adjusting the growth conditions.

As a final remark, it is essential to make a more efficient and sustainable use of the natural resources that the planet offers us, not wasting high-quality nutritional sources such as *Salicornia.* Soil salinisation imposes already a constraint on Portugal's agricultural potential, an issue which is only getting worse, wherefore the implementation of strategies that allow the recovery and enhancement of naturally or human-induced salinised areas is fundamental. [1] The cultivation of halophytes such as *Salicornia* is a strategy that has produced excellent results in several countries and needs to be seriously considered in Portugal.

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Effect of ultrasonic treatment on the physicochemical, bio-functional properties and digestibility of chayote (*Sechium edule (Jacq.) Swartz)* seed protein isolates

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Chayote (*Sechium edule* (Jacq.) Swartz) has gained widespread consuming acceptance and recognized by its nutritional and bioactive properties. This Cucurbitacea plant grows in tropical, subtropical, and warm regions around the world and is used in the food industry to produce purees, juices, jams, and alcoholic beverages. Chayote seeds contains approximately 5.50 % dw of protein content, mainly composed by essential amino acids, which indicates a good protein quality.[1] Preparations of plant protein isolates from different sources have been extensively studied; they are conventionally produced by alkaline or acid extraction/precipitation methods. However, extreme extraction conditions such as high temperature or high alkaline conditions could reduce the nutritive value of protein and may cause degradation of bioactive compounds. Hence, ultrasound as emerged as a promising environmental-friendly technology to increase the extraction efficiency of proteins and bioactive substances from plant, animal, and marine sources.

In this work, chayote seed protein isolates (CSPIs) were prepared by alkaline extraction (AE)[2] and ultrasonic-assisted extraction (UAE) using ultrasound probe (20 kHz) and ultrasound bath (40 kHz), and their physicochemical, functional properties and nutraceutical potential were investigated. For all treatments, protein solutions (10% w/v) were treated for 20 minutes. An ultrasonic processor (Sonic Vibracell, model VC 750, Newtown, CT, USA), comprising a 13 mm diameter tip, was used for probe sonication, while an ultrasonic bath (Selecta SA Barcelona, Spain) was used for the 40 kHz-ultrasound treatment. The protein content of chayote seed powder (CSp) and isolates (CSPIs) were determined according to modified Kjeldahl procedure (AoAC, 2000).[3]

The Size exclusion chromatography (SE-HPLC) profile of the CSPIs showed a significant reduction in the molecular weight and was similar for the three treatments (**Figure 1**). The sulfhydryl content increased after all treatments, indicating partial unfolding of proteins and reduction in the intermolecular interactions. The application of UAE significantly (p <0.05) improved the protein extraction yield and functional properties (protein solubility, turbidity, and emulsifying and foaming properties) of CSPI. This effect was more pronounced in probe sonication (20 kHz) rather than bath sonication (40 kHz). Also, the CSPI obtained by UAE-20 kHz contained 35.4% of proteins with a balanced amino acid profile, higher content of essential amino acids (344.6 – 387.0 mg/g of protein) and higher protein digestibility (81.52 – 91.58%). Besides, CSPI obtained by UAE-20 kHz exhibited higher phenolic content (8.25 mg GAE/g dw), higher antioxidant capacity, as measured by ABTS radical scavenging capacity assay, and higher anti-diabetic activity, (α-glucosidase inhibition of 74%, at 100 μg/mL) suggesting its potential as nutraceutical.

This study provided the first insight into the structural, functional, antioxidant, and antidiabetic potential of the unexplored chayote seeds. Overall, the results suggest that ultrasound technology contributed greatly to the corresponding bio-functional and nutritional properties of the chayote seed protein. Therefore, ultrasound is an effective technology that could be used in the food industry to valorize the bio-functional properties of chayote seed proteins.

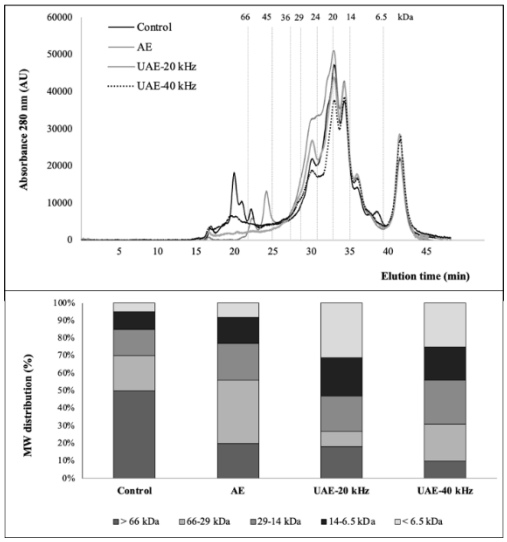

Figure 1: Size exclusion chromatography (SE-HPL) profiles of chayote seed protein isolates (CSPIs) under different treatment conditions: alkaline extraction (AE) and ultrasonic-assisted extraction using ultrasound probe (UAE-20 kHz) or ultrasound bath (UAE-40 kHz).

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Development of a clean label mayonnaise using fruit flour

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In the past few years there has been an exponential increase of people following plant-based diets, with the number of individuals identifying themselves as vegan rising 600% in the US between 2014 and 2017, and quadrupling in the UK between 2014 and 2018.[1] Another food trend that has been rising in the past decade is clean label food, meaning that consumers are searching for shorter and simpler ingredient lists, composed of familiar ingredients, and that are minimally processed.[2] Hence, the consumer's increasing demand for more natural, healthier, nutritionally complete, and sustainable foods led to the need for reformulation of foods like mayonnaise.

This work is part of the cLabel+ project and was developed with Casa Mendes Gonçalves. The main goals are the development of vegan mayonnaise using vegetable proteins as natural emulsifiers, but also the incorporation of fruit flour to achieve a clean label product. Therefore, ingredients like sugar, colorants, and preservatives are replaced by different fruit flours, namely apple, pear, nectarine, and peach flour, and its impact is evaluated regarding structure, bioactivity, and sensory profile.

Fruit flours, obtained from fruit by-products, are regarded as clean-label ingredients which provide health benefits because of their fiber and antioxidant content while contributing to a circularity in the supply chain by using resources that are available and are underutilized. Through use of such ingredients, it is possible to incorporate bioactive compounds like carotenoids and phenolic acids in the new mayonnaises therefore achieving adequate stability and shelf-life in the final products, while enhancing the product's color, increasing potential health benefits and improving its commercial appeal.[3] Texture enhancing capacity is due to its dietary fiber content, which impacts the water holding and retention capacity, oil holding capacity, foam capacity and stability. Adding fruit flour to liquid or semi-solid food products will improve their viscosity, even when added in small amounts.

The mayonnaises were prepared according to Casa Mendes Gonçalves' procedures with some modifications: replacement of egg by 1.5% (w/w) of lupin and faba proteins, and the fruit flour was incorporated to substitute the ingredients mentioned above. Texture (TPA), and rheology (SAOS) measurements were performed to evaluate the impact of the fruit flour on mechanical properties. The bioactivity was analysed through the determination of antioxidant capacity (FRAP and DPPH assays) and total phenolic compounds content. Color and pH of mayonnaises were also assessed.

Results showed that mayonnaises produced with the incorporation of fruit flour had better structure parameters and bioactivity compared to the control mayonnaise (mayonnaise without fruit flour). The incorporation of different fruit flours into mayonnaise increases the antioxidant potential, even though there is a loss of the antioxidant effect caused by the complex matrix. Through the results obtained by FRAP analyses (**Figure 1**), it is possible to conclude that the flour with the highest antioxidant potential is pear flour, 106.13 mg/100g, and the one with the lowest values are the ingredients found in control mayonnaise (sugar, potassium sorbate and colorant) with 3.62 mg/100g. Comparing all mayonnaises, the one with the greatest antioxidant effect is pear mayonnaise and the lowest is standard mayonnaise with values of 12.06 mg/100g and 5.5 mg/100g, respectively. Pear flour is significantly distinct ($p < 0.05$) from all flours, but mayonnaises with the addition of fruit flour are all similar ($p > 0.05$). Compared to the standard, these mayonnaises didn't show naked-eye differences in color ($\Delta E^* < 5$) and pH value is closer to the target than in the control mayonnaise. Nectarine flour showed the most promising results in terms of texture and bioactivity.

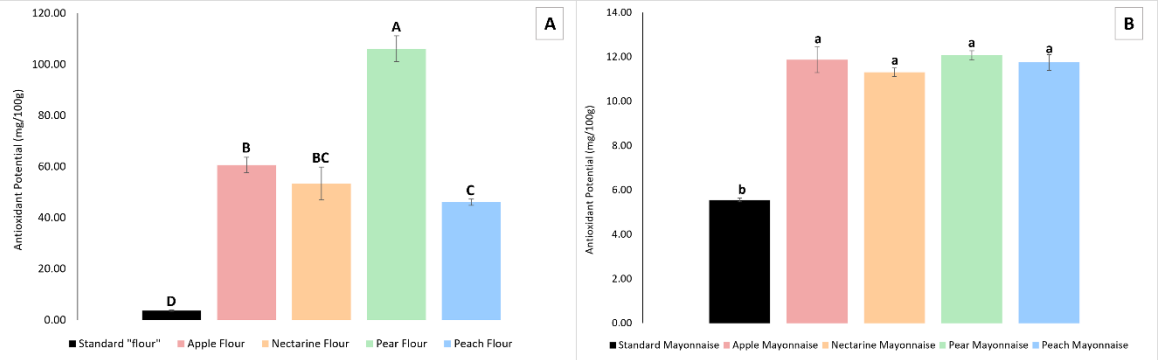

Figure 1: Antioxidant potential based on FRAP analyses from fruit flours and standard "flour" (sugar, potassium sorbate and colorant) (A) and mayonnaises (B).

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In vitro and *in vivo* antioxidant activity of 3D snacks enriched with different microalgae species

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Most commercially available natural antioxidants are derived from terrestrial plants. In the last decades, microalgae have been shown to be a promising potential as an alternative source of antioxidants. Several reports have been focused on the enrichment of food products with microalgae to evaluate their impact on fighting against reactive oxygen species (ROS). The most commonly used assays to evaluate the antioxidant activity of microalgae-enriched foods are *in vitro* assays. Overall, the results are very heterogeneous depending on the species of microalgae studied and the tests used to measure antioxidant activity. Protocols of these assays vary from one study to another, notably in terms of the extraction method, the solvents used, the reaction time and the concentrations tested. Yeast *Saccharomyces cerevisiae* is an appropriate model organism for fundamental eukaryotic cellular processes such as cell antioxidative activity related to bioactive compounds. *S. cerevisiae* cells in the stationary phase resemble cells of multicellular organisms namely in terms of cellular damage accumulation over time and presence of a defense mechanism as higher eukaryotes.

In the present work, 3D printed snacks were enriched with a 10 % level of incorporation of four different species of microalgae (*Chlorella vulgaris*, *Nannochloropsis gaditana*, *Haematococcus pluvialis* and *Phaeodactylum tricornutum*) and one cyanobacteria (*Arthrospira platensis*) – commonly known as "spirulina" and reported as a microalgae species in the present work – based on a previously optimized recipe[1]. Antioxidant activity of snacks was investigated based on both *in vivo* and *in vitro* methods.

The aim of this study was to analyse the antioxidant potential of 3D snacks extracts enriched with different microalgae species, based on *in vitro* and *in vivo* assays (**Figure 1**). Analyses were carried out on baked snacks and crushed into powder.

The *in vivo* antioxidant activity was performed according to Niccolai et al. (2020).[2] Extracts were prepared by adding 5 g of powdered snacks to 50 mL of sterilized water. The extraction was performed by sonicating samples (MicrosonTM XL2000, Misonix Inc., Farmingdale, New York, USA), for 10 min, at the maximum power (frequency 20 kHz, power 130 W). After sonication supernatants were recovered by centrifugation (13000 rpm, 15 min) and extract concentrations were set at 15 mg/mL. *In vivo* antioxidant activity was measured as intracellular oxidation of yeast cells of *S. cerevisiae*, used as a model organism. Yeast biomass was obtained from the Culture Collection of Industrial Microorganisms (University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia). The cultivation of *S. cerevisiae* was carried out in YEPD medium at 28 °C and 220 rpm until the stationary phase was achieved. At the stationary phase, yeast cells were then incubated with snacks extracts at 220 rpm, for 2 h, at 28 °C. Intercellular oxidation was determined by using 2',7'-dichlorofluorescein (H2DCF) which reacts with oxidants, thus revealing the presence of reactive oxygen species (ROS). Results were expressed as means of the relative fluorescence intensity.

The *in vitro* antioxidant activity was investigated through the radical scavenging activity (RSA) of extracts, based on the 2,2-dyphenyl-1-picrylhydrazyl (DPPH) assay, described by Rajauria et al. (2013). Results were expressed in RSA (%). Additionally, to the DPPH assay total phenolic compounds (TPC) were also determined, according to Ganesan et al. (2008), based on the Folin Ciocalteu assay. Results were expressed in gallic acid equivalents (mg GAE/ g snack).

Results showed that snacks enriched with microalgae presented higher antioxidant activities than control snack. Snacks enriched with *H. pluvialis* presented the highest antioxidant activity, for both *in vivo* and *in vitro*, as well as the highest content in phenolic compounds. Overall, results indicate differences in antioxidant activity of snacks between *in vitro* and *in vivo* studies. However, results obtained from TPC presented a high correlation (0.855) with i*n vivo* studies. Previous studies showed that intracellular oxidation is highly dependent on the individual molecular structure of bioactive compounds present in extracts.[3]

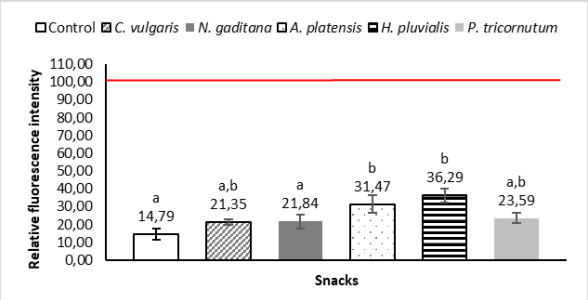

Figure 1: *In vivo* antioxidant activity of snacks.

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Development of low-fat vegan emulsions with the incorporation of citrus fiber

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Currently, all over the world, consumers are getting increasingly concerned about the quality of the food they eat and the health consequences that may arise from its consumption.

The rising concern with the transparency and the origin of food has turned consumer's attention to labels. However, the use of additives of artificial origin or whose meaning is unknown to most consumers makes it difficult to understand labels and often results in purchase inhibition. For this reason, trends in the food industry have gained great relevance, leading the industry to reinvent their products and look for clean label solutions for their products, making them low-fat, vegan, with fewer additives and with increasingly natural and recognizable ingredients.

Mayonnaise is often cited for health-related issues due to its high content of cholesterol and fat[1]. It is in this field of action that the development of this product arises. Mainly from the need to reduce the amount of fat in mayonnaise and to transform it into a healthier product, a 3% oil, totally vegan product was developed with citrus fiber as a fat substitute. In this emulsion, fibers are used as a clean label alternative to additives, such as hydrocolloids, usually used to impart viscosity[2].

This work is part of the cLabel+ project, PO CI/ANI/ 46080/2019: Innovative natural, nutritious and consumer-oriented clean label foods, and results from the collaboration with Casa Mendes Gonçalves. To fulfil the proposed goals, different amounts of vegetable protein (a mixture of fava bean protein with lupine protein) and citrus fiber were used to stabilize low-fat systems. The emulsions were compared to the company's commercial emulsion (with 25% oil), used as standard.

To test the influence of protein and fiber interaction in the emulsions, an RSM (Response Surface Methodology) was performed, with protein and fiber content as independent variables. Elastic Modulus (G') at 1Hz, Plateau Modulus (G^0_N), pH, firmness, adhesiveness and cohesiveness were the parameters selected as dependent variables. This experimental design provided 12 test conditions, with protein concentrations varying between 0 and 12g, and fiber concentrations varying between 0 and 7g. In Figure 1 it is possible to evaluate the appearance of the mayonnaise obtained under specific experimental conditions.

Of the 12 samples under study, three remained liquid and demonstrated a clear separation between phases (RSM 1, 3 and 7), four produced a product of thick and pasty consistency (RSM 5, 4, 2 and 8), and five were close to the conventional characteristics of a typical mayonnaise (RSM 6, 9, 10, 11 and 12).

Regarding sensory properties, all samples presented a sandy texture, with the samples: RSM 6, 9, 10, 11 and 12 presenting this characteristic to a lesser extent. Despite this, RSM 6 has a sandier texture compared to the others, a characteristic considered inappropriate for this type of product.

Through the study of the responses of the dependent variables (rheology parameters and texture profile analysis), it was possible to perceive that the protein does not have a significant influence on the quality of the emulsion, unlike the fiber, which showed to be preponderant in emulsion formation and stability.

a)

	Decoded Matrix	
	Protein	**Fiber**
RSM 1	1.76	0.43
RSM 2	1.76	6.57
RSM 3	10.24	0.43
RSM 4	10.24	6.57
RSM 5	0.00	3.50
RSM 6	12.00	3.50
RSM 7	6.00	0.00
RSM 8	6.00	7.00
RSM 9	6.00	3.50
RSM 10	6.00	3.50
RSM 11	6.00	3.50
RSM 12	6.00	3.50

b)

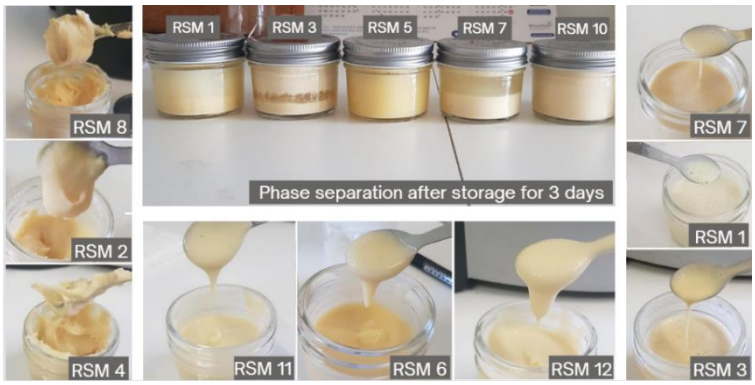

Figure 6: a) Decoded Matrix. b) Visual aspect of the experiments made for RSM. On the left, the mayonnaises with a pasty consistency. In the center, above, the clear separation between the phases of some samples after 3 days of storage. In the center, below, the samples most similar to conventional mayonnaise. On the right, samples with liquid consistency.

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Chemical profile and nutraceutical features of cape gooseberry fruit and fruiting calyx

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The Solanaceae *Physalis peruviana* L. is a South American native plant commonly known as cape gooseberry. Widely spread in the last century, today it is cultivated or grows wild across the world in tropical, subtropical and even temperate regions. A notable feature of this species is the formation of a large, papery husk derived from the calyx, which covers the fruit. The cape gooseberry fruit is mainly consumed fresh but can be transformed into derived products such as jam, sauces, syrups, marmalades, snacks, and beverages.[1] It is also used in folk medicine for the treatment of some diseases and health conditions, as well as its fruiting calyx.[1] Thus, given the increasing use and popularity of this fruit, it is important to go further in its nutritional and phytochemical composition, as well as its bioactive properties. While there is more information about the fruit than about its calyx, this is an important by-product of cape gooseberry processing that deserves further investigation as a source of bioactive compounds.[2]
This work aimed to characterize the chemical composition and nutraceutical properties of cape gooseberry fruit and its calyx. The fruit was characterized for its proximate composition (moisture, fat, protein, ash, and total dietary fiber; available carbohydrates were calculated by difference) following official methods of food analysis and free sugars, organic acids, fatty acids, and tocopherols were quantified by different chromatographic techniques. Hydroethanolic extracts of fruit and calyx were then prepared by solid/liquid extraction and used to characterize the phenolic profiles by the HPLC-DAD-ESI/MS^n technique. Both extracts were also used to evaluate the *in vitro* antioxidant activity, through cell-based lipid peroxidation and oxidative hemolysis inhibition assays, and antimicrobial effects against foodborne bacteria and fungi, using serial microdilution methods.[3]
The nutritional analysis revealed that the cape gooseberry fruit contains high levels of reducing sugars and dietary fiber. The fat content was relatively low and composed mostly of polyunsaturated fatty acids. Interesting amounts of tocopherols and ascorbic acid were also detected, thus highlighting the nutritional value of this fruit. Regarding the phenolic composition, caffeoyl-hexoside and apigenin derivatives were identified in the fruit extract, while phenolic acids and quercetin-type flavonoids predominated in the calyx extract. The latter showed greater capacity to inhibit lipid peroxidation and oxidative hemolysis than the fruit extract, and it was also more effective against most of the tested foodborne microorganisms. Overall, cape gooseberry proved to be an exotic functional fruit with a balanced nutritional profile and high antioxidant activity. In turn, its fruiting calyx showed potential to be upcycled into bioactive ingredients for food and nutraceutical application, among others.

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Nutritional and bioactive traits of Kweli® red raspberry cultivated in Portugal

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Red raspberry (*Rubus idaeus* L.) is an increasingly popular food in contemporary diets due to its freshness, organoleptic features, nutritional value, and health claims. From the nutritional point of view, red raspberry has been described as containing vitamins, minerals, soluble fiber, reducing sugars, citric acid, and phenolic compounds.[1] Anthocyanins are of particular interest in this fruit, since these pigments provide the characteristic red-purple color, as well as bioactive properties.[1,2] The demand for raspberries has risen sharply in Europe and North America and, among the existing cultivars, Kweli® is one of the most productive and suitable for growing in moderate and Mediterranean climates. High levels of ellagitannins, anthocyanins, and vitamin C have been described in this cultivar and its phytochemical content has been correlated with antioxidant properties.[1,2] Still, little else is known about the nutritional composition of Kweli®. Therefore, this work was performed to characterize the nutritional and chemical composition of this red raspberry cultivar gown in northern Portugal and assess its *in vitro* antioxidant and antimicrobial activities. Fresh fruits at commercial maturity were harvested and immediately lyophilized. Its proximate composition was determined by official method of food analysis and the concentrations of free sugars, organic acids, tocopherols, and fatty acids were evaluated by different chromatographic techniques.[3] Anthocyanins were identified in an hydroethanolic extract prepared by solid-liquid extraction, which was also used to evaluate bioactive properties.[3] The antioxidant activity was evaluated *via* inhibition of β-carotene bleaching, formation of thiobarbituric acid reactive substances (TBARS), and oxidative hemolysis.[3] The antimicrobial activity was tested against foodborne bacterial and fungal strains by microdilution methods.[3] Moisture (approximately 83%) and carbohydrates (16.12%), of which fructose (2.42%) and glucose (2.13%), were major constituents of Kweli® red raspberry, followed by ash (0.66%) and protein (0.18%). The fat content was quite low and consisted mainly by unsaturated fatty acids (58%), with a prevalence of oleic acid. High levels of citric (2.7%) and ascorbic (17 mg/100 g) acids and tocopherols (1.92 mg/100 g) were also detected. The anthocyanins (4.51 mg/g extract) cyanidin-*O*-hexoside and mostly cyanidin-*O*-sophoroside were identified in the hydroethanolic extract, which was able to inhibit in some extent the formation of TBARS, oxidative hemolysis, and β-carotene bleaching. In turn, the extract was more effective than the food additive E224 against *Bacillus cereus*. Overall, these results highlighted the nutritional quality of Kweli® red raspberry and may be useful to complete food composition databases. Therefore, the inclusion of this berry in a daily diet can be helpful to obtain nutrients and antioxidants and bring health benefits.

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Hypocholesterolemic functional food based in polysaccharides: from structure to function

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Polysaccharides from different sources have been related with hypocholesterolemic potential. Several cholesterol lowering mechanisms have been identified, namely a) sequestration of bile salts at intestinal lumen decreasing cholesterol bioaccessibility, b) increase viscosity at intestinal lumen limiting diffusion of bile salts micelles containing cholesterol and its delivery to intestinal epithelium; c) affect short chain fatty acid production by polysaccharide microbiota fermentation, influencing cholesterol endogenous production at liver.[1] In order to develop optimized hypocholesterolemic food ingredients, a better understanding of structure-function relationships of polysaccharides must be addressed. Soluble polysaccharides in hydrophilic matrices, may be particularly relevant because they can be taken after the major meals of the day, where a higher content of cholesterol is present in intestinal lumen (either from diet or discharged from the gallbladder), being the optimum intake time to be most effective and may broader the current functional food offer (mainly focused in dairy products and cereals). In this work, polysaccharides from several food sources (e.g. coffee, algae, and mushrooms) and by-products (e.g. apple pomace, and crustaceans exoskeleton) with hydrophilic character were studied to evaluate their effect on cholesterol bioaccessibility. The hypocholesterolemic effect was evaluated using an *in vitro* intestinal model containing bile salt (glycodeoxycholic acid, GDCA) and cholesterol, allowing to determine bile salt sequestration by the different polysaccharides and the decrease of cholesterol solubility in the bile salt micelles using a quantitative NMR methodology.[2] The polysaccharides with different structural features, such as i) non-charged, arabinogalactans and galactomannans (coffee), laminarans (algae), β-glucans (mushrooms) and arabinans (apple pomace); ii) negatively charged, fucoidan (algae); iii) positively charged, chitooligosaccharides (commercially available), were chemically characterized regarding sugar composition and glycosidic linkages. Non-charged polysaccharides, galactomannans and arabinogalactans, showed to have bile salt sequestration capacity, contrary to laminarans and β-glucans. Negatively charged fucoidan, showed to affect bile salt sequestration being this effect higher to less charged polysaccharide. Positively charged chitooligosaccharides showed the highest potential to sequestrate bile salts (negatively charged at physiological pH) and affect cholesterol bioaccessibility, being similar to a pharmaceutical cationic resin currently used as hypocholesterolemic agent. The binding of bile salts to chitooligosaccharides was shown to be dependent both on electrostatic and hydrophobic interactions shown by solid state NMR and zeta potential. This work highlights polysaccharides structural features and their influence on hypocholesterolemic function, with relevance for the development of functional food.

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Brown macroalgae-rich extracts as potential food ingredients: a holistic extraction approach

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Currently, there has been a growing demand for innovative, sustainable, healthy and clean-label food products. In this regard, marine macroalgae (or seaweeds) have recently attracted interests as alternative renewable feedstocks due to their large biomass yields and fast growth rates, as well as due to their balanced composition in nutrients and exclusive bioactive compounds related to health promotion (1). In particular, brown phyla stand out for their abundance in phenolics (e.g. phlorotannins), carotenoid pigments (e.g. fucoxanthin) and interesting polysaccharides (e.g. fucoidans, laminarans, and alginates) which are claimed to exert promising bioactivities, including antioxidant and immunomodulatory effects.[1] Such properties make these macroalgae-target compounds attractive for the development of added-value functional foods. Nevertheless, the strong odour and taste inherent to macroalgae limits its application as food ingredient, therefore using extracts seems to be a good strategy to take advantage of its great value.[2] In this context, the present work aimed to obtain economically-affordable extracts rich in specific target compounds from two European brown macroalgae *Laminaria digitata* and *Fucus vesiculosus* to be potentially used as food ingredients in food formulations.

To achieve this, a holistic and sequential solid-liquid extraction methodology was developed and optimized for both macroalgae species. Briefly, it was first performed an extraction either with cold water or hydroethanolic mixtures (96% and 70%) to recover phlorotannins and fucoxanthin. Following that, both macroalgae residues were extracted with hot water to recover water-soluble polysaccharides, followed by precipitation with 2% $CaCl_2$ to recover alginates. In *F. vesiculosus*, cold water extract (38%) revealed higher yields comparatively to EtOH extractions (9 – 18%). Using cold water at the beginning of the process allowed the recovery of water-soluble phlorotannins (0.2%), mannitol (5%) and branched laminarans (1.1%), while sequential 70% EtOH extraction yielded the highest fucoxanthin recovery (1.3%). Contrarily, using 96% EtOH allowed to obtain phlorotannins-richer extracts (0.4%), but almost no fucoxanthin was detected. In addition, structural differences between water- and hydroethanolic-soluble phlorotannins have been tentatively characterized by HPLC-DAD-ESI-MS^n. Sequential extraction with hot water allowed to obtain extracts containing fucoidans (1 – 3%) and laminarans (0.1 – 0.7%), while precipitated calcium alginates accounted for 1 to 4%.

Regarding *L. digitata*, the recovery of phlorotannins with 96% EtOH was approximately 75% higher than those from *F. vesiculosus* in the same conditions, while almost no fucoxanthin was detected. Likewise, sequential hot water extraction revealed the presence of low amounts of fucoidans (1.5%) with almost no laminarans quantified, as well as the recovery of 4% calcium alginates. Besides, sugar analysis to final extraction residues from both macroalgae revealed that still contained higher amounts of alginates. Therefore, they were extracted following an industrial alginates extraction procedure which briefly includes acidification, alkaline extraction with Na_2CO_3 and ethanol precipitation. The results revealed that *L. digitata* (17%) is indeed a better source of alginates comparatively to *F. vesiculosus* (6 – 9%). Overall, this work allowed the development of a holistic extraction procedure to obtain economic-affordable brown macroalgae-rich extracts by using simple techniques and environmental-friendly solvents, thus enabling less residues production close to the much recommended zero waste systems. Also, the several obtained food-grade extracts rich in specific target bioactive compounds from brown macroalgae open up opportunities to be applied as food ingredients in the formulation of new functional foods.

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Integrated Bioprocess for Structured Lipids, Functional Sugars, and Glucose Production using Olive Pomaces

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The use of local agro-industrial residues, to produce high added-value products, is a challenge to obtain economically sustainable and environmentally friendly industrial processes. Olive pomace is a by-product generated in large amounts, from the olive oil extraction mills. This residue mostly consists of lignocellulosic materials but also contains residual oil (3-4.5 %, wet basis), proteins, and other compounds. Olive pomace oil is obtained by solvent extraction (*n*-hexane) and after refining, is used for edible purposes. Extracted olive pomace (EOP) typically has cellulose contents in the range of 13.8-30.0%, 18.5-32.2% hemicelluloses and 30.0-41.6% lignin.[1]

The aim of this study was the valorisation of olive pomaces from Portuguese cultivars, 'Galega Vulgar' and 'Cobrançosa', to obtain added-value compounds, namely: (i) xylo-oligosaccharides (XOS) with recognized prebiotic activity; (ii) glucose, and (iii) structured lipids for food/pharmaceutical applications. An integrated green and sustainable process based on biocatalysis and thermal treatments, was used. Therefore, crude oils from Galega and Cobrançosa pomaces from fruits with different ripening indexes (RI; Cobrançosa: RI = 2.5 to 4.7; Galega: RI = 1.8 to 4.8) were extracted with *n*-hexane. The obtained EOP were submitted to autohydrolysis to obtain a liquid stream containing hemicellulose-derived compounds (mainly xylans), and the residual solid fraction rich in cellulose and lignin. The hydrothermal treatment (autohydrolysis) conditions were optimized by Response Surface Methodology, following a central composite rotatable design (CCRD), as a function of temperature (T: 142-198°C) and time (t: 48-132 min), corresponding to severity factor (SF) values ranging from 3.2 to 4.9. Autohydrolysis with SF equal or higher than 4.0 produced higher sugar yields, with maximum values around 180 g glucose equivalent/kg EOP for SF of 4.7 (190°C/120 min) or 4.9 (198 °C/90min). These values were similar for both cultivars and did not dependent on the ripening stage of the olives. Maximum oligosaccharide (OS) yields of 98% were obtained by autohydrolysis with SF of 4.0. The monosaccharides were mostly xylose: 55.8-67.7 % in Galega, and 50.4-69.0 % in Cobrançosa liquors. Therefore, the production of bioactive xylo-oligosaccharides (XOS) from olive pomaces showed to mainly depend on the hydrothermal conditions used.

The solid residue obtained from autohydrolysis was submitted to enzymatic hydrolysis (saccharification) using the following commercial enzyme preparations containing cellulases and beta-glucosidases: Saczyme Yield, Ultimase BWL 40 and Celluclast 1.5 L. Saczyme and Ultimase were active even when high pomace loads of 30% were used, reaching 80 and 90% of glucan conversion after 5 h reaction.[1] The obtained glucose can be further used for fermentation or other industrial uses.

Moreover, acidic crude olive pomace oils from industrial origin (free fatty acid contents from 3.4 to 20 %) were used as raw material to produce low-calorie dietetic structured lipids (SL), which are novel lipids with improved functional and bioactive properties, for food and pharmaceutical uses. SL were obtained either by acidolysis of these acidic crude oils with medium-chain fatty acids (C8:0 or C10:0) or interesterification with their ethyl esters, catalysed by immobilized sn-1,3 regioselective lipases, in solvent-free media, in batch and continuous bioreactors.[2,3] High SL yields, and high operational stability of the biocatalysts were observed even when high acidic crude oils were used. This is of utmost importance to decrease costs related with oil refining and biocatalyst.

The promising results obtained showed the feasibility of this approach as a green sustainable process for olive pomace valorization.

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The effects of dried halophyte as a salt substitute: a preliminary randomized study

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Previous studies have demonstrated that excessive salt intake is strongly associated with arterial hypertension, vascular dysfunction and overall risk of cardiovascular diseases. [1]
The aim of this study was to evaluate the dried halophyte plant effectiveness as a salt-substitute, addressing its effect on the cardiovascular function in healthy young individuals.
Thirty healthy participants, aged 18 to 26 years, were randomized into two groups: the control group (CG) and the intervention group (IG). The IG used halophyte powder as a salt substitute for cooking, and the CG used regular salt, for a period of one month. A baseline evaluation was performed before the participants started the intervention phase, and was repeated after a 30 days intervention period. Each evaluation included blood pressure (BP) measurement, carotid-femoral pulse wave velocity (PWV), carotid pulse wave analysis (PWA) and blood samples were also collected for analysis. Sodium excretion was measured at baseline and after intervention through urine collection and analysis.
The preliminary results (**Figure 1**) showed that sodium excretion was unchanged between-moments in the CG, but significantly decreased after intervention in the IG. The reduction in sodium excretion in the IG was followed by a significant reduction in brachial and aortic systolic and diastolic blood pressure, and also in PWV. No significant changes were observed in the CG in terms of cardiovascular and biochemical parameters.

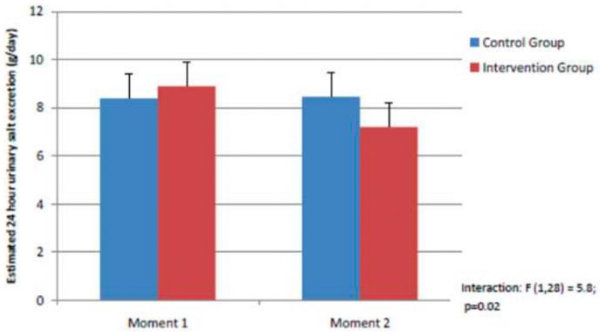

Figure 1: Comparison of daily saline excretion between groups at the two assessment times (Moment 1: Pre-intervention, Moment 2: Post-intervention)

Conclusions indicate that the consumption of halophyte dehydrate is beneficial to the cardiovascular system at several levels: reduction in peripheral and central BP, improved PWV, improved left ventricular function and coronary perfusion.
This halophyte has already been tested for the use in the production of several processed foods *e.g.* crackers [2], butter [3] and bread known for their high levels of sodium.
The halophytes can therefore be targeted as a promising substitute for common table salt, although further studies are needed to thoroughly ascertain the overall benefits of dried halophytes, particularly concerning their effect on BP and vascular protection.

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Acylated and polyacylated anthocyanins as a pallet of natural colors

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Anthocyanins are colored pigments belonging to the phenolic group that comprise a diverse group of intensely colored pigments responsible for red, purple, and blue colors in fruits and vegetables. They are water soluble, which facilitates their incorporation into aqueous food systems. Besides the color attributes, interest in anthocyanins has intensified due to their health benefits as antioxidant, anti-inflammatory, anti-mutagenic, anticancer, and neuroprotective effects. However, these compounds are strongly affected by different factors such as pH, temperature and light, which restrict their use as natural colorants, for example in food systems.[1] Anthocyanins whose glycosyl moieties are acylated by hydroxycinnamic acids residues (e.g. coumaric, ferulic and sinapinic acids) are known to exhibit more stable colors than nonacylated precursors.[2] This improvement in stabilization is achieved through π-π stacking interactions between the hydroxycinnamic acid and anthocyanidin chromophore, which can involve individual anthocyanin molecules (intramolecular copigmentation) or noncovalent dimers (self-association) and possibly higher oligomers.[3]

This work proposes the use of a green extraction methodology to obtain acylated and polyacylated anthocyanin extracts from two different edible sources, namely the red cabbage (RC) and the blue butterfly pea flowers (BPF) using hot water. Chemical composition of extracts in terms of sugars, lipids, proteins, anthocyanins and other phenolic compounds was determined using different methodologies. The color stability of an aqueous solution of each extract was also evaluated over time at different pH values (**Figure 1**), and it was observed that the color of solutions was practically unchanged over a period of 24 for hours at room temperature. Over time, the color intensity of all solutions started to decrease with the reduction being more pronounced for RC

extract when compared with BPF. For pH 4, the pH observed in many food matrices, the color intensity of BPF extract reduced approximately 36% and RC extract 47% after 21 days at room temperature. These results show that the increase of the complexity on anthocyanins structure yields not only in a higher color stability of these pigments but also in the possibility of obtaining different color hues from red to blue as it is clearly demonstrated in **Figure 1** (right). Knowing that color plays a key role in establishing consumer acceptability of food, and also that there is a current need in the substitution of synthetic colorants by natural ones, acylated and polyacylated anthocyanins can be the key to obtain a pallet of natural colors for the development of innovative functional foods.

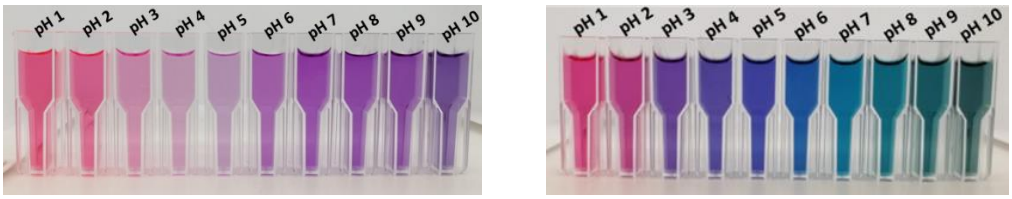

Figure 1: Color expression of acylated anthocyanins at different pH values in extracts of red cabbage (left) and blue butterfly pea flowers (right).

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Quimiometria na Ciência dos Alimentos

FT-Raman methodology applied to study the effect of seasoning time of Fino *Sherry Casks*® in Brandy de Jerez elaboration.

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Brandy de Jerez is a grape-derived spirit produced in the Southern Spanish area known as Marco de Jerez. The Technical File of the Geographical Indication under this name indicates that it must have a minimum alcoholic strength of 36% vol. (usually between 36–45% vol.); it has to be obtained from wine spirits and distillates and it must be aged in oak barrels with capacity of less than 1000 L, previously seasoned with Sherry wine and following a traditional dynamic ageing system used in the Sherry area known as *Criaderas and Solera*.

Brandy de Jerez has a number of specific organoleptic characteristics that distinguish it from other spirits. These characteristics are given to the brandy by the barrels where it is aged. This is because the barrels used to make Brandy de Jerez must have previously contained some type of Sherry wine, i.e., Fino, Amontillado, Oloroso, or Pedro Ximénez. This conditioning process is called *seasoning* and must be carried out following the specifications of the Technical File that regulates their production, is then referred to as *Sherry Cask*®. The Sherry wine must remain in the barrel for at least 12 months, in order to obtain the designation of *Sherry Cask*®. However, there are no studies that corroborate that 12 months is the optimal seasoning time or related to differences observed for and longer or shorter seasoning time. Fino Sherry wine is one of the Sherry wines used for *Sherry Cask*® seasoning. It is a dry wine, obtained by biological ageing under the action of flor yeast. Fino Sherry wine is characterised by having a pale color, dryness, slight acidity and sharp aromas with hints of almonds[1].

During ageing, the organoleptic profile of the Brandies de Jerez improves and develops not only because of the wood, but also because of the Sherry wine that the casks have previously contained. Nowadays, spectroscopy techniques are increasingly used in the analysis of food and beverages because they are fast, not expensive, non-invasive, without sample treatment and environmentally friendly. Although there are no studies on the analysis of Brandy de Jerez with FT-Raman spectroscopy, moreover this technique has recently been used to monitor other brandies[2]. In this work FT-Raman spectroscopy was applied to Brandies de Jerez aged in barrels that had been seasoned with Fino Sherry wine for different ageing times. The obtained spectra are similar to those proposed for other brandies[2] and show greater differences according to the ageing time (**Figure 1**). The region from 1610 cm^{-1} to 785 cm^{-1} seems to be the most influential part in distinguishing of the Fino Brandy de Jerez samples.

The peaks appearing in the region shown in **Figure 1** are due to the $–CH_2$, $–CH_3$ bending (also influenced by the ethanol content in the samples); H-C-H bending modes; C-O stretching vibration (associated with the presence of ethanol and methanol) and CH_3 rocking vibrations and C-C stretching. These bands could be influenced by the presence of organic acids present in Brandy de Jerez due to the *Sherry Cask*®. Brandy de Jerez is the only brandy that contains wine-derived organic acids in its composition, and the concentration of these acids could be different depending on the type of *Sherry Cask*® in which it is aged, so the amount of these types of compounds could be a distinguishing feature between the samples[3].

A chemometric study confirms that the Brandies de Jerez studied in this work are different according to the seasoning time that the barrels have previously received. FT-Raman is a potential technique to distinguish between the seasoning time of the barrels in the elaboration of Brandy de Jerez, which affects in its quality and sensory profile. It could be a very interesting technique for quality control, since that does not take much time to obtain good results and that could be used by companies to control their products.

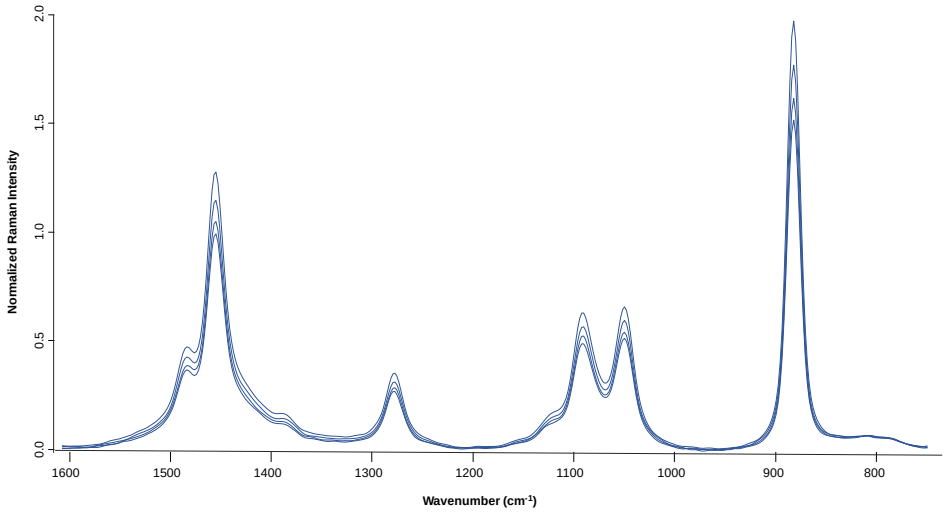

Figure 1. FT-Raman spectra of a representative Brandy de Jerez sample.

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Vibrational spectroscopy applied to *Arbutus unedo* fruit spirit characterization

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Arbutus unedo spirit (AUS) is a typical beverage of Mediterranean countries, which is usually produced without wood ageing. However, nowadays there is a trend related to the innovation and search for new market options related to this beverage. This work aimed to identify a technique to distinguish the AUS aged with different times and different toasting levels by an easier and cheaper way. For this propose the AUS, aged for three and six months with oak wood (*Quercus robur* L.) submitted to three different toasting levels (light, medium and medium plus) were analyzed. Concerning its chemical composition, identified by GC-MS and quantified by GC-FID, and its sensory analysis, a previously work [1] showed that is quite easy to distinguish these modalities, and the best one was produced using oak wood with medium toasting levels during three months of ageing. Concerning these results, all samples were analyzed using FTIR-ATR, FT-RAMAN and NIR to understand the faster methodologies to differentiate the studied ASU sprits.
The spectra of AUS samples were obtained in each equipment according to the following methods:

1) FTIR-ATR – with a Bruker spectrometer (Alpha, Bruker Optic GmbH, Germany), equipped with a flow-through cell with controlled temperature, with 128 scans per spectrum at a spectral resolution of 8 cm^{-1} in the range of 4000 to 450 cm^{-1};
2) FT-RAMAN –with a spectrometer (Bruker, MultiRAM, Germany) equipped with a Ge Diode detector, an integrated 1064 nm and Nd:YAG laser with a maximum output power of 500 mW. The spectra were collected with 100 scans per spectrum at a spectral resolution of 8 cm^{-1} in the wavenumber range from 3500 to 70 cm^{-1};
3) NIR –using a NIR spectrometer (MPA Bruker, Germany) in a transmitted light mode with 1 mm quartz cells. The samples were measured with an 8 cm^{-1} spectral resolution and 32 scans in the wavenumber range of 12,500 to 4000 cm^{-1}.

Appropriate chemometric tools were applied to extract information from the spectral data. Principal component analyses (PCA) of spectra were used to distinguish between the different groups of samples included in this experimental design. This analysis was made using the Unscrambler® X, version: 10.5.46461.632 (CAMO Software AS, Oslo, Norway). For spectral acquisition and first evaluation the software OPUS®, version: 7.5.18 (Bruker Optik, Germany) was used.
The spectra (Figure 1) were similar do those reported for AUS spirit [1] and for other similar spirits [2,3]. The observed bands are in accordance with the previously works and display a strong influence of the different compounds (mainly alcohols) present in this matrix.
Spectroscopic techniques, namely FTIR–ATR, were applied to discriminate the different beverages produced. The results highlighted an increase in *Arbutus unedo* spirit's quality with the wood contact, mainly based on the sensory attributes.
As conclusion all techniques are able to distinguish AUS It was possible to identify the potentiality of FTIR-ATR to distinguish AUS ageing time with oak wood and the three different toasting levels. However, the most accurate results were obtained using the FTIR-ATR technique.

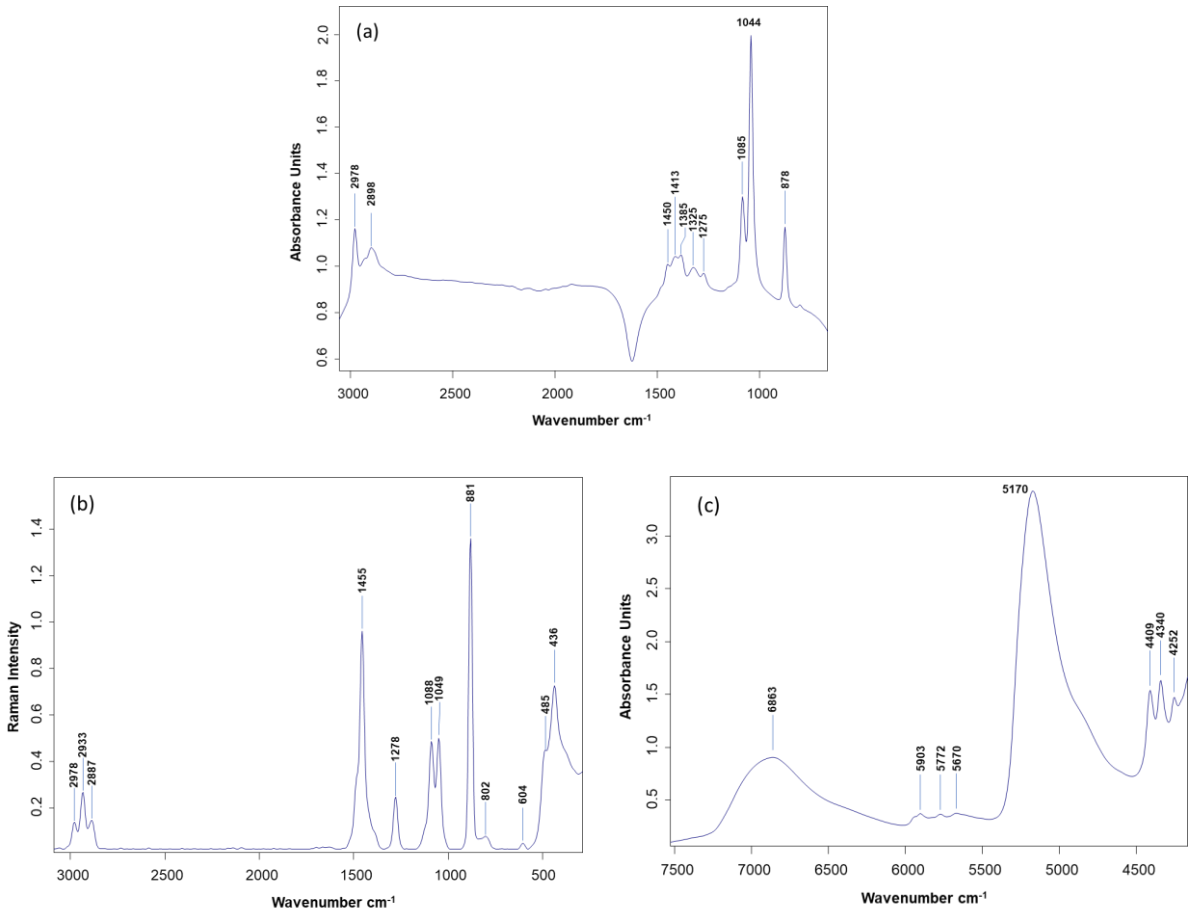

Figure 1: FTIR-ATR (a), FT-Raman (b) and NIR (c) spectrum of AUS samples.

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Posters Communications

Química Alimentar: Estrutura, Composição e Qualidade Alimentar

Changes of the physicochemical characteristics of aged wine spirits during the storage in bottle

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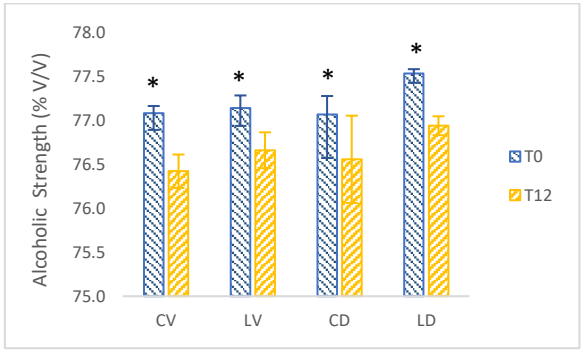
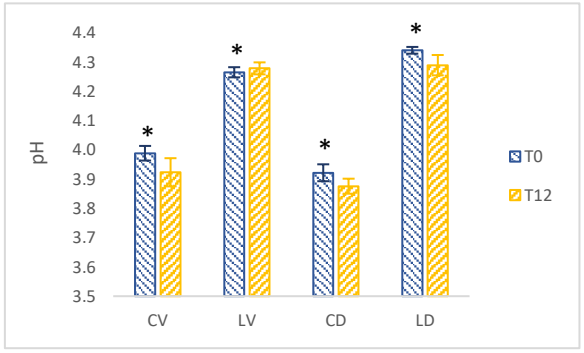
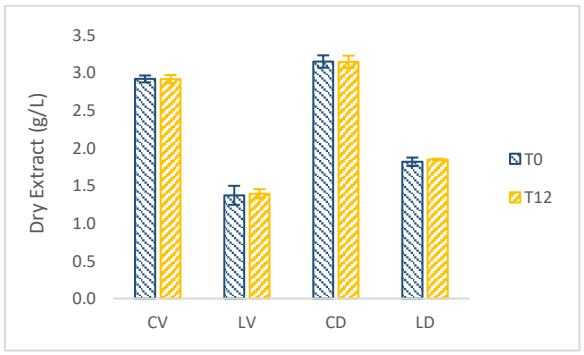
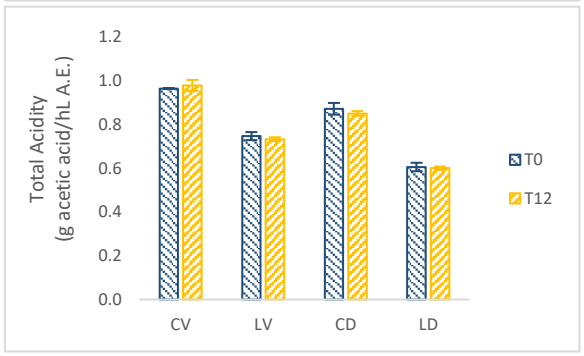
The wine spirit (WS) is a specific beverage obtained by the distillation of wine. Initially, the wine distillate is essentially made up of ethanol and water, and its physicochemical characteristics mainly result from the wine and distillation system used. During the ageing process, the distillate contacts with the wood and, with the mediation of oxygen, organic compounds are extracted from the wood and undergo several reactions over time, thus altering the sensory and physicochemical characteristics of this spirit beverage. Traditionally, ageing is carried out in barrels, which is an expensive and time-consuming technology.[1] To overcome these drawbacks, alternative technologies for a sustainable ageing of WS using wood staves combined with micro-oxygenation (MOX) has been studied by our team. In the Project CENTRO-04-3928-FEDER-000001, the wine distillate was aged for 18 months in 250 L wooden barrels of chestnut and Limousin oak (traditional technology), and in 1000 L stainless steel tanks with staves of the same kinds of wood combined with MOX - flow 2 mL/L/month (alternative technology) to compare the physicochemical characteristics acquired by the WS as a result of the different ageing technologies.[2] The alcoholic strength by distillation was analysed by electronic densimetry, pH by potenciometry, dry extract by weighing the residue left by evaporation at 100 ºC and total, fixed and volatile acidity by colorimetric titration. The low molecular weight phenolic compounds were analysed by a HPLC method developed and validated in our laboratory, the total phenolics index was determined by the absorbance measurement at 280 nm, the chromatic characteristics were determined according to the CIELab method and the dissolved oxygen was monitored *in situ* during the ageing period. At the end of this project, it was found that the WS's ageing was faster by the alternative technology, which also allowed obtaining high quality WSs compared to the traditional one. Within the scope of the Project Centro-04-3928-FEDER-000028, our team is studying the storage in bottle of such aged WSs in order to assess whether or not their physicochemical characteristics are preserved during this step. In this work, results of the basic chemical parameters were examined. Thus, eight aged WSs (n=2) from four ageing modalities [chestnut barrels (CV), Limousin oak barrels (LV), stainless-steel tanks with chestnut staves and MOX (CD) and stainless-steel tanks with Limousin oak staves and MOX (LD)] were bottled on the same day in amber glass bottles of 750 mL (n=2). The bottles were stored in the cellar of the INIAV-Polo de Dois Portos (Dois Portos, Portugal). Sampling was performed at the beginning and after 12 months of storage and the following parameters were analysed in duplicate: alcoholic strength, pH, dry extract and acidity (total, fixed and volatile). Among the studied parameters, only the alcoholic strength and pH were influenced by the storage in bottle. The ANOVA results revealed that they decreased significantly after 12 months (**Figure 1**). Some factors, including the closure and the permeability of the stopper (such as the effective diffusion coefficient) may have contributed to low values of the alcoholic strength[3] (reduction of 1% in the WSs from all modalities); therefore, it should be considered as a loss of the system and as a result of the physicochemical phenomena that may occur during storage in bottle. Conversely, the oxidation and condensation reactions of low molecular weight compounds may cause the decrease of pH value, but without increasing the total acidity. In summary, the differences between the WSs from the four ageing modalities (kinds of wood combined with technologies) remained during the storage in bottle, suggesting that the wine spirit quality was preserved. Further studies are required to understand the overall effect of this production step on the aged WSs' features.


Alcoholic Strength (% V/V)
78.0
77.5
77.0
76.5
76.0
75.5
75.0
CV
LV
CD
LD
T0
T12


pH
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
CV
LV
CD
LD
T0
T12


Dry Extract (g/L)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CV
LV
CD
LD
T0
T12


Total Acidity
(g acetic acid/hL A.E.)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CV
LV
CD
LD
T0
T12

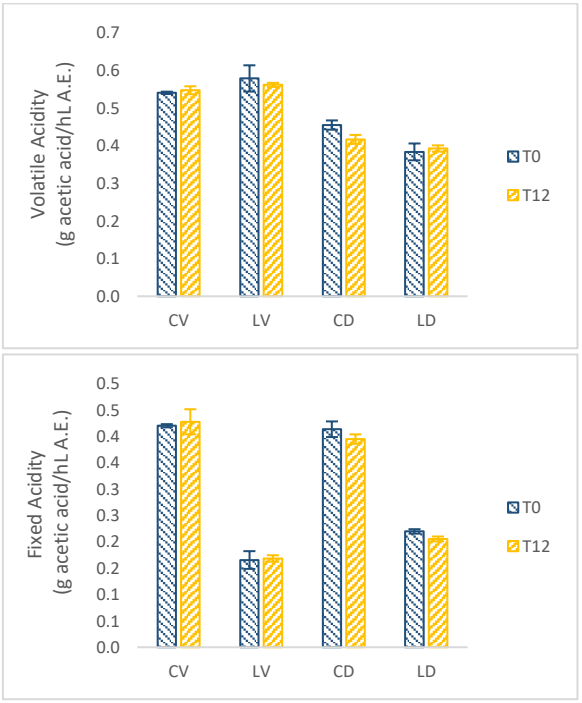


Figure 1: Physicochemical characteristics of the aged WSs after 12 months of storage in bottle (CV – chestnut barrels, LV – Limousin oak barrels, CD - stainless-steel tanks with chestnut staves and MOX, LD - stainless-steel tanks with Limousin oak staves and MOX). A.E. – absolute ethanol. The one-way ANOVA (significance of means comparison by Fisher's test; $p < 0.05$).

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Fatty Acid composition of *Brassica rapa* landraces from Portugal

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Brassica rapa is an important crop adapted to agricultural settings worldwide, and with great morphological diversity in the organs used for consumption. A variety of different agricultural morphotypes forms has been selected for use as oilseeds, leafy vegetables - baby leaves, turnip greens, turnip tops, and turnips.
The three well-defined groups, root, leafy vegetable and oilseed types of *B. rapa* occur throughout the eco-geographic range of *B. Rapa.*
The major cultivars of *Brassica* oilseed crops, i.e., *B. napus, B. juncea*, and *B. rapa*, have an average oil content ranging from 45 to 50% and are the world's third most important source for vegetable oil (1). The five major fatty acids present in the oil extracted from the seeds of the genus *Brassica* are palmitic (C16:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosenoic (C20:1) and erucic (C22:1) acids, with erucic reaching almost half (50%) of the total of the fatty acids.
The interest in the *Brassicaceae* as an oilseed mostly for industrial uses, is a consequence of the high erucic acid (EA) content in the oil, and the high protein meal left over after oil extraction. Canola bran has 34 to 38% protein and it is an excellent protein supplement in the formulation of cattle, pig, sheep and poultry feeds. Because of the importance placed on biodegradability, and renewability, there is an upward trend in producing chemical compounds for various industries utilizing plant-based feedstock. Erucic acid (*cis*-13-docosenoic acid) (C22:1) is a chemical ingredient used in industries to produce plastics, printing inks, food, personal care products, pharmaceuticals, and other products (2). EA is found only in seed oil from plants belonging to *Brassicaceae* and *Tropaeolaceae* families. Commercially, the global EA market is categorized based on its source, end-use industry, production region, application, and grade. Based on the grade, EA sources are segmented in two categories: EA content of 43–50% and EA content >50%.
High erucic acid makes the oil unsuited for human consumption (3).
To enable the use of the oil for human consumption, a variety was developed in the 1970's with low EA content, specifically for edible proposals. Canola oil is primarily used for edible purposes.
Canola was originally a trademark name of the Rapeseed Association of Canada, and the name was a condensation of "Can" from Canada and "OLA " meaning "Oil, low acid". It was developed through conventional crossbreeding of the rapeseed (*Brassica napus* L. and *Brassica rapa* L.) plant with unwanted traits removed. Once registered as a trademark in Canada in 1970s, canola has now been recognised and used internationally as a generic term for edible varieties of rapeseed with EA less than 2% in the oil. Before 1986 it was an industrial trademark, of an oil which should contain less than 2% erucic acid and each gram of air-dried solid seed component should have not more than 30 micromoles of glucosinolates.
The present study is aimed to evaluate the oil content and fatty acid composition profile of the Portuguese *Brassica rapa* germplasm, *ex situ* conserved at Portuguese Genebank (BPGV), and to investigate the variation for these traits among accessions from different sites in Portugal.
This information can be promising to improve oil content and fatty acid composition in breeding programs and to find the most promising genotypes for industrial purposes.

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doi: 10.2903/j.efsa.2016.4593

Nutritional and chemical profile of grape pomace generated in red wine production

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The estimated world production of grapes in 2018 was over 79 million tones, with approximately 75% of the total produced grapes being destinated for wine production, of which 20-30% represent waste. These residues are known as grape pomace andare formed by skins, remaining pulp, seeds, and stalks. Due to its chemical composition, if an effective management of these residues is not carried out, numerous environmental problems, such as groundwater and surface water pollution, in addition to the consumption of oxygen in the soil and groundwater, can harm the entire ecosystem in which they are found [1–3]. Therefore, studying applications for these residues has become a relevant subject, closely related to industry and academia. Also, currently, there is a growing demand for healthy and natural food ingredients that can replace the synthetic ones currently used, which could be obtained from this kind of residues. In this context, the objective of the present work was to analyze the nutritional and chemical profile of grape pomace generated in the production of red wine to evaluate its suitability as source of value-added compounds for the development of new products.
Regarding the nutritional value, it was found that the grape pomace had a high percentage of moisture and protein. At the sametime, it had a low percentage of total fat and ash, as well as low carbohydrate and energy content. As for the chemical composition, four organic acids were identified, with a predominance of quinic acid. Regarding fatty acids, twelve different molecules were identified, with linoleic acid as the most abundant, followed by oleic and palmitic acids. A high amount of fiber and two isoforms of tocopherols were also identified, with a high concentration of α-tocopherol. Regarding minerals, the high amount of iron stands out, followed by manganese and copper, and low levels of magnesium and sodium. These results demonstrate that the studied residues have good chemical characteristics and can be used as raw material for the developmentof new products with interesting nutritional and chemical features.

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Quantification of mineral elements in leafy vegetables from Portuguese markets

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Vegetables can contain different mineral elements in a wide range of concentrations, as a consequence of its presence in the water or soil in which the vegetable is grown but also as a result of human activity (agricultural and industrial). The ingestion of the edible part of vegetables is a very important source of these mineral elements for humans since some of them are nutrients (macro and micronutrients) that cannot be synthesized and are essential to normal metabolism function. However, its ingestion in doses above the recommended values may present toxicity for the human body. Some other elements can also be present and are toxics for humans (such as Cd, Pb, As and Sn) and can accumulate in organisms by ingestion of contaminated vegetables.

The main objective of the present study was to evaluate the mineral composition (Cu, Zn, Fe, Mn, Na, K, Ca, Mg, P, S, V, Li, Co, Cr and Ni) of six different species of green leafy vegetables (rocket, cabbage, spinach, turnip leaves, swiss chard and watercress) available in national markets from different origins and producers. Toxic elements (Cd, Pb, As) were also determined in order to assess their possible presence in these plant products, since they can be harmful to human health above regulated limits for non-essential elements.[1]

Mineral content of the vegetable samples was categorized according to species, origin and cultivation method since these factors can influence the mineral composition. Analytical determinations of the mineral content of the vegetable samples were performed by ICP-AES after acid digestion.

The results indicate that potassium is the macroelement with the highest average content and iron is the microelement with the highest content, in all vegetables. Some of the differences that were found might be related to the mineral composition of the soil where it was grown and also of the irrigation water. The values vary significantly between different vegetable species as some of them are known to be richer in specific elements. They also show some differences when comparing different origins and different cultivation methods (conventional and organic).

The influence of human activity also has to be considered as it can contribute to the presence of toxic elements and may be one possible source of potentially toxic elements in the soil and irrigation water and consequently its present in leafy vegetables that can be available for consumption. Regarding the toxic elements Cd and Pb the results were compared with the existing limits in European regulations[2, 3] and in all cases were lower than those legislated. On average, the Cd levels in vegetables are slightly higher in those produced in conventional farming in comparison to organic farming (Figure 1a) although this is not observed in relation to Pb, where the average values are similar in relation to both farming techniques (Figure 1b).

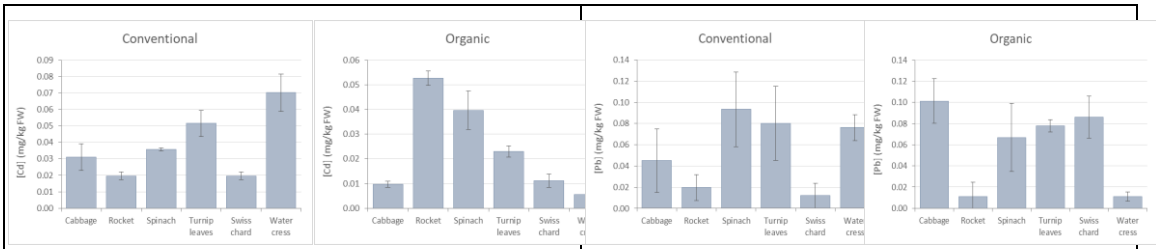

Figure 1: Cadmium (a) and lead (b) contents in different vegetables in relation to farming techniques (conventional and organic)

Funding: This work was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food, Research Unit.

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Variation of the chemical composition of red wine (Alicante Bouschet and Syrah) during the first eight months of maturation in new and reused oak wood barrels.

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Storage in wood barrels to acquire different aromas and stabilize colloidal colour matter is an important stage in the maturation of high-quality red wines or the ageing process[1]. Wood specie, their toasting level and the barrel usage (new or in a second or third maturation process) are vitally important factors because they are correlated with the wine evolution and consequently with their final quality and aroma profile. The natural micro-oxygenation through the wood pores and the barrel lid is also essential because it improves polymerization and condensation reactions between flavonoid compounds in wine, incrementing their organoleptic characteristics[2].

This study aimed to follow the variation of the quality of a wine composed of a blend of the cultivars 'Alicante Bouschet' and 'Syrah' during the maturation process in French oak barrels produced with wood from Bertranges ou Tronçais region, comparing new barrels with third time reused barrel in the wine maturation. Four barrels (two of each modality) were used with a medium toasting level. This study was performed during the first eight months of the maturation process, and the wines were sampled every two months.

The chemical composition was monitored over time while measuring the acetic acid, alcohol, citric acid, density, fructose, glucose, glycerol, lactic acid, malic acid, pH, saccharose, tartaric acid, total acidity, and total sugars with a Fourier transform infrared spectroscopy (FTIR) with Attenuated Total Reflection (ATR) (Bruker spectrometer, Alpha) using a diamond crystal. The FTIR-ATR used was equipped with a flow-through cell with controlled temperature. The injection of water cleaned the cell in the flow-through cell, and the background was also measured with distilled water for every ten samples.

To evaluate the differences and the interaction between wood barrels and maturation time, Analyses of Variance (ANOVA) with two factors (barrel [2 levels] and maturation time [4 levels]) were performed. A Principal Component Analysis (PCA) was also performed to study the relationship between variables and samples.

According to the ANOVA, both factors indicated a significant effect, in which the maturation time accounts for a higher percentage of variance. On PCA, the first two components explain a percentage of variance higher than 70%. Lactic acid, saccharose, citric acid and alcohol content had an opposite variation in relation to total acidity, fructose, acetic acid, malic acid, total sugar, glucose content and density.

The results showed a similar evolution of the wine's chemical composition, despite some differences concerning the time of maturation and the kind of barrel used. Using the spectral information, it was also possible to distinguish the different times of the maturation process and the different kinds of barrels.

Keywords: Red wine; Wine maturation; Oak barrels; reused barrel.

Acknowledgements: This work has been supported by the "Projeto Estratégico de Apoio à Fileira do Vinho na Região Centro" (CENTRO-04-3928-FEDER-000028) and by National Funds through FCT—Foundation for Science and Technology under the Project UIDB/00239/2020 [CEF].

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Grape composition during ripening, in two cultivars and different sites of "Beira Interior" region

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The grape composition along the ripening period will determine the must characteristics and, consequently, wine quality. To study the grape chemical composition during the ripening period, about 60 samples with 100-200 grapes per sample were collected and analysed for total soluble solids (TSS), total acidity, total polyphenols, colour intensity and tint, in 'Síria' and 'Aragonês' (syn. 'Tinta Roriz'; 'Tempranillo') cultivars, and in three different locations of the "Beira Interior" region of Portugal. TSS was evaluated by refractometry and total acidity was determined by acid-base titration. Total polyphenols index ($A_{280\ nm}$), colour intensity ($A_{420+520+620\ nm}$) and tint ($A_{420/520\ nm}$), were evaluated by UV/VIS spectroscopy.

Using the thermal time (sum of temperatures ≥ 10ºC from 1st of January) to represent the natural conditions influencing vine ecophysiology during the ripening period, the results showed a similar trend for increasing TSS and decreasing total acidity and total polyphenols, despite some differences between locations. Those differences agreed in general with the differences of vines location, more northern (cold) or more southern (warm) ones. Local differences in total polyphenols were more pronounced than in TSS or total acidity. The colour intensity and tint of the 'Aragonês' red grape generally increased until the middle of the ripening period and then decreased until the harvesting. The differences between sites were not pronounced, except for tint in the southernmost vineyard. In the white grape 'Síria', colour intensity and tint have shown any regular pattern with thermal time.

In conclusion, it seems that grape characteristics, as sugars and acidity, are primary influenced by general environmental conditions, while others, as polyphenols, colour intensity and tint, are rather depending on specific or local factors.

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Cynara cardunculus L. flowers enzymatic profiles and ewe's cheese yield

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Abstract

Cynara cardunculus L. flowers have been used, in the form of an aqueous extract as vegetable coagulant, in the production of Protected Designation of Origin (PDO) ewe's cheeses in the Mediterranean Basin. The presence of aspartic proteases, cardosins, promote specific proteolysis in milk coagulation and cheese ripening. Nine aspartic proteinases have been identified in *C. cardunculus* flower extracts, which have been assigned different names, cardosins A–H [1]. Cardosins A and B specifically proteolyze at the surface of casein micelles in the early phases of milk coagulation. Cardosin A has a high specific proteolytic action on K-casein resulting in less softer textures, whereas cardosin B has a low proteolytic action on K-casein, and a higher non-specific proteolytic action responsible for non-firm inner cheese texture [1]. Lower ratios between specific milk clotting activity and the non-specific enzymatic proteolytic coagulant activity have been associated to lower cheese yields [2].

In the present work, we evaluated the influence of the extract of flowers from two individual *C. cardunculus* plants (Cyn_AB, presence of cardosina A, clearly predominant, and cardosin B; Cyn_B, presence of cardosin B and absence of the typical cardosin A) on ewe cheesemaking parameters and cheese yield.

The characterization of the cardosin profiles was confirmed by native-PAGE (12,5%), a technique that allows the distinction of the presence or absence of the typical cardosin A. Milk-clotting activity (MCA) was accessed by visual observation according to ISO 23058/IDF 199 (ISO/IDF, 2006). The ewe's cheeses were manufactured in artisanal cheese manufacturer in Serpa, Portugal. Physicochemical parameters of milk and whey were evaluated, time to curd firmness (CF) was registered and the cheese yield accessed during the 30 days ripening time.

The main results showed that the initial cheese yield (kg/L) per treatment differed, but there were no differences at the end of the ripening period, suggesting that the enzymatic profile of *C. cardunculus* flowers does affect the cheese yield.

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Interaction of Sodium Caseinate with Caffeic Acid by Fluorescence Spectroscopy Analysis

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Although Cow's milk is of great interest due to the nutritional value, milk proteins are responsible for about 90% of food allergie.[1] Sodium caseinate (Cas) is a dairy product, obtained from casein treated with a strong base under heating.[2] Cas is widely used in processed foods to increase the protein content.[2] Caffeic acid (CA) is a polyphenol generated by the secondary metabolism of some vegetables. It is considered a highly potent natural antioxidant in the human diet. CA plays an important role as an inhibitor of lipid oxidative damage in emulsion systems, scavenging free radicals and chelating active transition metals.[3]

In this work, the effect of Cas interactions with CA on the caseinate structure was studied by fluorescence spectroscopy.

Fluorescence emission spectra were recorded (295 nm to 450 nm with excitation at 280 nm) at room temperature on FluoroMax-4 (Horiba Jobin Yvon - Edison, NJ USA). The Cas concentration was kept constant (5 µM), and the CA concentration varied between 10 µM and 72.5 µM. Samples were prepared in 50 µM phosphate buffer, pH 7.40 and incubated 10 minutes at 310 K before recording the spectra.

The fluorescence spectra of the Cas solution and the Cas-CA solutions (**Figure 1**) indicate that the fluorescence of Cas tryptophan decreases with increasing concentration of CA, which acts as a quencher. To clarify the fluorescence quenching mechanism, the Stern-Volmer quenching constant (K_{sv}) was calculated. In addition, the binding constant, K_b, for Cas-CA was obtained from the Lineweaver-Burk equation. K_q is higher than the maximum scatter collision quenching constant of the biomolecule ($K_q = 2.0 \times 10^{10}$ M^{-1} s^{-1}), which indicates that a static quenching mechanism is the main responsible of Cas fluorescence quenching, resulting in the formation of a complex with a K_b value of the order of magnitude of other complexes between proteins and phenolic compounds (**Table 1**).

Therefore, the results indicate that there was a change in the tertiary structure of the protein, which suggests a possible strategy to alter the allergenicity of these milk proteins.

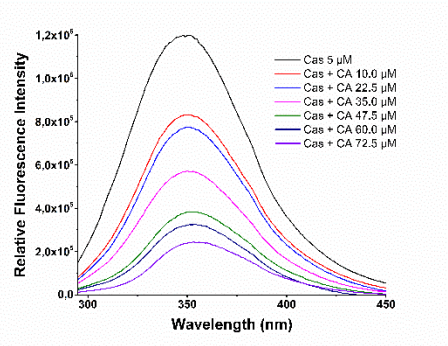

Figure 1: Fluorescence spectra of Cas (5 µM) at 310 K with CA at pH7.40 solutions.

Table 1: The Stern-Volmer (K_{sv}), quenching (K_q) and binding (K_b) constants between Cas (5 µM) and CA ($K_q = K_{sv}/10^{-8}$).

CA	K_{sv} (M^{-1}) x10^4	K_q (M^{-1} s^{-1}) x10^{12}	K_b (M^{-1}) x10^4
	5.65	5.65	4.22

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Quantification and characterization of polyphenol content in apple products

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Polyphenols result from the secondary metabolism of plants and they have gained great attention because of their widely proven bioactivities (1,2). Chemically, polyphenols have multiple phenol units, which confer antioxidant properties. Free radicals can damage the lipids of cell membranes and/or disrupt the structure of nucleic acids (3). Due to their antiradical activity, polyphenols can revert the oxidative stress promoted by free radicals. Indeed, they promote defense against several pathologies such as cancer, microbial infections, cardiovascular disease, help control symptoms of menopause, and many others(1–3). Among polyphenol-rich sources apples stand out lowering the level of cholesterol and triglycerides preventing cancer, atherosclerosis, diabetes mellitus, and Alzheimer's(2).

Apple, the major fruit consumed in Portugal, has been described with more than 60 polyphenol compounds highlighting the dihydrochalcone phloridzin and the flavonoid quercetin by their high bioactivities (1). On one side, phloridzin has been described as an inhibitor of glucose transport in the blood, and on the other side, quercetin can stimulate the production of osteoblasts and decrease the activity of osteoclasts presenting anti-inflammatory properties that help the treatment of rheumatoid arthritis (1). Polyphenol content in apples depends on geographical origin or variety. Furthermore, processing techniques would increase, decrease, or modify their profile in polyphenol compounds(3). This study aims at understanding the effect of apple processing in the polyphenol profile to obtain juice and puree depending on 1) centrifugation and 2) temperature. The samples were prepared at a national food industry company as follows: from the same raw apple puree, this sample was 1.1) centrifugated at 4400/10 rpm, and 1.2) centrifugated at 4400/35rpm giving turbid juices 1.1. and 1.2. Juice 1.2 was then clarified by filtration at 2.1) 60°C and 2.2) 40°C. To quantify and characterize the polyphenol profile, juices were then purified by Solid Phase Extraction (SPE) C18 Column to remove sugars and other interferent compounds. Turbid juices (1.1. and 1.2) were centrifuged before the SPE procedure. To analyze the bounded polyphenols, the obtained precipitates were further homogenized by ultra-turrax with 20% formic acid in methanol followed by basic and acid hydrolysis. The total polyphenol content was assessed by the colorimetric assay Folin-Ciocalteu and the polyphenol profile was characterized by high-performance chromatography coupled to UV-vis and tandem-mass spectrometry.

The juices 1.1 and 1.2, have the lowest content of total polyphenols, 0.470±0.025 mg/mL and 0.770±0.024 mg/mL, respectively. The lower content in polyphenols could be explained by polyphenol compounds being trapped in the precipitates collected by centrifugation. So, the increase in the differential velocity of centrifugation seems to result in higher soluble polyphenol concentration. Regarding the clarified juices (2.1 and 2.2) results show that the major content of polyphenols was for the clarified juice at 40°C with 1.081 ±0.130 mg/mL, while clarified juice at 60°C had 0.763 ±0.018 mg/mL (statistical different). This result seems to indicate that temperature leads to a decrease in polyphenols content. Ongoing analyses are exploring the polyphenols present in the precipitates. Regarding the polyphenols profile, the main polyphenols were chlorogenic acid and procyanidins.

In conclusion, filtration and centrifugations may affect the polyphenol content. The results suggested that apple fruit matrices can offer a high content of polyphenol compounds depending on the process, and in the end, their consumption can contribute to promoting health benefits.

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Method validation for determination of amino acids in feedstuffs by HPLC

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A method for the quantification of amino acids by reverse phase-high resolution liquid chromatography (RP-HPLC) with ultraviolet-visible detector (UV-Vis) in the range 190-600 nm was developed adapting a procedure used for wine and beer[1]. In this way, it was possible to include the amino acid composition in the nutritional tables of several by-products (https://www.subpromais.pt/conteudo2.php?idm=15).

With this method derivatized compounds are obtained, with high stability and good resolution in chromatographic analysis, allowing the use of RP-HPLC-UV-VIS equipment instead of a fluorescence detector in the simultaneous determination of amino acids in different types of feedstuffs.

Amino acids were identified according to their retention time and quantification was performed at 280 nm using the internal standard method (**Figure 1**).

Method validation included the determination of the following analytical performance characteristics for 17 amino acids in different feeds: the limit of detection calculated on a barley grain sample ranged from 0.29 $g.kg^{-1}$ (alanine) to 0.78 $g.kg^{-1}$ (histidine); recovery rates on a sample of tomato pomace giving values in a range of 94 % (isoleucine) and 107 % (valine); relative standard deviation of repeatability and intermediate precision ranged from 0.9 % (phenylalanine) to 3.1 % (cysteine) and from 3.8 % (lysine) to 8.5 % (proline), respectively; the combined uncertainty of the amino acids was from 1.8 % (tyrosine) to 6.8 % (valine).

The performance characteristics are similar to those referred in the bibliography[2,3]. The developed HPLC-UV-Vis procedure is therefore a suitable and efficient method for the determination of amino acids in feedstuffs.

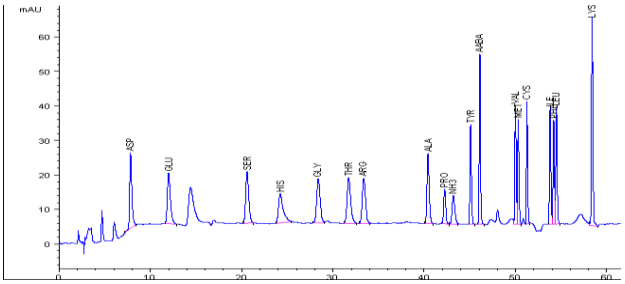

Figure 1: Chromatogram of amino acid standard analyzed at 280 nm

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Pulsed electric field technology as pretreatment to enhance strawberries (*Fragaria ananassa*) drying efficiency and physicochemical quality

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Strawberries (*Fragaria ananassa*) are highly appreciated as a seasonal fruit in innumerous countries around the world. Official data reported that the worldwide production of strawberry in 2020 was around 8.86 million tons.[1] However, strawberries are one of the most delicate and perishable fruits with a very high respiration rate, weight loss and susceptibility to fungal attack.[2] Currently, the best drying method applied for strawberries is freeze-drying. Nonetheless, freeze-vacuum drying is an extremely energy-consuming operation. In order to overcome this problem, the effect of pulsed electric field (PEF) at low electric fields has been evaluated in different foods such as tomato, apple and strawberry. When PEF is applied the biological membrane is electrically pierced and loses its permeability temporarily or permanently, which can allow for improving drying and freezing processes.[3]

This study evaluates PEF technology as pretreatment to enhance the effectiveness of strawberry drying and improve fruit's properties.

Fresh strawberries (var. Savana) were obtained at an industrial unit (Horta Grande Agrifood Company) of Abrançalha de Cima, Abrantes. Strawberry fruits (≈10 kg) were produced in semi-hydroponics conditions and manually harvested. The fruits were pretreated with PEF (treatment was applied using a bipolar pulse protocol) and dried by freeze-drying (at a pressure of 0.06 mbar). Afterwards physicochemical characteristics such as, ascorbic acid, color, pH, a_w, rehydration, and texture were measured. Also, morphological analysis by scanning electron microscopy was made. Comparisons were made between fruits dried by conventional hot-air drying and freeze-drying, with and without PEF pretreatment. The conditions of different drying processes are described in (**Figure 1**). Before freeze-drying, the samples were frozen using a rapid air-blast freezer (−35 °C). The temperature inside the sample geometrical centre was measured with a thermocouple. Finally, the dried strawberries were packaged in anti-humidity metallized bags and stored at room temperature for subsequent analysis. The freezing and drying time were also evaluated. Furthermore, consumer-based sensory evaluation was conducted using (i) acceptability and (ii) preference tests. All parameters were analyzed in dried strawberries slices. The results were compared by analysis of variance (ANOVA). The Tukey's test at a significance level of 5 % was used as a post-hoc test using the GraphPad Prism v6. Ink software. When comparing PEF treated and PEF untreated samples which will be freeze dried afterwards, the results showed that PEF pretreatment has the potential to reduce freezing in 5 %. Furthermore, PEF treatment combined with freeze-drying reduces drying time in 34 % (**Figure 1**) when compared with freeze-drying treatment alone as a control. As a consequence, total energy consumption, which is a critical industrial and economic aspect, is also reduced. In fact, in PEF treated fruit tissues, the heat and mass transfer processes are enhanced when compared to non-treated tissue, due to electroporation.[3] Also, PEF treated samples were characterized by high structural quality and high rehydration rate. Nonetheless, all samples pretreated with PEF when compared to freeze-drying samples alone, maintained ascorbic acid content. Moreover, color and texture analysis showed similar results. In addition, the sensory analysis indicated that freeze dried strawberries with and without PEF pretreatment had similar consumer acceptance, mainly through the preserved of the fresh strawberries' odor, color and flavor. However, fruit pretreated by PEF had the highest preference.

In conclusion, the application of PEF treatment before freeze-drying allows greater productivity and sustainability at the industrial level drying process and ensures greater preference for the product by the consumer. Based on the promising results obtained, the scale-up of this application to an industrial scale should be addressed.

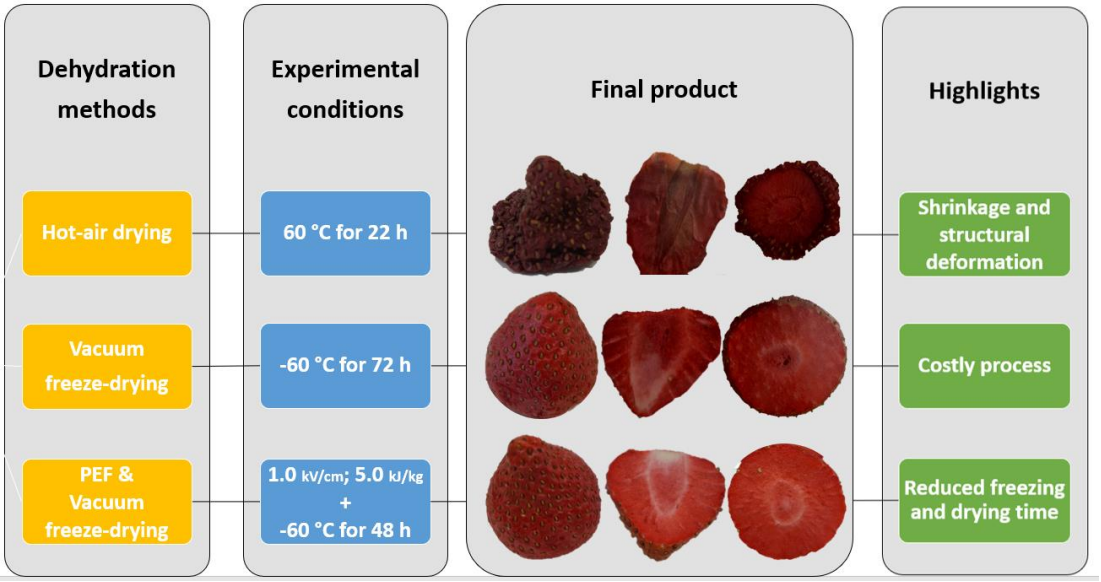

Figure 1: Methods and results of strawberries drying by different methods such as hot-air drying, conventional freeze-drying and PEF combined with vacuum drying.

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Shared co-first authorship: The second author contributed equally as the first author.

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Comparative evaluation of regional olive cultivars for potential transformation into table olives

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The Mediterranean region, due to its soil and climate conditions is favorable to the development of the olive tree (*Olea europaea* L.), with more than 90% of the world's production of olives and oil. Table olives, processed from healthy olive fruits, are appreciated and consumed for their sensory, chemical, and nutritional characteristics, playing an important role in the Mediterranean diet. From a nutritional point of view, in addition to the quality of their fat, which is mostly monounsaturated, they have an appreciable amount of natural antioxidants, especially phenolic compounds, tocopherols, and some vitamins and minerals[1,2].
Traditionally, in the various regions of Portugal, fruits of different cultivars, adapted to each region, are used for the processing of table olives, namely cv. Negrinha de Freixo in Trás-os-Montes and the cv. Azeitoneira in Alentejo. The suitability of the fruits required for this process is largely determined by the attractive characteristics of the fruit and by a set of criteria, such as size, taste, shape, firmness, good pulp/stone ratio, ease of detachment of the pulp from the respective pit and low levels of fat[3]. All these attributes are crucial to the success of the technological process to which the fruits are submitted to make them edible. This work aimed to characterize nine fruit regional olive cultivars for potential transformation into table olives: Judiaga, Galego de Évora, Carrasquenha de Elvas, Gama, Conserva de Elvas, Ocal, Azeitoneira, Maçanilha de Tavira and Galega. The fruits of each cultivar, from the INIAV/Elvas experimental field, were evaluated against morphological (biometrics, mass, pulp/stone ratio) and physicochemical (moisture, fat content, and pulp firmness) parameters. The results were submitted to Cluster analysis with the aim of grouping the olive cultivars according to the quality attributes analyzed.
Marked differences were shown between the cultivars, namely in terms of fruit morphology (size and mass), the pulp/stone ratio (maximum of 6.6 for Judiaga and minimum of 2.6 for Galega Vulgar), and firmness (80 N for Judiaga and 28 N for Azeitoneira). Two distinct groups were identified, the first is constituted only by the cv Judiaga and the second is where the remaining olive cultivars are grouped. The fruits of cv Judiaga showed the best suitability for transformation into table olives.

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Application of an electronic nose to differentiate extra virgin olive oils according to the geographical origin: Côa *versus* Douro regions

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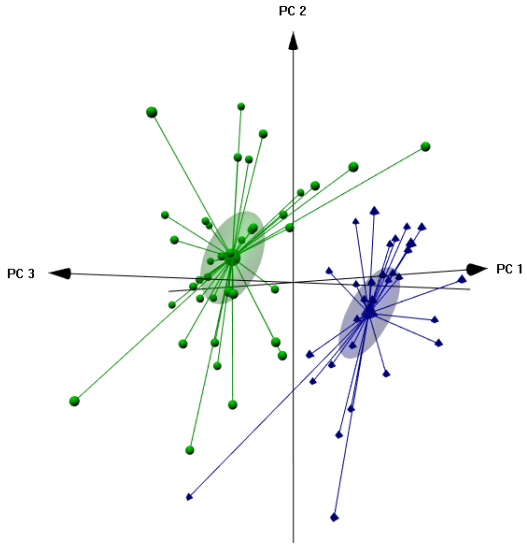
Olive oils produced in the Douro's region (north of Portugal) are greatly appreciated due to their specific chemical-sensory characteristics, which can be associated to the region's "Terroir". Thus, for olive oil producers and consumers, the capability to guarantee the authenticity of the geographical origin of these oils is of most importance. In this sense, this study aimed to evaluate the possibility of using an electronic nose (E-nose), with nine metal oxide semiconductors (MOS), as a non-invasive analytical tool to recognize the geographical origin of olive oils from two geographical sub-regions: "Côa" and "Douro". For each region, 40 different oils were collected, and the chemical quality (acidity, peroxide value and extinction coefficients) and sensory profiles were evaluated. From the initial samples, only those fulfilling the legal thresholds of extra virgin olive oil (EVOO) category were further used [1], being kept 38 and 31 different EVOO from "Côa" and "Douro" regions, respectively. For the majority of the oils, 9 olfactory attributes were perceived by the panellists (fruity – green or ripe; apple; banana; tomato and tomato leaves; dry fruits; cabbage; fresh and dry herbs) and 14 gustatory attributes (sweet; bitter; pungent; fruity – green or ripe; apple; banana; tomato and tomato leaves; dry fruits; cabbage; fresh and dry herbs; plum; olive leaves). Olive oils from "Douro" region were more sweet and had greater intensities of fruity-ripe, banana and dry herbs sensations. On the other hand, "Côa" olive oils were bitter and pungent showing higher intensities of fruity-green, tomato and tomato leaves, cabbage, fresh herbs and olive leaves sensations. Considering the differences perceived at the sensory level, namely at the olfactory profile, a lab-made E-nose [2] was applied. The results showed that this sensor-based device could identify the geographical origin of the studied olive oils. In fact, the principal component analysis (PCA) of the average response curve of the sensors' signals (resistance data) enabled a satisfactory unsupervised differentiation of the 69 olive oils according to the two geographical regions studied (Figure 1). The satisfactory performance could



be tentatively attributed to the abovementioned different olfactory profiles found for the oils, depending on their origin.

Figure 1: E-nose-MOS performance: 3D PCA plot (PC1: 61%; PC2: 23%; and, PC3: 4%) regarding the unsupervised differentiation of "Côa" olive oils (green filled regions) and "Douro" olive oils (blue filled triangles), based on the average resistance response curves of the 9 MOS sensors comprised on the sensor device.

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region: rediscovering the past to valorise the future" (ref. COA/BRB/0035/2019). Nuno Rodrigues thanks to National funding by FCT-Foundation for Science and Technology, P.I., through the institutional scientific employment program-contract.

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Blend olive oils: mixing two monocultivar olive oils (Cobrançosa and Arbequina) for improving sensory and chemical characteristics of olive oils

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All olive cultivars may be used to produce olive oils. Each cultivar has a unique organoleptic and chemical profile that could be influenced by the environmental and agronomic aspects of the production as well as its extraction technics. The blend of the different cultivars is a common technique, especially in the olive oils extracted from olives proceeded from traditional olive groves, where different cultivars are grown side by side. Monocultivar olive oils that are usually produced on intensive and high-intensive olive groves often presented a higher quality but are less complex from an organoleptic standpoint than blended olive oils. On the other hand, certain monocultivar olive oils, a few months after extraction, exhibit characteristics like high spice and pungent content that consumers find less appetizing, whereas other olive oils are quite sweet and fruity. The usefulness of blending different olive oils with different characteristics can create a more balanced olive oil from a chemical and sensory point of view. For example, cultivars like Cobrançosa (one of the most cultivated Portuguese olive cultivar), with strong sensory attributes after extraction, keep their organoleptic properties considerably better than other cultivars like Arbequina. Arbequina olive oils (a widespread cultivar) are known to present low phenolic content (natural antioxidant compounds), consequently presenting a more limited shelf-life with soft sensory attributes. [1] Cobrançosa olive oils are very rich in polyphenols and presented a higher bitter and pungent flavor[2], with a higher shelf-life. The blend of the oils of these two cultivars can result in a balance between pleasantness and shelf-life. To do this it is necessary to have a deep knowledge of the characteristics of each cultivar and the interaction between them, due to not all mixtures can create a balanced and graceful blend. In this regard, the present work aims to evaluate the organoleptic and chemical characteristics of the blend between Cobrançosa and Arbequina cultivars varying their percentages. Ratios of 80:20, 60:40, 40:60, and 20:80 (*cv.* Cobrançosa: *cv.* Arbequina) were chosen and placed in the dark at room temperature (25ºC) for different periods of 0, 2, 4 and 6 months to be studied. Quality parameters (acidity, peroxide value, UV specific extinction coefficients), descriptive sensory analysis, volatile compounds, oxidative stability by Rancimat technique, total phenol content, and antioxidant activity by DPPH method were used to assess the alterations that occurred (**Figure 1**). *Cv.* Arbequina and the blend 20:80 (*cv.* Cobrançosa: *cv.* Arbequina) presented the highest loss of antioxidant activity. In terms of quality parameters, the blend 20:80 (*cv.* Cobrançosa: *cv.* Arbequina) showed the most significant differences, at the end of 2 months already observed the highest increase of peroxide value (≈33%), K_{232} (≈28%), and K_{268} (≈26%). During the 6 months, it was observed a reduction of the total volatile amounts, together with the positive sensory attributes fruity, green, bitter, and pungent as expected. Although, the blend 20:80 (*cv.* Cobrançosa: *cv.* Arbequina), at the end of the 2 months, already present the most significant loss of the aldehyde compounds (*E)*-2-hexenal and 2-methylpent-4-enal and a higher increase of alcohol compound (*E*)-hex-2-en-1-ol. The present work allowed concluding that in the different blends studied, despite being classified as extra-virgin, the peroxides and conjugated dienes were significantly higher while there was a reduction in antioxidant capacity as well as in phenolic compounds and oxidative stability. Globally, according to chemical and sensory analysis preformed the mixture of 20:80 (*cv.* Cobrançosa: *cv.* Arbequina) was the most unbalanced blend after 6 months of mixture.

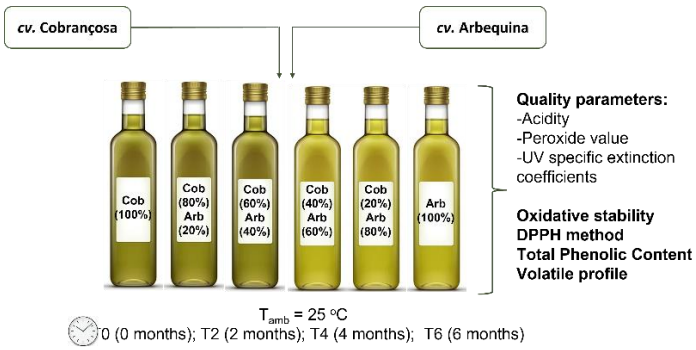

Figure 1. Experimental scheme.

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Endogenous Algarve pomegranate to improve nutritional benefits in the development of a pie

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Sustainable development involves the consumption of local food products, to reduce energy spending and pollution[1]. The Algarve region, due to its climatic conditions, is rich in food products with high bioactive compounds and nutritional value. In this work, a pie was developed using endogenous products from the Algarve region, such as carob flour, almond flour, honey, orange zest and pomegranate. Pomegranate has become popular for its medicinal properties and its nutritional benefit in the human diet[2]. Unfortunately, it is a seasonal fruit and seldom used in the food industry. Many pomegranates are not even harvested despite their high content of bioactive compounds. In this work, the pomegranate juice was dehydrated at 35 ºC, for 4 days, into an air circulation hothouse, to maintain its pink colour and to be usable at any time during the year. The pomegranate was used to produce a beautifully coloured topping for the pie, and to improve its nutritional value.

Variables in this study were the presence of polyphenolic compounds, analysed by the Folin-Ciocalteu method[3], the antioxidant activity estimation by DPPH and ABTS[3], and a sensorial assessment in 150 secondary-school Portuguese students. In the sensory assessment, basic tastes (acid, bitter, sweet, and salty) are rated by participants on a scale of 1 to 5, where 1 is imperceptible and 5 is very noticeable. Nutritional composition for the pie was also determined using validated food composition tables.

Analyses were performed in triplicate. The content of phenolic compounds was 84.8 ± 10.2 mg/g of pie without pomegranate. Dehydrated pomegranate present 260.4 ± 3.6 mg/g. For each serving of pie, set at 100 g, 0.5 g of dehydrated pomegranate topping is added, prompting an increase of about 15% in phenolic compounds. Similar increases were obtained for antioxidant power when using dehydrated pomegranate topping over the pie.

The sensory assessment shows a contrast between salty and sweet, with a slight complement of acid and bitter, which are also perceptible, although with less intensity. **Figure 1** represents the average of the 150 responses for each of the basic tastes.

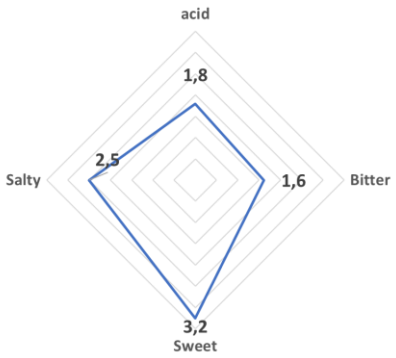

Figure 1: Spider diagram that summarizes the pie basic tastes average responses of 150 tasters, where 1 represent imperceptible and 5 very noticeable

To complement the sensory assessment, 97.3% of participants (n=147) stated that they would consume this pie in a restaurant.

The caloric intake from one serving of pie is 567 kcal, putting it on par with other desserts meant to be served on special occasions.

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Sensory and chemical characteristic of white monovarietal wines produced from varieties more adapted to abiotic stress

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The search and study of varieties that are better adapted to climatic variations is one of the strategies to help the wine sector to overcome the challenges that will arise in the future. Within this framework and within the scope of the WineClimAdapt project (project code PDR2020-101-031010), the adaptability of around 100 white grape varieties was evaluated in the hottest and driest region of Portugal and the twenty five best suited varieties were used to produce monovarietal white wines. The vineyard (Herdade do Esporão, Alentejo, Portugal) was submitted to deficit irrigation (Water stress coefficient-Ks≈0.5) from pea size to maturation and grapes from 2021 harvest were vinified in the INIAV experimental cellar in duplicate and the wines produced were subjected to physical-chemical and sensory analysis.

The fifty wines were profiled by the tasters panel, based on the intensity evaluation of several sensory attributes (four visual; eight olfactory and five gustatory attributes), using a structured scale (0-no perception to 10-highest perception). The tasters were asked to rate also the overall quality of the wines, from 0 to 20. At the same time, the wines were analysed concerning their alcohol strength, fixed, total and volatile acidity, density, pH, reduced sugars and the absence of malolactic fermentation was confirmed.

The multidimensional analysis (Principal component analysis) of all the results shown a discrimination of the wine samples. The variables with major contribute for that separation were pH, acidity, odour attributes such as floral and fruity notes and some gustatory attributes (body and persistence). The results pointed out significant differences (from the ANOVA analysis) across the overall quality of the wines with higher significant values rated to the wines produced from Fernão Pires, Sarigo, Parellada, Riesling, Chenin, Dona Branca and Cercial grapes. Additionally, some of these white wines exhibited high values of acidity (**Figure 1**) which is very interesting for a hot grapevine region.

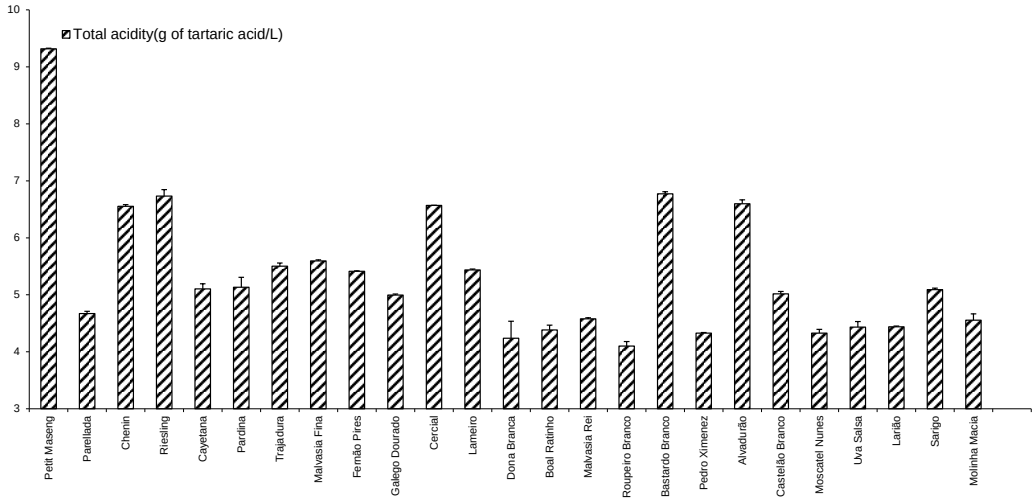

Figure 1. Average values and corresponding standard deviation for total acidity of the twenty-five monovarietal wines

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Influence of pH on lipid oxidation, lipolysis and proteolysis susceptibility of pork dry cured sausage

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Lipid oxidation is considered as one of the main causes of meat products quality deterioration, affecting its nutritional, sensory and technological characteristics, and also causing adverse effects on consumer health. The presence of unsaturated lipids, heme pigments and other oxidizing metals in muscle tissue makes meat susceptible to oxidative deterioration, resulting in discoloration, development of undesirable off-flavors, formation of toxic compounds and the consequent loss of shelf life and endogenous nutrients (Falowo et al., 2014). The economic implications for the meat industry make the control of the oxidative process a real challenge in this sector. Lipid oxidation depends on multiple intrinsic and extrinsic factors and on the balance between anti- and pro-oxidant compounds. The composition of fatty acids and the presence of pro-oxidant enzymes are relevant intrinsic factors, as they constitute the main substrates for the lipid oxidation. Differently, the development of lipolysis and proteolysis related to fatty acids and amino acids release, respectively, is seen as crucial biochemical phenomena associated to the sensorial quality profile achievement. Muscle and adipose tissue proteins and lipids are subject to intense biochemical reactions, observed as substantial increases in non-protein nitrogen /free amino acids and free fatty acids concentrations respectively. All these changes are associated to diverse enzyme systems which may remain active and stable even after 8 months of processing/storage. For each enzyme involved (cathepsines B, D, H, L, aminopeptidases, acid estearase and lysossomal acid lipase) the effect of processing parameters, namely the pH can be determinant (falling from near 6.0 to values varying between 5.0 to 4.8). While variations found in free amino acids patterns do not affect sensory scores significantly the hydrolytic and oxidative changes in the lipid fraction during the early ripening stages are the source of flavouring compounds (carbonyl compounds). Muscle pH is recognized as an important factor influencing the rate and extent of meat lipid oxidation. Several studies have shown an inverse relationship between pH and lipid oxidation in meat. Higher pH values are associated with inhibition of lipid oxidation in pork. The pH decline facilitates the oxidation of the muscles components as H+ may promote the redox cycle of myoglobin and its pro-oxidant action. The aim of the present study was to evaluate the effect of pH on lipid oxidation, lipolysis and proteolysis susceptibility in pork dry cured sausage.

Nineteen pork cured meat products were produced in the pilot plant of INIAV and kept vacuum-packed at 4ºC until analysis. The pH of the salami was evaluated with a 654-pH meter (Methrom Herisau, Switzerland) equipped with a combined pH glass electrode (Metler Toledo, Switzerland). Lipid oxidation was evaluated through malondialdehyde determination by reverse-phase high-performance liquid chromatography (HPLC) as described by Roseiro et al. (2017). Acidity and free amino acids nitrogen (FAAN) were determined according to Santos et al. (2019) in order to evaluate the lipolysis and proteolysis, respectively. Acidity was expressed as oleic acid content. The products were grouped in 3 classes of pH up to 5.4; pH between 5.5 to 5.79 and pH between 5.8 to 6.0.

The results evidenced an inverse relationship between malonaldehyde content and the pH value of the products, with those having a pH range 5.8 to 6.0 presenting a significantly lower mean value (0.28 mg/kg), almost 2-fold, than counterparts with the pH $\leq$ 5.4 (0.45 mg/kg). In relation to the lipolysis and proteolysis indicators they both showed a direct relationship with the final pH of products but, the way they progress differed. While the FAAN content increased continuously a long the pH interval considered in the present study, the acidity value incremented significantly in products with the intermediate values when compared to counterparts with pH $\leq$ 5.4 but kept the content practically unchanged in those with pH between 5.8 to 6.0.

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Vitamin A and vitamin D contents of cow's milk and plant-based milk alternatives: preliminary comparison

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Cow's milk provides macro and micronutrients to the growth and development of the human body. However, consumers increased their interest in plant-based milk alternatives (PBMA) due to allergy to cow's milk, lactose intolerance, or as consequence of lifestyle changes. PBMA are perceived by consumers as healthy alternatives to cow's milk, however, some authors expressed their concerns regarding shifting cow's milk consumption to PBMA since the nutritional composition is quite variable between different PBMA types and even within the same type.[1-3] Therefore, the present study intends to present a preliminary comparison between cow's milk and PBMA vitamin A and vitamin D contents.

In this study 10 samples of cow's milk (half-skimmed and ultra-pasteurized) and 40 samples of PBMA (soy, oat, rice, and almond), all bought on the Portuguese market, were analyzed and used for comparison. Vitamin A and D were determined by normal phase in high-performance liquid chromatography (HPLC), using an ultraviolet detector for vitamin D determination (wavelength 265 nm) and a fluorescence detector for vitamin A determination (excitation/emission wavelengths of 325nm/475 nm). The data analysis was performed using the general linear model (GLM) procedure of statistical analysis system (SAS).

The vitamin D content did not significantly differ ($P>0.05$) between PBMA (averaging 0.64 µg/100 ml), while cow's milk showed the significantly ($P=0.005$) lowest value (0.23 µg/100 ml). However, this value was not significantly different from those presented by soy PBMA and rice PBMA (0.62 µg/100 ml and 0.75 µg/100 ml, respectively). Regarding vitamin A, no significant differences ($P=0.269$) were found between the contents of all beverages analyzed, averaging 21.7 µg/100 ml.

Due to their important role in vision (vitamin A) and bone health (vitamin D), an adequate daily intake of these vitamins is crucial. Therefore, considering the vitamins A and D contents reported above, the daily consumption of 100 ml of cow's milk by an adult man ensures 5.39% of vitamin A and 1.52% of vitamin D, while 100 ml of all PBMA ensures around 3.42% of the daily needs of vitamin A and 5.37% of the daily needs of vitamin D. The high vitamin D content observed on PBMA is probably the result of PBMA fortification. Additionally, some attention should be paid to PBMA vitamin contents as equal vitamin contents does not guarantee nutritional similarity due to differences in their bioavailability.[3]

The preliminary results presented herein showed that some PBMA may have equal or higher vitamin contents than cow's milk. However, some precaution should be paid since the nutritional similarity is not guarantee due to differences in bioavailability. Therefore, more studies are needed to determine the bioavailability of PBMA and establish if PBMA are or not substitutes of cow's milk.

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Olfactometric approach to screen the odorant compounds profile in red monovarietal wines produced from varieties more adapted to abiotic stress

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Two main strategies can be followed in order to reduce the damages caused by climate change in the wine sector, the mitigation (of the factors that cause climate change) and the adaptation (use of cultural techniques to limit damages caused by the phenomenon in question). One of the adaptation techniques proposed by researchers and which seems to give very promising results consists to explore within the viticultural germoplasm those varieties which are better adapted to the scenarios of the exacerbation of abiotic stress.
This work framed in the project WineClimAdapt project (project code PDR2020-101-031010) intend to evaluate the sensory characteristics of several monovarietal wines produced from very different varieties such as Petit Verdot, Marselan, Merlot, Touriga Franca, Syrah, Vinhão, Bobal, Preto Martinho, Corropio, Trincadeira, Tinta Caiada, Alfrocheiro, Alicante Bouschet and Touriga Nacional, growing in Esporão vineyard (Alentejo, Portugal).
Each grape variety was vinified at small scale in duplicate and the wines were evaluated by a sensory panel, which rated several sensory attributes (visual, olfactory and gustatory). The wines revealed interesting sensory quality but with a different sensory profile. Previous studies pointed out also differences in wines anthocyanin composition.[1] The volatile composition of these wines was studied in this work by Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) (**Figure 1**) and the odorant compounds were screened by Gas Chromatography Olfactometry (GCO) (**Figure 2**) in order to point out the key odorants. In the GCO analysis it was used the frequency of detection in order to ranking the impact of the compounds in the aroma.

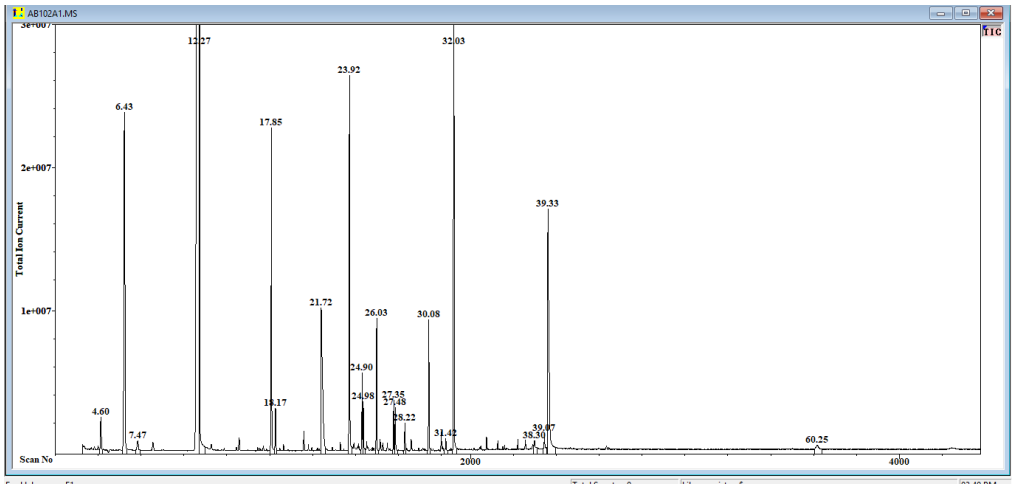

Figure 1: Total ion current (TIC) chromatogram of an Alicante Bouschet wine extract

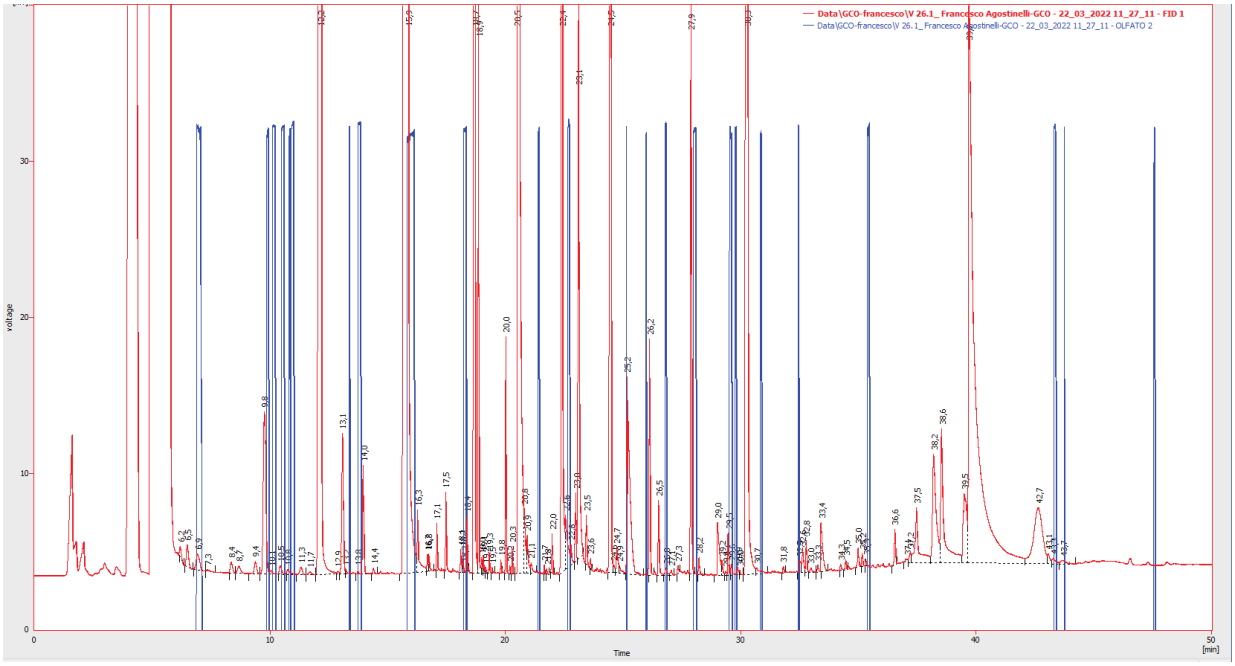

Figure 2: Chromatogram (GC-FID) and the corresponding aromagram (sniffer) of a Vinhão (V 26.1) wine extract

The obtained results pointed out a quite different GCO profile across the analysed wines in accordance with wines sensory results.

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Rheological properties of cranberry suspensions as affected by temperature and solid concentration

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Cranberry is a small fruit that grows in cold environmental conditions. The fruit is abundant in nutritional components, such as Vitamin C, Vitamin E, Vitamin K, and minerals, and many bioactive compounds with antioxidant properties, such as phenolic compounds, flavonoids (anthocyanins and flavonols), and tannins. These characteristics made cranberries recognized as important foods and healing agents (Nemzer et al., 2022). In Brazil, fresh cranberries are not widely available for consumption due to the climate of the country and usually are sold after processing. As pointed out by Nindo et al. (2007) it is important the careful design of equipment and operations for processing and handling to minimize the loss of physical and chemical quality attributes of processed foods made from berries. Controlling the oxygen and light exposure, the extension of heat, and churning during pumping are necessary to minimize the degradation of the beneficial compounds. In addition, the knowledge of the flow behavior of a suspension is of interest since it influences pumping, and mass and heat transfers (Nindo et al., 2005). Therefore, the present study aimed to determine the model that best represents the rheological behavior of cranberries suspensions and evaluate the effects of solids concentration and temperature in the rheological parameters of the selected model to provide important information for design improvements and control of product quality during some unit operations, as pumping and evaporation processes. Dehydrated cranberries were purchased in a local market and suspensions at concentrations of 10%, 11.45%, 15%, 18.54% e 20% (w/w) (independent variable X_1) were prepared by crushing the corresponding amount of fruit and water. Adapting the methodology of Nindo et al. (2007), using a concentric cylinder rotational viscometer (Brookfield LVDV – III ULTRA, USA) the rheological behavior of the suspensions was evaluated at 20, 25.82, 40, 54.18, and 60 °C (independent variable X_2). Four readings of ascending ramps (0 to 250 rpm) and four readings of descending ramps (250 to 0 rpm) were performed, changing the sample between one reading and another, to verify the reproducibility of the results. A 2 minutes step was selected to equilibrate the sample at the chosen temperature before data collection. Experimental data were adjusted to the following models: Newton ($\tau = \mu\dot{\gamma}$), Bingham ($\tau = \tau_0 + \mu_{pl}(\dot{\gamma})$), power law ($\tau = K\dot{\gamma}^n$), Herschel-Bulkley ($\tau = \tau_0 + K_H\dot{\gamma}^n$), Casson ($\tau^{0.5} = {K_{0c}}^{0,5} + K_c(\dot{\gamma})^{0.5}$), Sisko ($\eta_{ap} = \eta_{\infty s} + K_s(\dot{\gamma})^{n-1}$), and Mizrahi-Berk ($\tau^{0.5} = K_{0mb} + K_{mb}(\dot{\gamma})^n$).The adjustment of the data to the models was evaluated by the values of the coefficient of determination (R^2), the mean relative error ($P = \frac{100}{n}\sum_{i=1}^{N}\frac{|x_{exp}-x_{calc}|}{x_{exp}}$), the bias factor ($B_f = 10^{\left[\sum\frac{log\frac{x_{calc}}{x_{exp}}}{n}\right]}$), and the existence of initial shear stress (τ_0). The effects of X_1 and X_2 on the parameters of the Sisko model were evaluated using a 2^2 central composite rotatable design (CCRD) with three repetitions at the central point. The adequacy of the models was evaluated from the analysis of variance (ANOVA) at a confidence interval of 95% ($p \leq 0.05$). Within the evaluated models, Sisko was the one that best represented the experimental data with $R^2 > 0.994$, $P < 2.000\%$, and $B_f = 1.000$ for all the suspensions. The consistency coefficients (K_s) of the Sisko model vary from 0.243 ± 0.018 (X_1 = 11.45% and X_2 = 25.82 °C) to 0.796 ± 0.059 Pa s^n (X_1 = 20% and X_2 = 40 °C) with relative deviations from -30.6% (X_1 = 11.45% and X_2 = 25.82 °C) to 8.1% (X_1 = 15% and X_2 = 20 °C). The results indicated that increasing the concentration increased the consistency coefficients and this effect was significant and represented by: $K_s = 0.388 + 0.287X_1 + 0.146X_1^2$. The infinite-shear rate viscosity (η_∞) vary from 0.063 ± 0.007 (X_1 = 11.45% and X_2 = 54.18 °C) to 0.255 ± 0.017 Pa s (X_1 = 20% and X_2 = 40 °C) with relative deviations from -17.4% (X_1 = 11.45% and X_2 = 54.18 °C) to 11.8% (X_1 = 15% and X_2 = 40 °C). Both independent variables significantly affect η_∞. The temperature affected the parameter negatively and the concentration of the suspension influenced positively this index. The model representing these effects was: $\eta_\infty = 0.156 + 0.123X_1 - 0.037X_2$. The flow behavior index vary from 0,347 ± 0,107 (X_1 = 10% and X_2 = 40 °C) to 0,567 ± 0,095 (X_1 = 11.45% and X_2 = 25.82 °C), indicating that the suspensions presented non-Newtonian behavior. Any independent variable was significant for this parameter in the ranges studied. As a result, predicted values and relative deviations were not calculated, and a model wasn't obtained. The results pointed out that the pump of the suspensions should occur at higher values of temperature, because this variable negativelly affected η_∞.

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Olive oils quality from the Trás-os-Montes region: comparative analysis of the last three years

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Trás-os-Montes region is one important olive oil-producing in Portugal, and is characterized by a large number of low or medium producers that sell their olive oil. In general, it is recognized the high quality of the oils produced in this area, nevertheless, for the classification of its quality grade, the olive oils need to be subjected to physic-chemical and organoleptic assessment according to the European Regulation (EEC) No.2568/91[1]. For commercial classification, it is necessary to evaluate the parameter mentioned in the referred Regulation including free acidity, peroxide value, specific coefficient values at 232 and 268 nm, and organoleptic assessment, among others. According to the results of the different analyses, the oils will be classified into different commercial categories. In this sense, in this work, we proceeded to the comparative analysis of the quality, in terms of free acidity, peroxide value, and extinction coefficients in the ultraviolet and organoleptic assessment, of olive oils from local producers analyzed by the AgroBioTechnology Laboratory of the School of Agriculture of the Polytechnic Institute of Bragança, over three consecutive years (2020, 2021 and 2022). A total of 228 olive oils were analyzed for quality parameters and 154 oils were organoleptic assessmented. From the total of the analyzed samples (**Figure 1**), for free acidity in 2020, the values varied between 0.17 and 0.85%, with only one olive oil that above the limit set for Extra Virgin Olive Oil (≤ 0.8%). In 2021, the free acidity values varied between 0.11 and 1.92%, with 2 olive oils classified as Virgin Olive Oil (≤ 2%), and in 2022 the values varied between 0.17 and 1.64%, with 2 olive oils classified as Virgin Olive Oil. For the analysis of the peroxide value, in 2020 and 2022 the values were in under the limit established for the categories Extra Virgin Olive Oil and Virgin Olive Oil (≤ 20 mEq.O_2/kg of olive oil), between 1.66 and 15.81 mEq.O_2/kg of olive oil (2020), and 1.66 and 13.31 mEq.O_2/kg of olive oil (2022). However, in 2021, 5 samples were not within the limit established, being classified as Lampante Olive Oil. The results for specific coefficient values at 232 nm, varied between 0.93 and 2.97 in the year 2020, with two olive oils outside the limits for Extra Virgin Olive Oil (≤ 2.50) and Virgin Olive Oil (≤ 2.60), in 2021, 8 samples were above the allowed limit, being classified as Lampante Olive Oil, with values varied between 0.99 and 4.18. In the last year (2022) all samples were below the limit set for Extra Virgin Olive Oil (≤ 2.50) with values varied between 1.28 and 2.07. The results for specific coefficient values at 268 nm, in 2020, there was only one sample as Virgin Olive Oil (0.23), and the other samples were within the limit for Extra Virgin Olive Oil, with values between 0.08 and 0.19 (≤ 0.22). In 2021 there were 5 oils classified as Lampante Olive Oil, with values between 0.08 and 0.52. Lastly, in 2022, the samples were within the limit for Extra Virgin Olive Oil (0.09 and 0.19). The results obtained in the sensorial analysis of the olive oils (**Figure 2**), showed a decrease in the commercial classification of extra virgin olive oil and virgin olive oil over the years analysed. However, the median of fruitiness varied between 1.5 and 5.5 in the years 2020 and 2021, and between 2 and 5.5 in the current year (2022). In 2020, a total of 59% of olive oils were classified as Extra Virgin Olive Oil, 32% as Virgin Olive Oil, and 9% as Lampante Olive Oil. In 2021, 58% were classified as Extra Virgin Olive Oil, 30% as Virgin Olive Oil, and 12% as Lampante Olive Oil. In the last year of analysis (2022), there was a total of 30% of the olive oils classified as Extra Virgin Olive Oil, 42% as Virgin Olive Oil, and 28% as Lampante Olive Oil. The defects that predominated in the olive oils classified as Lampante were fusty, winey, musty, heated or burnt, and wood. These results confirm the necessity for the farmers to receive technical training in order to improve the quality of the oils that are already on the market.

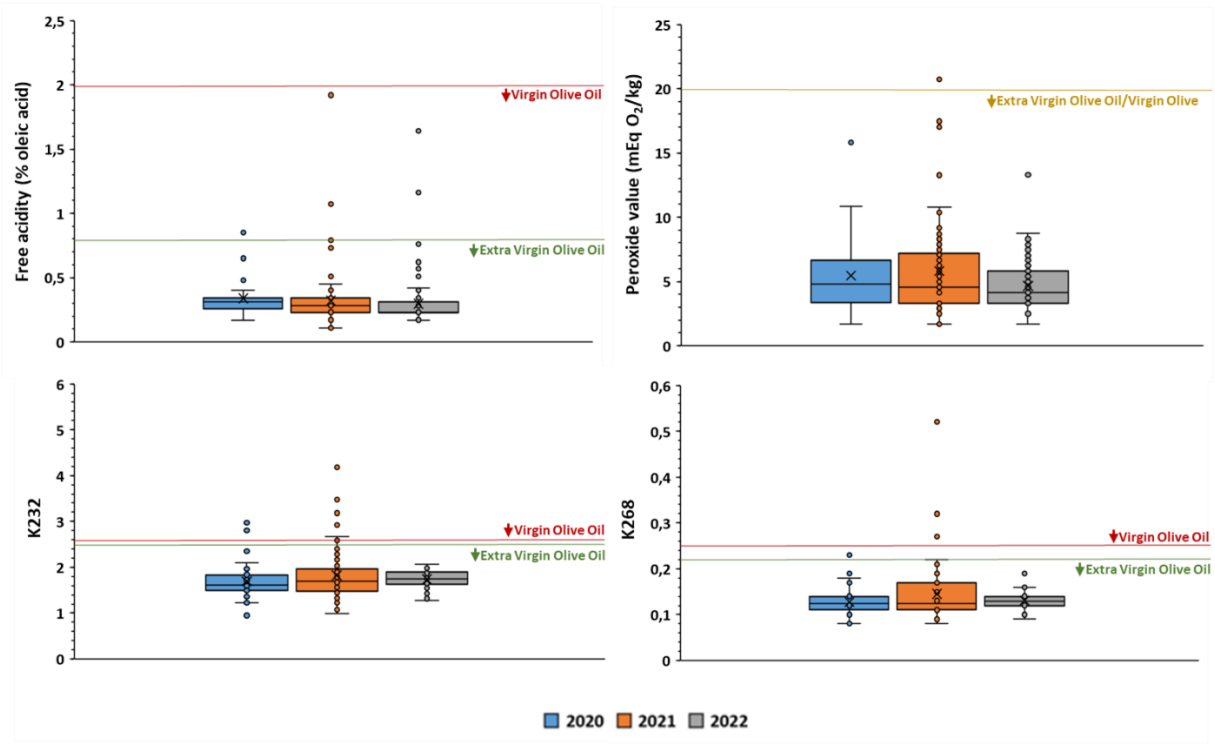

Figure 1: Physico-chemical parameters evaluated in olive oil samples according to the year (2020-2022).

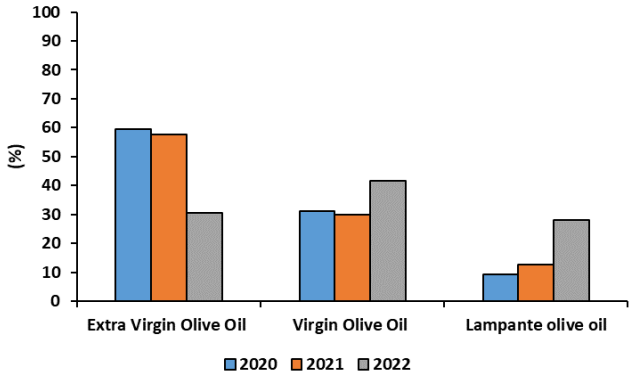

Figure 2: Distribution of olive oil commercial categories, by year, based on organoleptic assessment.

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Quality of tomatoes produced in a hydroponic system with nutritive solution of pre-treated agro-industrial wastewater

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The scarcity of water and food has been a growing problem in the world due to climate change [1], in this sense, the reuse of pre-treated agro-industrial wastewater for food production makes it possible to implement an integrated management of effluents in sustainable food production systems, according to the UN Sustainable Development Goals (SDGs).
The challenge of sustainable agriculture is also to recycle the waste produced and transform it into a valuable source of nutrients and water for agriculture. Pre-treated agro-industrial wastewater can be considered a source of nitrogen, phosphorus, potassium, organic matter, another macronutrients, and micronutrients for plant growth.
Cultivation in a hydroponic system represents an alternative to conventional cultivation, with advantages for both producers and consumers, namely in the production of quality products, with less water consumption and avoiding soil degradation [2].
In this sense, hydroponic systems fed by agro-industrial effluents from the production of cheese (CWW) pre-treated by immediate one-step lime precipitation process (IOSL) [3] allows the reuse of wastewater as a nutrient solution, avoiding its problematic discharge into municipal collectors, due to it's high organic load, and reducing the use of potable water for food production.
In this context, the objective of this work is to evaluate the quality of cherry tomatoes (*Solanum lycopersicum* L.) produced in a sustainable hydroponic system fed with a nutrient solution prepared from effluent of the production of cheese, which makes it possible to reduce the consumption of water and nutrients.
Three hydroponic drip systems (System 1 - hydroponic system without water depth, System 2 - hydroponic system with water depth and System 3- hydroponic system of deep-be) were developed for tomato crops that allowed free rooting of the crop where agronomic parameters were evaluated (number of fruits per plant, calibre, weight, firmness), physicochemical characteristics (pH, soluble solids (Brix), titration acidity, moisture and ash content), antioxidant compounds (vitamin C, β-carotene and lycopene) as well as sensory analysis (with a hedonic test to evaluate colour, smell, taste, texture, juiciness and overall acceptability).
It was observed that the cherry tomatoes produced by the developed hydroponic systems allowed the recovery of nutrients from agro-industrial effluents for food production, using only 25% of the amount of water and nutrients compared to conventional systems; produce higher quality and quantity of food and ensure the compatibility of reusing the nutrient solution at the end of the crop for soil irrigation.

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A look upon the adsorption of different families of polyphenols to salivary oral models: understanding the secondary mechanisms in astringency

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Polyphenols are abundant in the human diet due to widespread consumption of plant-derived food and beverage). Throughout the years, these types of compounds have been repeatedly associated to anti-cancer, anti-degenerative, anti-oxidant properties and cardiovascular protection [1]. On the other hand, they have also been highly related to non-pleasant organoleptic sensory attributes such as astringency and bitterness of tannin-rich foodstuffs. Astringency is described as a tactile sensation of puckering, tightening and dryness in the oral cavity, commonly induced for by phenolic compounds [2]. The major mechanism attributed to this phenomenon is the interaction between salivary proteins and polyphenols and respective formation of insoluble complexes that precipitate in the oral cavity. However, more recently, this research line is growing curious about the importance of secondary mechanisms in the perception of different subqualities of astringency. Currently, the disruption of salivary film, the aggregation and sedimentation of polyphenols in the mucosal pellicle, the interaction of polyphenols with the membrane of the oral cells and the implication of mechanoreceptors are examples of suggested secondary mechanisms in astringency. However, there is still little to no proof that can substantiate these theories. A recent study from our team as already shown some evidence that, depending on their structure, polyphenols may bind in different ways to the oral constituents and therefore elicit different mouthfeels [3].
Following those discoveries, in this study it was decided to further explore the secondary mechanisms in astringency. Oral models constituted by epithelial cells (HSC-3 or Caco-2), whole saliva and mucin, were applied to interact with three concentrations of a sensorial standard and different families of polyphenols: Aluminium Ammonium Sulfate Dodecahydrate (Alum; sensorial astringent standard; at 1 $g.L^{-1}$, 1.5 $g.L^{-1}$ or 2.75 $g.L^{-1}$), Grape seed extract (representative of procyanidins; at 0.2 $g.L^{-1}$, 0.6 $g.L^{-1}$ or 1 $g.L^{-1}$), Tannic acid (representative of hydrolysable tannins; at 0.1 $g.L^{-1}$, 0.3 $g.L^{-1}$ or 0.5 $g.L^{-1}$) and Green Tea Infusion (astringent food matrix rich in flavan-3-ols; at 1.2 $g.L^{-1}$, 2.4 $g.L^{-1}$ or 4.8 $g.L^{-1}$). After interaction, adsorption of polyphenols to the oral models was evaluated by colorimetric methods using Aluminon (for Alum), DMACA (for Grape Seed Extract and Green Tea infusion) and Folin-Ciocalteu (for Tannic acid). For Alum, results have shown that oral epithelial cells (HSC-3) had a four-fold to ten-fold increase in adsorbed compounds per cell layer area, compared to intestine epithelial cells (Caco-2). This stresses the importance of the cell-type used in the models, in order to form a well-structured mucosal pellicle, and the specificity of the interaction toward epithelia (different epithelia may interact in different ways depending on their origin). Also, the presence of saliva in oral epithelial models resulted in a higher adsorption of alum, up to 59%, when compared to models without that oral constituent (0.021 mg/cm^2; Adsorbed Alum per Cell Layer Area). Analyzing the interaction of Grape Seed Extract (GSE) and Tannic Acid (TA) with oral epithelial cell models, the resultant adsorbed compounds of GSE had up to an eight-fold decreased adsorption when compared to Alum (0.016 mg/cm^2), at similar initial concentrations. These results can be explained due to the nature of the compounds. Like manner to the polyphenols present in GSE and TA, Alum is known as a highly astringent compound, however it is not able to precipitate salivary proteins. Therefore, Alum higher adsorption by the oral cell-based models may explain its astringent abilities, thus supporting the importance of secondary mechanisms in astringency. Moreover, Green Tea Infusion (GTI) presented a different behavior when compared to the other compounds or polyphenol families. The results have shown that a higher compound adsorption was achieved in the models where saliva was not present, reaching up to a maximum of a two-fold increase. This could be explained by the smaller structure of the polyphenol compounds when compared to GSE or TA. GTI is majorly composed by catechins which have a much lower degree of polymerization and could allow those compounds to have a higher potential to penetrate or aggregate with the mucosal pellicle when salivary proteins are not present in that biological matrix. In the end, these results brought further insights to astringency studies, highlighting the importance in considering the interrelationship between all mechanisms to better evaluate and associate astringency subqualities.

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PDO Serpa cheese volatiles and descriptive analysis

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Volatile compounds of cheese come from the decomposition of the main constituents of milk including lactose, citrate, lipids, and proteins. These compounds are responsible not only for the characteristic flavour of the different varieties of cheese, but also for off-flavours, resulting for the formation of undesired compounds. Serpa cheese is a Portuguese Protected Denomination of Origin (PDO) cheese, produced from raw milk of sheep native region. A panel testing to evaluate cheese sensory characteristics and their correlation with the volatile profile and the physical-chemical properties applies quantitative descriptive analysis (QDA). Flavour evaluation using descriptive sensory analysis is a powerful sensory tool for cheese flavour research. It involves effective consumer testing to assess cheese flavour acceptability, preference, and consumer perception [1]. Although descriptive sensory analysis cannot provide analytical data on specific flavour compounds and flavour profiles, linking instrumental analysis with descriptive sensory analysis could effectively characterize specific flavours significantly influencing consumer acceptability where important flavour attributes were perceived and that directly correlate to consumer preferences.

In the present work, the volatile profile of ten Serpa cheeses was analysed by accredited panellists of Protected Denomination of Origin Serpa cheese, in the Sensory Analysis Laboratory of Polytechnic Institute of Beja. All cheeses submitted to a panel which sensory analysis respected the minimum total score of 14.0 in the total sensory parameter's classification and a minimum score of 4.0 in the Taste and Aroma will be accepted for PDO "Certification". This preliminary work intends to identify compounds that may be responsible for the sensory characteristics of different Serpa cheese when analysed by a sensory panel and by Solid Phase Microextraction, followed by Gas Chromatography-Mass Spectrometry, SPME-GC-MS. A set of ten samples of Serpa Cheeses, with 30 days maturation was used in this study. The sensory analysis of the cheeses was carried out by twelve accredited panellists of PDO Serpa cheese, for the following sensory attributes, in accord with the Specifications Book of Serpa Cheese: crust; shape and consistence; texture and paste color; taste and aroma. In the same cheeses, the volatile compounds were subjected to solid phase micro extraction (SPME) followed by Gas Chromatograpghy and Mass Spectrometry (GC/MS) to separate and identify the volatile compounds that mostly contribute to the bouquet of aromas in this kind of cheese.

In terms of sensory results, four of the cheeses had taste and aroma from 4.25 to 5.25 and a total of score from 14.7 to 17.45, which means these cheeses had score for certification demands. Three of the cheeses had in taste and aroma less than 4.0, but still the total score was from 14.0 to 14.7 and other three of the cheeses had no minimum scores in both taste and aroma as well as in the total score, therefore this six cheeses had not reached the minimum scores for certification. For that reason, these six cheeses were considered defective and the remaining four as non-defective, and in view of these results, all were set out for volatile compounds 'analysis. In relation to the results from the analysis by SPME-GC-MS, 144 compounds distributed in the following families were identified: esters; acids; alcohols, ketones; aromatic hydrocarbons; lactones, aldehydes, ethers; terpenes, alkanes; amides, furanones; sulfones, trisulphides, thiolane, nitrile and diol. In the defective cheeses, a greater number of compounds was identified, 143/144, while in non-defective cheeses only 123/144 appeared. Acids, alcohols, ketones, and esters were predominant volatile compounds in all the cheeses and therefore were involved in the basic cheese aroma of these ewes' raw milk PDO cheeses. In the cheese worst classified by sensory analysis, a great imbalance was observed between the group of acids, namely propanoic, butanoic and pentanoic acids, and the other families present, which may have given rise to unpleasant cheese flavours, compromising the PDO classification. This is corroborated by literature [2] where it was described that acids in high proportions, such as propanoic, pentanoic, octanoic and decanoic, led to acid and/or rancid off-flavour, as well as some minor odorant compounds, such as phenol for faecal off-flavour. These results show that both the quantification of odour-active compounds and its sensory odour description are necessary to detect off-flavours in cheese.

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Contribution to the chemical characterization of Algarve grapes and wines native's varieties "Negra Mole" and "Crato Branco"

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The Algarve wine region is in the southernmost of Portugal and it is characterized by climatic conditions, namely the Atlantic Ocean maritime influence, the presence of mountains in the north and the approximately 3000 hours of sunshine per year. However, the most important factor in the identity and character of Algarve wines is the diversity of its viticulturally heritage. The identity of Algarve wines is the native grape varieties, the red grape variety "Negra Mole" and the white grape variety "Crato Branco" or "Síria". The "Negra Mole" was the basis for the famous *clarete* and *palhete* Algarve wines[1]. This variety has a peculiarity of containing red, white and rose berries in the same grapes bunch. If a gently pressed is used a fresh white wine can be obtained. An intense press originates a fruity and fresh light rose wine. When macerated for a long time, light red wines are found. "Crato Branco", is a white native grape variety that produces fruity wines with citrus aromas. Most of the wines produced by this variety are fortified and originate unique wines in Portugal. According to some authors, the quality of a wine is directly related to the chemical composition of the grapes. Grapes are considered one of the largest sources of phenolic compounds compared to other fruits and vegetables. Different grape varieties result in different organoleptic and sensory characteristics (flavour and colour). The presence of certain organic acids in the grapes also affects the organoleptic characteristics of the wines. The main objective of the present work was to study the organic acids and phenolic compounds in these two autoctone grape varieties.

Tartaric, malic, lactic, gallic, vinylic, caffeic, shikimic and citric acids, catechin anthocyanins and resveratrol were analysing using reverse-phase high efficiency liquid chromatography at different wavelengths[2,3].

"Negra Mole" variety having the highest concentrations of tartaric acid (387.9 ± 0.6 mg/kg dry weight) and "Crato Branco" the highest content of malic acid (141.2 ± 0.2 mg/kg dry weight). The amounts of shikimic acid and citric acid detected in the two native grape varieties were very low. The highest amount of resveratrol (3.54 ± 0.01 µg/g dry weight) and anthocyanins as expected, was also found in "Negra Mole" grapes skin (0.07 ± 0.01 mg/g dry weight). In general, the red grape variety "Negra Mole" has a higher content of phenolic acids and the "Crato Branco" grape variety has a higher content of organic acids. In the Algarve wines studied, it was found that the red wines obtained from the "Negra Mole" grape variety presented a higher quantity of tartaric acid (6.13± 0.03 g/L) as found in grapes from the same variety, **figure 1**.

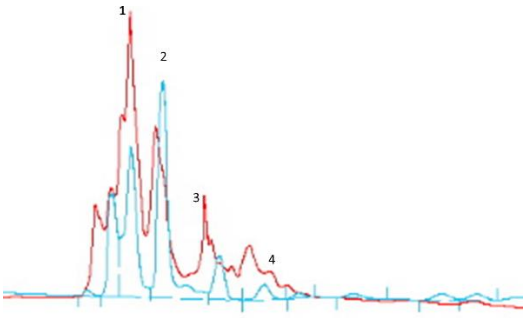

Figure: 1 - *"Negra Mole" chromatogram grape (blue) and wine (red). 1 – tartaric acid, 2 – malic acid, 3- lactic acid e 4 – citric acid*

The amount of malic acid in the analysed red and white Algarve wines were very similar. As observed in the grapes, the concentrations of shikimic and citric acid in the wines were not detected or were detected in very low concentrations, almost inexistent. Anthocyanins were only quantified in rose and red wines produced from the "Negra Mole" grape variety and their concentration was higher in red wines (420.56±0.01 mg/L). In rose wines, the anthocyanins content can vary from 1 to 13 mg/L depending on the production technology used. Curiously, the highest amount of resveratrol was not detected in the red and rose wines as it happens with the grapes but in the white wines produced from the white "Crato Branco" variety (9.94±0.01 mg/l).

Phenolic compounds play an important role in the quality of wine, particularly on colour and astringency.[4] A phenolic compound more detailed study is necessary to improve the knowledge of these grape varieties and the wines produced by them.

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Atlantic Yellowfin tuna loin protein content and quality

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Tuna is an important commodity worldwide. In the European Union, the yearly *per capita* consumption of tuna reaches 3.14 kg, which represents about 13% of the total consumption of fish. Among tuna, the Yellowfin tuna (YFT; *Thunnus albacares*) is one of the most important species, being accountable for 28.3% of the global tuna catches. This study aims to evaluate the Atlantic YFT loin protein content and quality.

A total of 15 specimens of YFT were used in this trial. The specimens were harvested in the Atlantic Ocean, within the FAO fishing area 34, in 2020. The left loins were removed, vacuum packed, frozen, transported to FMV laboratory and processed after defrosting. The Atlantic YFT loins were evaluated for total protein, and bioactive peptides contents and amino acid (AA) profile. Total protein content was determined by the Kjeldhal method, using the conversion factor of 6.25. The AA profile was determinate by reverse phase HPLC as previously described by Quaresma et al. 2022.[1] Bioactive peptides (anserine, carnosine and creatine,) were evaluated by HPLC according to the method described in detail by Mora et al. 2007.[2] The amino acid score (AAS) and the essential amino acid index (EAAI) were calculated using the formulas presented below and in accordance with the reference protein amino acid pattern previously established by WHO/FAO/UNU.[3]

$$\text{AAS} = \frac{\text{mg de amino acids in 1g de protein in evaluation}}{\text{mg de amino acids in the reference protein}} \times 100$$

$$EAAI = 100 * \sqrt[n]{\frac{Aa1_c}{Aa1_{rp}} \times \frac{Aa2_c}{Aa2_{rp}} \times \ldots \times \frac{Aan_c}{Aan_{rp}}}$$

n: number of essential amino acids
c: protein in analysis
rp: reference protein

Data on total protein, total AA and bioactive peptides contents, AA partial sums, AA ratios are displayed in Table 1, while the AA profile is presented in Table 2, together with the AAS for essential AA. The total AA content (usually considered the true protein content) averaged 20.5 g/100 g of loin representing just 71.5% of the total protein value determined by the Kjeldhal method. The AA profile is dominated by the essential AA (43% of total AA; 8.95 g/100 g of loin), followed by the dispensable AA (34% of total AA; 7.1 g/100 g of loin) and the conditional essential AA (22% of total AA; 4.47 g/100 g of loin). Among individual AA, the glutamic acid (14.96%), was the predominant AA, followed by glycine (12.27%), histidine (12.16%), aspartic acid (8.40%) and serine (8.20%). Beyond AA, it was possible to identify and quantify three bioactive peptides, namely, anserine (572 mg/100 g of loin), creatine (323 mg/100 g of loin) and carnosine (17.5 mg/100 g of loin).

The essential AA index (EAAI) of the YFT loin was 31.85, being classified as inadequate for human nutrition, since a protein source with a EAAI value below 75% is classified as inadequate for humans. The assessment of the amino acid score (AAS) for each essential AA allowed the identification of eight limiting amino acids (LAA) in Yellowfin loin protein, the ones presenting a value below 100%, namely: tryptophan (12.9%), leucine (16.0%), methionine (22.4%), isoleucine (23.2%), valine (25.7%), phenylalanine (26.9%), lysine (38.0%) and threonine (69.1). The identification of the LAA is an alternative approach to evaluate protein quality of most foods, and the YFT loin presents eight LAA out of nine essential AA, and tryptophan is the most LAA with an AAS of just 12.9%

The YFT loin protein presented an EAAI of just 31.85% being classified as inadequate for humans, while the determination of the amino acid score (AAS) identified 8 limiting amino acids (LAA) out of 9 essential AA. Beyond this negative attributes, the YFT loin presents an high total AA contents and remarkable contents of anserine and creatine.was classified as inadequate, the lowest classification associated with the EAAI, while tryptophan with a AAS of just 12.9% was classified as the most limiting AA.

Table 1 – Total protein and total AA contents, partial sums of prime AA classes, protein and AA ratios, and bioactive peptide contents

Atlantic Ocean YFT	
Total proteín[1]	28.68
Total AA[1]	21.52
Partial sums	
Σ Dispensables[1]	7.10
Σ C. essentials[1]	4.47
Σ Essentials[1]	8.95
Bioactive peptides	
Anserine[2]	571.6
Carnosine[2]	17.49
Creatine[2]	322.6
Ratios	
Total AA / Total proteín[3]	0.75
DAA/T[4]	0.33
CIAA/T[5]	0.21
EAA/T[6]	0.42
EAA/DAA[7]	1.26

[1] expressed in g/100 g of loin;

[2] expressed in mg/100 g of loin;

[3] Total AA/total protein contentes;

[4] Σ Dispensable AA/total AA;

[5] Σ Condicional essential AA/total AA;

[6] Σ Essential AA/total AA;

[7] Σ Essential AA/Σ Dispensable AA

Table 2 – Amino acid contents (expressed as g/100 g of YFT and as % of total AA content) and amino acid score (AAS)

Atlantic Ocean YFT			
	g/100 g[1]	%	AAS
Essentials			
Histidine	2.62	12.17	174.5
Isoleucine	0.70	3.25	23.21
Leucine	0.94	4.37	15.96
Lysine	1.71	7.95	38.01
Methionine	0.49	2.28	22.35
Fenilalanine	0.82	3.81	26.89
Treonine	1.59	7.39	69.10
Triptofan	0.08	0.37	12.89
Valine	1.00	4.65	25.67
Condicional Essentials			
Arginine	0.84	3.90	--
Glycin	2.64	12.27	--
Proline	0.79	3.67	
Tirosine	0.20	0.93	--
Dispensables			
Alanine	0.07	0.33	--
Aspartic acid	1.81	8.41	--
Cysteíne	0.24	1.12	
Glutamic acid	3.22	14.96	--
Serine	1.76	8.18	--

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The Atlantic yellowfin tuna (*Thunnus albacares*) fatty acid signature

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Tuna is an important commodity worldwide. In the European Union, the yearly *per capita* consumption of tuna reaches 3.14 kg, which represents nearly 13% of the total consumption of fish. Among tuna, the Yellowfin tuna (*Thunnus albacares*) is one of the most important species, being accountable for 28.3% of the global tuna catches. The Yellowfin tuna (YFT) is a species of great economic importance, since its meat is quite versatile being consumed raw, cooked, smoked, and canned[1].

To evaluate the YFT loin (white muscle) lipid content and fatty acid profile.

A total of 15 specimens of YFT were used. The specimens were harvested in the Atlantic Ocean, within the FAO fishing area 34, in 2020. The left loins were removed, vacuum packed and frozen, transported to FMV laboratory and processed after defrosting. The quantification of total lipid (TL) content was performed according to the Folch's procedure, using dichloromethane rather than chloroform and the fatty acids profile determined according to a well-established methodology [2].

The nutritional quality of YFT based on the fatty acid profile was assessed considering the nutritional quality indices (peroxidability index (PI), atherogenicity index (AI), thrombogenicity index (TI), and the FA ratios (polyunsaturated/saturated fatty acids (PUFA/SFA), n-3/n-6 and hypocholesterolaemic/hypercholesterolaemic (h/H)), calculated according to the equations presented below.

PI = (% monoenoic × 0.025) + (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 4) + (% pentaenoic × 6) + (% hexaenoic × 8);

AI=(C12:0+(4×C14:0) + C16:0)/[(ΣMUFA+Σ(n−6) + Σ(n−3)];

TI=(C14:0+C16:0+C18:0)/[(0.5×ΣMUFA)+(0.5×(n−6))+(3×n−3)+(n−3/n−6)];

h/H=[(C18:1n−9+C18:2n-6+C18:3n-3+C20:4n−6+C20:5n-3+C22:5n−3+C22:6n-3)/ (C14:0 + C16:0)];

PUFA/SFA = ΣPUFA/ΣSFA; n-3/n-6 = [(Σn-3)/(Σn-6)].

Data on TL content, fatty acid (FA) profile, FA partial sums, FA ratios and lipid quality indices are depicted in Table 1.

The YFT loin total lipid content averaged 0.85 g/100 g of muscle. The FA profile was dominated by the polyunsaturated fatty acids (PUFA; 48% of total FA) followed by the saturated (SFA; 37%) and the monounsaturated (MUFA; 15% of). Among PUFA, the n-3 and n-6 families were accountable for 88% and 12% of total PUFA, respectively.

Regarding the detailed FA profile, the docosahexaenoic acid (DHA) was the predominant FA (38.4% of total FA), followed by palmitic (23.1%), oleic (12.5%), stearic (10.5%), arachidonic (4.75%) eicosapentaenoic (EPA; 3.7%), palmitoleic (1.3%) and myristic acids (1.1%). Together these 8 FA were accountable for 95.4% of total FA, while the remaining 20 FA were responsible for minor contents (all below 1% of total FA).

The PUFA/SFA, n3/n6 and h/H ratio averaged 1.3, 7.12 and 2.51, respectively, while the AI, TI and PI averaged 0.44, 0.25 and 351, correspondingly.

The YFT FA profile presents favourable n3/n6 and h/H ratios and the PUFA/SFA reveals an equilibrium between these groups. The AI and TI are compromised due to tuna´s high content in SFA, particularly myristic, palmitic and stearic acids. Whereas, the PI reveals a high predisposition to oxidation due to their high unsaturation degree.

Considering the health-promoting properties of long chain n-3 PUFA, as EPA and DHA, an evaluation of the FA profile would be incomplete without the evaluation of YFT loin content in these two FA. The YFT loin total PUFA content averaged 171 mg/100 g of muscle while EPA plus DHA represents 87.2% of total PUFA, i.e., 149 mg of EPA plus DHA in 100 g of muscle. Therefore, 100 g of the Atlantic YFT provides around 60% of the recommended daily intake of EPA plus DHA (settled in 250 mg of EPA plus DHA)[3].

Fatty acid profile (expressed as % of total FA		Partial sums. ratios and índices	
C10:0	0.34	Partial sums (expressed as mg/100 g of loin)	
C14:0	1.13	Σ SFA	132.3
C15:0	0.42	Σ MUFA	55.6
C16:0	23.1	Σ PUFA	171.1
C17:0	0.72	Σ n-3 PUFA	150.0
C18:0	10.5	Σ n-6 PUFA	21.1
C20:0	0.14	Partial sums (expressed as % of total FA	
C21:0	0.03	Σ SFA	36.7
C22:0	010	Σ MUFA	14.9
C23:0	0.05	Σ PUFA	48.3
C24:0	0.22	Σ n-3 PUFA	42.4
C16:1 n-7	1.30	Σ n-6 PUFA	5.98
C17:1 n-7	0.07	FA ratios	
C18:1 n-11	0.08	PUFA/SFA	1.322
C18:1 n-9	12.5	n3/n6	7.122
C20:1n-9	0.42	h/H	2.510
C22:1 n-9	0.04	Nutritional indices	
C24:1 n-9	0.51	Atherogenicity índex	0.436
C18:2 n-6	0.90	Thrombogenicity índex	0.253
C18:3 n-6	0.03	Peroxidability index	350.6
C20:2 n-6	0.22		
C20:3 n-6	0.10		
C20:4 n-6	4.75		
C22:2 n-6	0.02		
C18:3 n-3	0.19		
C20:3 n-3	0.09		
C20:5 n-3	3.72		
C22:6 n-3	38.4		

Table 1- YFT loin fatty acid profile (expressed as % of total FA), fatty acid partial sums (expressed either as mg/100 g of loin and as % of total FA)

SFA - saturated fatty acids. MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids;
h/H - hypocholesterolaemic/hypercholesterolaemic ratio.

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Valorization of endogenous wild fruits in Alto Minho region, Northern of Portugal: Centesimal composition and fatty acids profile of *Myrtus communis* L.

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The interest in wild species has been gradually increasing due to its rich nutritional composition which is very important for the proper functioning of the organism. The diversity of plant bioactive compounds is not only beneficial in a food level but also establishes advantages for future applications in several industrial sectors such as food, pharmaceutical and cosmetics. In fact, wild species which have been used since antiquity as a source of food, cosmetic products, and medicines are the large reservoir of plants with potential for multiple uses. *Myrtus communis* L. is an endemic medicinal plant widely distributed in the Mediterranean area. The myrtle is a very aromatic plant, due to the high content of essential oil in its leaves, flowers and fruits. The essential oils from the leaves have been the subject of many chemical and pharmacological studies. The oils extracted from the berries by distillation are used in the flavor and fragrance industries. [1,2] The berries of *Myrtus communis* (**Figure 1**) are very astringent and used as condiment to replace pepper.

This work aims to contribute to the valorization of the natural heritage, wild fruits of Alto Minho region, in the Northern of Portugal, and raise awareness of the need for preservation of endogenous species. This study evaluated the nutritional composition of *M. communis* fruits.

The berries were collected at random from different *M. communis* scrubs, were harvested in the municipality of Ponte de Lima (Viana do Castelo district) in September 2019. Moisture and ash content were quantified according to the ISO recommended standards 1442:1997 and ISO 936:1998, respectively. Kjeldahl method (ISO 937:1978) was used for the quantification of protein content while total fat content was quantified following the Soxhlet method. Carbohydrates were determined by calculation and the energy value was calculated according to the conversion factors indicated by Annex XIV to Regulation (EU) n.º 1169/2011 of the European Parliament and the Council of 25 October 2011. The fatty acid profile was determined according to the procedure described by Cruz et al., 2013.[3]

A high moisture content (64.29 ± 0.43 %) and a significant crude fiber content (14.09 ± 5.86 %) and carbohydrates (30.82 ± 0.52 %) were found in the fruits. However, low levels of total ash (1.80 ± 0.11 %), fat (0.38 ± 0.02 %) and protein (2.71 ± 0.15 %) were recorded. For the energy level, a value of 620.81 ± 15.22 kcal/100 g was determined in the studied samples. The fatty acid profile of myrtle fruits is represented by saturated (14.63 ± 0.51 %), monounsaturated (9.12 ± 0.16 %) and polyunsaturated (76.25 ± 0.67 %) fatty acids. The nutritional characterization herein presented suggests that myrtle fruit is an energetic fruit with carbohydrates as the most abundant macronutrient. The high fiber content and fatty acid profile, rich in polyunsaturated fatty acids, especially Linoleic (LA, C18:2) and α-Linolenic (ALA, C18:3n3) acids evidence the nutritional value interest of this wild berry.

Figure 1: *Myrtus communis* L.

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Characterization and Valorisation of endogenous wild berries in Northern Portugal: Centesimal composition and fatty acids profile of Tramazeira (*Sorbus aucuparia* L.)

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In recent years, the characterization of several wild berries has deserved special attention given to the benefits they can bring to human health. Thus, the consumption of berries has increased in view of the increasing demand by consumers for plant-based products with functional properties. Even though some wild species have already been highly studied, *Sorbus aucuparia* (L.) lacks detailed characterization. *S. aucuparia* is a relatively rare autochthonous species found in Portugal mainly in higher altitude areas, namely in the Gerês, Cabreira, Larouco, Montesinho, Roboredo and Estrela mountains, with edible berries and great ornamental and landscape potential. From the point of view of use, the berries are used to make drinks, jams, jellies and infusions to combat and prevent diseases[1,2]. This work aims to contribute to the valorisation of the natural heritage, wild fruits of Alto Minho region, in the Northern of Portugal, and raise awareness of the need for the preservation of endogenous species. This study evaluated the nutritional composition of *Sorbus aucuparia* fruits.

The berries (approximately 250 berries collected at random from different *S. aucuparia* trees) were harvested in the municipality of Terras de Bouro (Braga district) in August 2020. pH was measured using a digital portable pH-meter, moisture and ash content were estimated according to the ISO recommended standard method (ISO 1442:1997 and ISO 936:1978, respectively). Kjeldahl method (ISO 937:1978) was used for the quantification of protein content, while total fat content was quantified following the Soxhlet (behr ED) method with ether petroleum solvent. Carbohydrates were determined by calculation and the energy value was calculated according to the conversion factors indicated by Annex XIV to Regulation (EU) n.º 1169/2011 of the European Parliament and the Council of 25 October 2011. The fatty acid profile was determined according to the procedure described by Cruz et al. (2013).[3]

The berries have an acidic pH (3.65), high moisture content (62.57 ± 0.64%), and a significant carbohydrates content (29.76 ± 0.57%) were found. However, low levels of total ash (1.27 ± 0.15%), protein (4.13 ± 0.05%), fat (2.27 ± 0.03%), and crude fiber content (6.84 ± 0.05%) were recorded. For the energy level, a value of 169.67 ± 1.68 kcal/100 g was determined in the studied samples. The fatty acid profile of *S. aucuparia* berries is represented by saturated (17.52 ± 0.14%), monounsaturated (26.31 ± 0.11%) and polyunsaturated (56.16 ± 0.12%) fatty acids.

This study contributed to the knowledge of Portuguese endogenous species in the sense of valuing endogenous resources and evaluating their production and commercialization potential, fostering the dynamics of local populations.

The nutritional characterization herein presented suggest that *S. aucuparia* is an energetic fruit with carbohydrates as the most abundant macronutrient. The results show that the berries have a good proportion of essential fatty acids (C18:2n-6, linoleic acid and C18:3n-3, α-linolenic acid), highlighting the nutritional value interest of this wild berry.

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Physicochemical composition of eggs from Portuguese Autochthonous Poultry Breeds

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The conservation of autochthonous breeds is a topic of great importance for the maintenance of local animal genetic resources, and fundamental for sustainability policies in rural areas. In Portugal, there are four autochthonous chicken breeds: “Amarela”, “Branca”, “Pedrês Portuguesa” and “Preta Lusitânica”, all of them classified as endangered.[1,2]
As in other developed countries, in Portugal, eggs production depends on commercial hybrids, characterized by high productivity. However, concerns with food safety, sustainability, and animal welfare have reinforced the interest of consumers in poultry products produced by extensive farming methods.[2]
Integrated in a project of valorisation and characterization of the products of the four autochthonous Portuguese poultry breeds, this work aims to contribute to the valorisation of the natural heritage, and raise awareness of the need for the preservation of local animal genetic resources. This study evaluated the physicochemical composition of the eggs from four autochthonous Portuguese poultry breeds.
The pH value, moisture, ash, protein, fat and mineral content of the yolk and albumen in 240 eggs, 60 per breed, was estimated. pH was measured using a digital portable pH-meter, moisture and ash were estimated according to the ISO recommended standard method (ISO 1442:1997 and ISO 936:1978). Protein content was determined according to Kjeldahl method (ISO 937:1978), while total fat content was quantified following the Soxhlet (behr ED) method with ether petroleum solvent. The mineral composition (phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), and zinc (Zn)) were determined in samples freeze-dried according to the procedure described by Vale et al. (2015).[3]
The results show that the physicochemical composition and mineral content of eggs differ between breeds and egg constituents (yolk and albumen). Moisture, ash, and protein contents differ between breeds, presenting significant differences in albumen ($p \leq 0.05$) with a commercial genotype, ash and protein contents were higher in the native breeds.
Albumen shows pH, moisture, ash, and protein contents higher than the yolk physicochemical parameters. Potassium, magnesium, and sodium contents were also higher in albumen ($p \leq 0.05$). The remaining minerals mean values were higher in the yolk ($p \leq 0.05$). Similar to the physicochemical composition, minerals also differed between breeds and egg constituents ($p \leq 0.05$). K, Ca, Fe, and Zn contents were superior in native breeds when compared to the commercial genotype ($p \leq 0.05$).
The characterization of local products is highly relevant to the preservation of species and knowledge of product quality characteristics. The results of the present work aim to contribute to the study of the physicochemical variability of eggs between the different production systems in order to consolidate the superior quality characteristics compared to conventional methods.

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Analysis of biometric parameters of the pine cone and pine nuts of *Pinus pinea* L. from Viseu Dão Lafões region

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Pine nuts from stone pine (*Pinus pinea* L.) are edible seeds highly appreciated worldwide and represent one of the most valuable non-wood products obtained from the Mediterranean Basin forests.[1] In Portugal, the area cultivated with stone pine has been increasing considerably and the latest data point to an occupation of around 193,600 hectares and a pine cone production of around 330 thousand tons per year.[2] More than 50% of the national production is located in the south of Portugal in the region of Alcácer do Sal and therefore few information is available regarding the economic profitability of pine nuts from other regions of the country.[3]
The purpose of this study was to collect biometric data and physico-chemical parameters in order to characterize the pine cone and pine nuts in the region of Viseu Dão Lafões.
Studies were conducted with pine cones from three different populations of *P. pinea* trees: i) trees grafted from selected local plants; ii) trees grafted from Alcácer do Sal plants; iii) local trees without grafting. The analysis of morphological parameters of pine cones, in-shell pine nuts and kernels was carried out using a digital caliper and was also recorded using an image analysis open source software (ImageJ, Bethesda, MD, USA). The length, diameter, and the mass of pine cone; the number and mass of in-shell pine nuts per pine cone; the length, width, and thickness of in-shell pine nuts and kernels were measured. Regarding the physico-chemical parameters of the kernel, moisture content was determined using a halogen moisture analyzer, protein content was estimated by the Kjeldahl method, total fat was extracted with n-hexane using a Soxhlet apparatus, and color parameters L, a,b were measured using a digital chromameter.
The data collected in this work is an important contribution to promote the attractiveness of investment in stone pine in the region.

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Hydroponic systems for lettuce cultivation using treated olive mill wastewater

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The growing water scarcity in the world has been arising a variety of problems for the past years, one of them being food shortage. According to the UN's Sustainable Development Goals, water scarcity affects more than 40% of the world's population and over 800 million people are undernourished. In the other hand, it is known that the Mediterranean countries produce around 30 million m^3 of wastewaters annually from olive oil production, and since there is no efficient and safe treatment or use for these olive mill wastewaters (OMW) yet, another worry arises, as the OMW can be very pollutant due to its high organic load, resulting from the content in phenolic and organic compounds, and the low biodegradability.

To try to fight these problems, this work focuses on growing lettuce (*lactuca sativa crispa* variety) in a more sustainable way, through hydroponic system, using treated OMW as feed solution. Hydroponics is an alternative to traditional farming that provides faster crop growth, higher productivity, higher water efficiency and less need for fertilizers, thus being considered more efficient and environmentally friendly.

The hydroponic system used in this work was the Nutrient Film Technique (NFT), where two rows with 11 plants each were filled with expanded clay as a supporting material. One row is fed with a control solution composed by KH_2PO_4, $MgSO_4$, KNO_3, $CaNO_3$ and N-P-K solution, and the other with a nutrient solution made up of supplemented previously treated OMW. The raw OMW used for this assay was collected from a two-phase olive oil production mill and treated through application of a sustainable, fast and eco-innovative technique, named immediate one-step lime precipitation process (IOSLP)[1].

The lettuce growth parameters are monitored regularly, and the nutrient feed solutions were replaced every 15 days. After 7 weeks, the lettuces were harvested and morphologically, chemically and sensorially analysed[2].

After the first analyses were complete, it was possible to concluded that growing lettuce through NFT using treated OMW by IOSLP, as a nutrient source is an effective way to both reuse OMW and fight water and food scarcity (**Figure 1**). Moreover, so far there were no significative differences between each group of lettuces, other than the fresh shoot mass, where the lettuce fed by treated OMW showed lower values than the control ones.

This way, the reuse of treated OMW, by IOSLP, makes it possible to implement an integrated management of effluents in sustainable food production systems, also contributing to a circular economy.

Figure 1: Lettuce grown in the NFT hydroponic system fed with control nutrient solution (right side) and with OMW treated by IOSLP as a nutrient source (left side)

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Valorization of waste and by-products from wine industry: chemical composition of grape skin, seed and stalk of *loureiro* and *espadeiro* varieties from the vinho verde region

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The wine sector, one of the most important agro-industrial activities, is highlighted worldwide for its multimillion-dollar market and its historical, traditional and cultural value, considering the amount of land dedicated to the cultivation of the vineyard that makes it one of the main agricultural activities of the Human Being.[1,2] Given the current situation and the growing awareness of societies on sustainability issues, it is essential to strengthen the transition from a linear economy to a circular one, with the objective of balancing the use and management of finite natural resources. The adoption of a circular model was identified as essential to the achievement of sustainable development goals.[3]

The wine industry, after processing the grape to obtain the final product, generates several by-products and residues, such as grape seeds and grape skins, which can be transformed into products of added value, both from a nutritional and industrial viewpoint.

This work aims to contribute to definition a strategy for the recovery of waste from the wine sector and the promotion of circular bioeconomy, evaluating the potential of wine by-products and residues (grape seeds, grape pomace and grape stalk) for the development of new products.

After the separation, disinfection, drying and stabilized storage of the by-products, the physicochemical parameters were determined. Moisture and ash content were quantified according to the ISO recommended standards 1442:1997 and ISO 936:1998, respectively. Kjeldahl method (ISO 937:1978) was used for the quantification of protein content while total fat content was quantified following the Soxhlet method. The pH of the samples was measured using a digital portable pH-meter. Neutral Detergent (NDF) and acid detergent fiber (ADF) were determined according to the Van Soest methodology.

When comparing the by-products, previously referred, from *Loureiro* and *Espadeiro* varieties, there were significant differences ($p \leq 0.05$) in the ash (3.27 vs 2.74%), fat (13.95 vs 10.61%) and ADF (61.68 vs 47.91%) contents of the grape seeds. Regarding grape skins parameters, only pH value (3.67 vs 3.51) presented significant differences ($p \leq 0.05$). In stalk physicochemical parameters, it was possible to observe significant differences ($p \leq 0.05$) in ash content (3.75 vs 3.36%), total fat (1.31 vs 2.76%), ADF (37.91 vs 50.74%) and protein (7.11 vs 5.31%), for *Loureiro* and *Espadeiro* varieties, respectively.

The grape seeds have shown to be rich in fat and fiber, which can be valued, namely, by extracting its oil, since the beneficial effects of grape seeds oil on human health, namely cardiovascular effects, and the importance of fiber in food, are already well known. However, other food applications can be developed, namely in the form of seed flour for bakery and pastry industry.

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Influence of the type of cocoa beans on phase transition, rheology, and fat bloom formation of dark chocolates

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Fatbloom formation is an undesirable defect that affects the quality and shelf life of chocolate. This defect brings a change in the appearance and texture of the chocolate, which not compromise the food safety but will reduce its commercial value and consumer acceptance. As a result, controlling fatbloom formation is a challenge in chocolate production. This work presents itself as a contribution to the improvement of the control of chocolate production [1]. The purpose of this study was the evaluation of the influence of cooling conditions on fat bloom formation analysing the thermal profile, texture, appearance and digital imaging on dark chocolates samples, produced with using Chuncho, Piura Blanco and São Tomé cocoa beans. The evaluation of cooling kinetics was an indicator for the study of the development of the crystal network during the solidification of cocoa butter [2]. Through thermography, the thermal infrared radiation emitted during the cooling of chocolate was converted into temperature. In this way, it is possible to use a thermal camera (Figure 1) that captures this radiation and translates it into a thermographic image. The pixels that are part of the resulting image use colors to represent the temperature at that particular point in the image. With the thermography technique, it is possible to know the different temperatures within a thermal image, being possible to follow their evolution during the cooling of the chocolate.

Figure 1: Thermal camera

The hardness of the chocolate was evaluated using a texturometer, at 20ºC and after 20 days storage time Because of the limitations that colorimetric methods and a panel of tasters can present in the detection of fatbloom formation [3], the digital image captured with a digital camera was used to detect the changes that occur in the chocolate after 20 days of storage at 20ºC.

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Organic acids profile of water kefir beverages produced from pineapple waste extract

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The rinds, crowns and central axis of the pineapple are considered rejects by the fruit pulp industry. However, these residues contain nutrients, including hydrolysable sugars that can be used to produce fermented products such as water kefir beverages. Water kefir grains are made up of a consortium of lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeast that live in a stable symbiotic relationship. The main metabolites produced during the fermentation of water kefir are organic acids and ethanol. Organic acids are related to the protective effect of kefir beverages, in addition to being important for the sensory quality of the product.[1] Therefore, the objective of this research was to develop and evaluate the organic acid profile of two beverages fermented by water kefir grains, using pineapple residue extract, with and without guarana extract for 21 days at 4 °C.

To prepare the pineapple extract, the pineapple waste and water were mixed with a proportion of 2:1 (m/m), then 1.3% (m/m) of fresh mint was added to the mixture. All ingredients were mixed in a blender for 2 min. The final mixture was filtered and transferred to glass bottles. The bottles were pasteurized at 60 °C for 60 min and then cooled to 25 °C. To elaborate the formulation with guarana extract, 0.15% (m/m) guarana extract was added to the pineapple waste extract. To prepare the beverages, brown sugar was dissolved in water (10%, w/v). The sugar solution was sterilized and cooled to 25 °C. Three formulations were prepared: control (containing only the sugar solution) (FC), with the addition of pineapple waste extract (F1) and with the addition of pineapple waste extract containing guarana extract (F2). The fermentation process was carried out in two stages as described by Bueno et al.[1] Then the beverages were stored at 4 °C ± 1 °C in a refrigerator. Analyses were performed every 7 days for 21 days. For the analysis of organic acids, samples were diluted in a proportion of 1:3, followed by vortexing for one minute. Subsequently, the diluted sample was centrifuged at 15,000 rpm for 15 minutes. The supernatants were passed through Sep-Pak C18® and filtered through a 0.22 µm PVDF filter membrane. Organic acids were determined by High Performance Liquid Chromatography (HPLC) as described by Bueno et al.[1] The standards used were citric, malic, lactic, succinic, and acetic acids.

The variations found are attributed mainly to the addition of pineapple wastes (with and without guarana extracts) in the second fermentation step, as well as by the action of the diversified microbiota of the kefir grains capable of producing such acids.[1] In the beginning of second fermentation stage, citric acid was the major contributor in F1 and F2 beverages, probably derived from pineapple waste extracts. During storage, the profiles of all organic acid of the beverages reflected the metabolic heterogeneity of LAB and AAB. F1 formulation showed a progressive increase with the highest concentrations of acetic (2.46 ± 0.05 mg/mL), citric (4.83±0.04 mg/mL), lactic (1.57 ± 0.05 mg/mL), and succinic (1.53 ± 0.03 mg/mL) acids verified on day 14, and subsequent decrease ($p < 0.05$) in the last day of cold storage. Similarly, Fiorda et al.[2] produced honey-water kefir and obtained citric acid as the main (69.86 mg/mL) organic acid, while lactic, acetic, and succinic were detected in smaller proportions.

In contrast, for F2 formulation, the acids contents increased more slowly, reaching a maximum (acetic, 3.03 ± 0.05 mg/mL; citric, 2.89 ± 0.01 mg/mL; succinic, 0.83 ± 0.00 mg/mL) at the end of the evaluated period. Only for lactic acid, this trend was not observed. The oxidative metabolism of AAB is evidenced mainly by the high concentrations of acetic acid in F2 beverage (21 day). The decrease of all organic acid, in the final period of storage, is observed mainly in F1 and can be explained by overoxidation of these acids to CO_2 and H_2O by the most acid tolerant genera, *Acetobacter* and *Komagataeibacter*. The difference in organic acid concentrations between beverages can also be attributed to the addition of guarana extract in F2. A recent study reported that the bioactive compounds present in these extracts demonstrate antimicrobial properties.[3] Thus, the guarana extracts may be limiting the growth of microorganisms of F2 beverage, in addition to reducing the rates of organic acids production.

In conclusion, the use of alternative substrates, such as pineapple waste and guarana extract, for the development of kefir-fermented beverages, demonstrated to be an effective way to convert sugars into organic acids. Alternative substrates can help maintain and/or improve the food safety, nutrition, sensory and shelf-life properties of fermented beverages.

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Nutritional composition of omnivorous and carnivorous wild freshwater fish from Tagus River basin

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Portugal is one of the countries in the world with highest *per capita* fish consumption (59.9 kg/year) (**1**), mainly saltwater fish from Atlantic Northwest and marine aquaculture. However, in the interior of Portugal is traditional the consumption of wild freshwater fish (common carp [CC], iberian barbel [IB], crucian carp [CrC] and largemouth bass [LB]). These species are very interesting as game fishing and also very important in the local cuisine due to their characteristic flavor. In summer and autumn there are several regional gastronomic festivals related to freshwater fishes. Little is known about nutritional composition of the edible part of these fish species. The aim of this study was to evaluate the nutritional composition and fillet havy metal contamination of wild *Cyprinidae* family omnivorous freshwater fish, CC (*Cyprinus carpio* Linnaeus, 1758), IB (*Luciobarbus comizo* Steindachner, 1864) and CrC (*Carassius carassius* Linnaeus, 1758) (n=40 samples), and wild *Centrarchidae* family carnivorous freshwater fish LB (*Micropterus salmoides* Lacépède, 1802) (n=20 samples) from Tagus River basin lentic systems (hydroelectric power plants dams and small irrigation reservoirs). We concluded that the omnivorous fish had significantly higher levels of humidity, ash, Ca and fat and carnivorous fish had significantly higher levels of protein Na, Mg and K. All samples exhibit very low levels of heavy metals (Cd, Cr, Pb) below the apparatus limit of quantification (Cd LOQ<0.05; Cr LOQ<0.03; Pb LOQ<0.2) and fish legal values (Cd <0.05 mg/kg body weight, Pb <0.3 mg/kg body weight (**2**) and Cr <0.05-0.15 mg/kg body weight (**3**). These results may be a reference for further studies, primarily as a source of information for the consumers and for conservation through restocking (*L. comizo*), and also for the development of commercial projects of aquaculture (*C. carpio* and *M. salmoides*).

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Influence of the preservation method on the nutritional profile, antioxidant activity and free sugar content of coffee pulp

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During coffee production, most of the by-products are wasted and not used at all, so it is important to give them value to help to reduce waste and increase incomes. Coffee pulp is a by-product generated in the first step of the wet coffee processing. When improperly discarded to the environment in very high amounts, it raises environmental concerns due to its phytotoxic properties and high organic load.[1]

Having in view the use of this by-product for food purposes, this work aimed to study the influence of the preservation procedure (lyophilization or hot drying) on the nutritional composition, antioxidant activity (ferric reducing antioxidant assay and DPPH• inhibition capability) and sugar content (by HPLC-ELSD).

Samples of coffee pulp from *Coffea arabica* (caturra vermelha variety) were collected in S. Miguel, Azores, immediately after coffee depulping. The samples were lyophilized or dried at 75 °C during ~6 h in a thermostatized oven. In dry weight, no significant differences (p>0.05) were found between protein, ash, and fat contents of both processed samples. Nevertheless, soluble fiber was significantly higher (p<0.05) in dried coffee pulp (4.8 *vs* 1.7 g/100 g dw). In turn, available carbohydrates decrease with hot drying (25 *vs* 33 g/100 g dw), probably due to their direct involvement in browning reactions during the thermal treatment.

In what concerns to total phenolics and total flavonoids contents, the values were significantly higher (p<0.05) in lyophilized coffee pulp (30 vs 16 mg gallic acid eq./g; 35 vs 11 mg epicatechin eq./g, respectively). Consequently, this sample also showed significantly higher antioxidant activity by both methods. Fructose was the only free sugar that slightly increased (p<0.05) in the dried pulp.

In sum, the results show that coffee pulp is a great source of fiber, mainly insoluble one (~40%) and antioxidants. Nevertheless, the thermal procedure tested to dry the samples led to a 2-fold decrease of total phenolics and a 3-fold reduction of total flavonoids, which directly influenced the antioxidant activity of the sample. Based on this, lower temperatures should also be tested to find a compromise between time of drying and compounds preservation.

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Study of the amino acids profile of *Coffea canephora* silverskin from different geographical origins

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Coffea canephora represents about 35% of the world coffee production. Its main producers are in the Central and Western Africa, Asia (mainly Vietnam and Indonesia), and South America (Brazil).[1] During roasting, coffee silverskin, a by-product of this process, is obtained and separated to be discarded. Nevertheless, this by-product is of high value due to its nutritional and chemical composition, although it may depend on the geographical origin of the bean.[2] Recent approaches to silverskin valorization have been suggested to answer to the circular economy and sustainability issues and its richness in dietary fiber, protein, and antioxidants has been highlighted in literature.[1]

To explore differences among *Coffea canephora* silverskin from different geographical origins, this work aimed to study and compare the free and total amino acids profile of samples from different countries (Vietnam, Cameroon, Indonesia, and India). The samples were kindly provided by a national coffee roaster (BICAFÉ) after subjecting the individual green beans to a similar roasting procedure (210 °C, 10 min).

Free amino acids were extracted using a previously optimized solid-liquid extraction with deionized water at 40 ˚C, for 30 min.[3] To analyse total amino acids, hydrolyses were performed using 6 M HCl at 110 ˚C, for 24 h, or 4 M KOH at 110 ˚C, for 4 h (for tryptophan), after removing oxygen with a N_2 stream. The extracts and hydrolysates were submitted to an automatic pre-column online derivatization with OPA/3-MPA and FMOC, followed by RP-HPLC-FLD analysis[3]. The amino acids were identified by comparing their retention times with those of standards. Quantification was performed using norvaline as internal standard.

The total amount of free amino acids varied between 1302 µg/g for silverskin from Vietnam and 2182 µg/g for the Cameroon sample. Regarding the free profile, the main amino acids were aspartic acid (253-372 µg/g), glutamic acid (169-300 µg/g) and arginine (131-336 µg/g). In what concerns to total amino acids, they ranged between 101 mg/g for the sample from Indonesia and 119 mg/g for the Vietnam silverskin. Aspartic (12.3-16.9 mg/g) and glutamic acids (11.1-15.2 mg/g) were also the main amino acids when considering the complete profile. The important branched-chain amino acids (valine, isoleucine and leucine) are also present in substantial amounts (4.8-7.1 mg/g, 6.0-7.1 mg/g and 8.4-9.9 mg/g, respectively). Although in minor quantities, methionine and tryptophan were also present (0.8-2.1 mg/g and 0.9-1.3 mg/g). All the essential amino acids have also been identified.

The richness of silverskin in these specific amino acids suggests their potential to be used in food formulations or dietary supplements directed to the improvement of cognitive and physical performances.

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Influence of organic *vs*. conventional production on the nutritional profile and antioxidant activity of roasted coffee

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Coffee is one of the most popular beverages around the world, not only for its stimulating properties, but also for its special aroma. In the last years, the growing concern with the environment has contributed to the increased popularity of organic coffee. Coffee with this certification is grown in a sustainable way, without the use of any synthetic fertilizers.[1]
The main aim of this study was to investigate if the different cultivation practices (organic and conventional) may affect the nutritional composition and the antioxidant properties of coffee beans.
In this work, 11 samples of roasted arabica coffee from different geographical origins were studied (9 from conventional production and 2 with the organic certification).
The following parameters were determined: moisture, total protein, total lipids, and total ash contents by AOAC methods. Total carbohydrates were calculated by difference. Total phenolics content, the Ferric Reducing Antioxidant Power and DPPH• inhibition tests were also performed to assess the antioxidant properties of the samples.
The results show no significant differences (p>0.05) between the nutritional profile of organic and conventionally cultivated samples. All the beans presented an ash content of 4-5%, total fat content between 12-16%, total protein ranging from 11 to 13% and total carbohydrates of approximately 65%. Higher variations were observed for total phenolics (4.4-7.1 g chlorogenic acid equivalents per 100 g in conventional coffees, but also without significant differences compared to organic ones (~6 g chlorogenic acid equivalents per 100 g). The same conclusions were observed for the antioxidant activities observed with the two employed assays.
As conclusion, although the organic group contained few samples due to the lower availability of organic producers, this preliminary study shows no significant differences between the macroconstitutents analysed and the antioxidant properties of organic and conventionally roasted coffees. Nevertheless, safety concerns related with the use of synthetic chemicals should not be disregarded.

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Comparison of the amino acid profile in young and adult of wild boar male (*Sus scrofa*) meat

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Meat is one of the most important foods for humans, as it is a nutritious food with a high energy content. Such a product is very important because it is linked to the maintenance of a balanced and healthy growth, thus being essential for the development of the human being. Regarding its nutritional properties, meat is considered a rich source of essential amino acids.[1]

In general, compared with farmed animals, wild meat is characterized by a lower content of fat and cholesterol, higher levels of protein, essential amino acids, vitamins, minerals and unsaturated fatty acids.[2] Over the last years, there has been an increased interest in wild boar meat due to its high culinary value and organoleptic profile.

The aim of this work was to analyse and compare the amino acid composition of the meat from young and adult males of wild boar. The research involved the meat of 6 wild boars, young males (n=3) and adult males (n=3). Sampling of wild boars meat was collected during the hunting seasons 2018/2019 and 2019/2020, from different hunting grounds, located in continental Portugal. No animals were sacrificed for the purposes of this study. None of the authors was responsible for the death of any animals. All applicable institutional and/or national/international guidelines for the care and use of animals have been followed.

Total amino acids were determined based on the method described by Machado *et al*.[3] with slight modifications. Briefly, 3 ml of HCl (6 M) to 75 mg of sample. The mixture was subjected to a N_2 stream and, then, heated at 110 ˚C during 24 h. Alkaline hydrolysis was performed to determine tryptophan content using KOH (4 M) and 22 h of hydrolysis. The hydrolysates were submitted to an automatic pre-column online derivatization with OPA/3-MPA and FMOC, followed by RP-HPLC-FLD analysis.

In what concerns to the protein content (based on the sum of total amino acids), no significant differences (p>0.05) were found between the groups of different ages (young wild boar: 197-234 mg/g; adult wild boar: 196-251 mg/g). The amino acid profile was also very similar among both groups, being glutamic acid (33.5 mg/g *vs*. 32.2 mg/g), aspartic acid (21.7 mg/g *vs*. 20.7 mg/g), leucine (21.4 mg/g *vs.* 20.6 mg/g) and lysine (20.5 mg/g *vs*. 18.7 mg/g) the major compounds for adult and young animal meat, respectively (results expressed in fresh weight). The minor amino acids for all the samples were taurine (3.1 mg/g *vs*. 3.5 mg/g), tryptophan (2.5 mg/g *vs*. 2.4 mg/g) and hydroxyproline (1.1 mg/g *vs.* 2.1 mg/g), by the same order.

In conclusion, the age of the wild boar (young and adult) seems to have no major influence on the total amino acids amount.

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Nutritional profile and mineral content of Sonchus asper: a Wild Edible Plant from the Mediterranean area

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Agrobiodiversity as part of overall biodiversity can be defined as the variety of living forms within agricultural ecosystems and is strongly linked with diversity in food and agricultural production and, thus, with nutrition and human health. In addition to the diversity of common crop species, Mediterranean agrobiodiversity resources also include Wild Edible Plants (WEPs)[1]. *Sonchus asper* is considered a wild edible plant and is popularly known as spiny sow thistle. This species of European origin, but found on other continents, is traditionally harvested by local people from nature, to be consumed mainly sautéed with sauces and broths of the well-known Mediterranean diet[2]. The unrestrained collection of wild plants can cause serious environmental problems as well as health problems for the consumer, since these species grow spontaneously to maintain themselves and are not managed or cultivated, so there is no reproducibility in the nutritional contribution. Thus, the objective of the present study was to characterize the nutritional profile, mineral content, and energy of the leaves of two wild spiny sow thistle (SA1 and SA2), grown on different medium, using AOAC methods. The content of crude protein (AOAC, 991.02), total fat (AOAC, 989.05), total dietary fiber (AOAC, 991.43), ash (AOAC, 935.42) and carbohydrates (by difference) were evaluated. The mineral content was measured using atomic absorption spectrophotometry and the energy was calculated according to the equation: energy (kcal per 100 g) = 4 x (g protein + g carbohydrate) + 2 x (g total dietary fiber) + 9 x (g fat). In all nutritional parameters, wild *Sonchus asper* samples showed different values. In total fat, SA1 presented the highest amount (5.6 g/100 g dw) while SA2 presented 2.8 g/100 g dw. In terms of carbohydrates, SA2 showed twice the concentration when compared to SA1. In terms of crude protein and total dietary fiber, SA1 shows promising concentrations (15.96 and 41.6 g/100 g dw, respectively), while SA2 contained namely 11.86 and 37.45 g/100 dw. Regarding ash, the leaves of SA2 showed values lower than SA1. Although SA1 presented the highest concentrations of total fat, crude protein, total dietary fiber, and ash, the SA2 sample presented the highest energy contribution (304.5 kcal/100 g), possibly due to the significant presence of carbohydrates. The mineral content also showed great differences between SA1 and SA2, mainly with regard to the concentration (mg/g of dw) of manganese, with the sample SA1 showing a content of 30% higher when compared to SA2. For potassium, copper, and zinc, the sample SA2 showed the highest concentrations, on the other hand, SA1 showed high concentrations in the content of sodium, calcium, magnesium, and iron (9.4, 10.5, 3.2, 0.2 mg/g of dw, respectively). This distinct nutritional profile of the two wild thistles is possibly caused by the quality of the soil, since the primary metabolism depends on the edaphoclimatic conditions in which the plant grows, namely light, salinity, and temperature[3]. The preliminary results obtained point out the great differences in the nutritional value of spiny sow thistle, highlighting the need to implement an adequate cultivation system that enhances this species functional macro and micronutrients.

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Effects of the fertilization system on the chemical profile of *Ribes rubrum* L.

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Ribes rubrum L. fruits are widely consumed for their pleasant taste and nutritional features (**Figure 1**). These small red fruits are also considered superfoods, mostly due to their high content of phenolic compounds, fiber, iron, and vitamin C, among others, which confer them anti-inflammatory, antioxidant, antibacterial, depurative, and diuretic properties.[1] In fact, in the last years, there has been a reasonable increase of these fruits' consumption, with the growing interest of consumers in functional foods and the sustainability of their production. In this sense, organic and integrated production has been gaining expression, being seen as a way to enhance the quality of the fruits, rich in added-value antioxidant compounds, allowing to meet the most demanding consumers' expectations.[2]

Through this study, fruits produced conventionally and by applying a biological fertilizer were compared in terms of nutritional value (AOAC) and fatty acids (GC-FID), free sugars (HPLC-RI), organic acids (UFLC-PDA), tocopherols (HPLC-fluorescence), and phenolic compounds (HPLC-DAD/ESI-MS) composition. Moreover, the antioxidant properties of their hydroethanolic extracts (ethanol:water 80:20, v/v) were assessed by two cell-based methods (TBARS and OxHLIA). In general, higher levels of carbohydrates and energy, sucrose, polyunsaturated fatty acids and anthocyanins were found in the fruits grown in conventional agriculture. On the other hand, the fruits cultivated in biological mode showed higher concentrations of lipids, fructose and glucose, ascorbic acid, saturated and monounsaturated fatty acids, phenolic acids and flavonoids.

This difference in the chemical profile revealed to influence the bioactivity of these fruits, both in terms of inhibition of lipid peroxidation (TBARS) and oxidative hemolysis (OxHLIA), which were enhanced in the fruits produced in biological way.

The results obtained in the present study may serve as a basis for the definition of production parameters that best fit the culture of *R. rubrum*.

Figure 1: *Ribes rubrum* L. fruit. Source: Tropicos.org. Missouri Botanical Garden (http://www.tropicos.org/Name/29101108; accessed in 16/12/2019). Image source: http://www.tropicos.org/Image/100191795 8].

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"Pingo de Mel" fig as a rich source of phytochemicals with antioxidant and antimicrobial properties

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Green fig ("Pingo de Mel") is the most appreciated and produced fig variety in Portugal. These fruits are substantial sources of trace minerals (above all calcium, but also iron and potassium) and vitamins (mostly thiamin and riboflavin), also presenting a high number of essential amino acids and great contents of fibers and antioxidant phytochemicals (especially phenolic acids, flavonoids and carotenoids).[1,2] For this reason, the present work aimed to contribute to its valorisation through the study of its chemical composition and bioactive properties. A full characterisation of two parts of this fruit (peel and pulp) was carried out regarding their nutritional value (AOAC procedures), free sugars (HPLC-RI), organic acids (UFLC-PDA), tocopherols (HPLC-fluorescence), fatty acids (GC-FID), and phenolic (HPLC-DAD/ESI-MS) composition; as well as their bioactive properties (antioxidant and antibacterial). The peel revealed a higher energetic contribution than the pulp, with both samples presenting similar concentrations of protein. Four free sugars, five organic acids, the four isoforms of tocopherols, and twenty-three fatty acids were detected in the samples. Fifteen different phenolic compounds were found in the peel, while twelve were found in the pulp. Quercetin-3-*O*-rutinoside (rutin) was the main constituent of the peel, representing 33.8% of its phenolic content, followed by 5-*O*-cafeoilquinic acid and vanillic acid di-deoxyhexoside malonyl. Derivatives of caffeic acid, such as hexosides, were the main components of the pulp, followed by derivatives of vanillic acid and 5-*O*-cafeoilquinic acid. Both extracts showed promising antioxidant capacity; however, the peel showed significantly lower IC_{50} values than the pulp. The extracts showed almost identical antibacterial capacity and were more effective against *Staphylococcus aureus*, *Escherichia coli*, and *Morganella morganii*. These results showed the nutritional and bioactive potential of "Pingo de Mel" peel and pulp, with the peel revealing higher energetic value, phenolic compounds concentrations, and bioactive properties.

Figure 1: "Pingo de Mel" fig samples.

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Nutritional assessment of fresh, salted and soaked European catfish

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Portugal is one of the countries with the highest *per capita* seafood consumption in the world, and the higher *per capita* seafood consumption in Europe (59.9 kg/year) (**1**). One of the most consumed seafood products by the Portuguese population is soaked cod purchased this way 54% of the time and chilled in 38% of the cases (**2**). Salted and soaked cod is part of the country's tradition partly because it was accessible and cheap in the past. The European catfish (*Silurus glanis* Linnaeus, 1758) is an invasive fish species in Portugal and it is the largest-bodied European freshwater fish. Tagus River fisherman's report that more and more big fish of this species are currently being caught and the risks to native species are disease and parasite transmission, competition for benthic habitats and predation. Recent studies show that the European catfish caught in the Tagus River has high nutritional interest with low level of fat and very low levels of heavy metals (**3**), even though it is a lentic systems apex predator. As in the interior of Portugal is traditional the consumption of wild freshwater fish, a freshwater fish processing company (Conserveira do Interior Lda. - Bem Amanhado) is interested in knowing more about the salting processes of European catfish to produce salted catfish similar to those made with cod. The aim of this study was to evaluate the nutritional composition of fresh European catfish from the Tagus River (n=9 samples), some chemical parameters of salted catfish (n=9 samples) and the nutritional composition of soaked catfish (n=9 samples). As expected, we concluded that the catfish salting process reduces moisture ($p<0.05$) and increases salt and Na concentration ($p<0.05$). When we compare the nutritional composition of fresh European catfish with soaked catfish we verify soaked catfish has more energy, ash, protein, fat, saturated fatty acids and monounsaturated fatty acids ($p<0.05$) but lower polyunsaturated, n-3 and n-6 fatty acids ($p<0.05$).

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Additive, synergistic, and antagonistic effects of a mixture of fruit and vegetables

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The World Health Organization and the Food (WHO) and Agriculture Organization of the United Nations recommend a minimum consumption of 400 g of fruit and vegetables per day (excluding starchy tubers) and stress the importance of varying the type of food consumed, to obtain maximum benefits associated with these food groups. Low consumption of fruits and vegetables is among the top ten risk factors that contribute to global mortality. According to the data obtained by the last National Food and Physical Activity Survey, it was found that the average consumption of fruits and vegetables is approximately 418 g/day. Although this value is slightly above the WHO recommendations (> 400 g/day), more than half of the population (52.7%) does not meet it, which means that there is unequal consumption between different age groups and/or geographic locations.[1] The concern with the practice of a healthy lifestyle is increasing in the society. The demand for healthy, organic and "natural" food has grown in recent times, giving rise to a preference for eating foods in their natural state, such as detox juices, that are a mixture of fruit and vegetables. In this study five ingredients (spinach, carrot, apple, orange and kiwi) and their mixture, were evaluated concerning antioxidant activity, total phenolics, total flavonoids and vitamin C contents, to estimate the potential additive, synergistic or antagonistic effects of their mixture. In 2022, the ingredients were collected in the major supermarket chains of Lisbon, Portugal. Afterwards, for orange, carrots and kiwi, non-edible parts (peel and seeds) were removed, while for apple only the seeds were removed, and the peel was maintained. All the ingredients were washed and dried with tissue paper. Then, a portion of each ingredient was used for the analysis of the different parameters and a portion of the same ingredients was used to prepare the mixture. For the mixture the following quantities were used: 100 g of spinach, 40 g of orange, 20 g of carrot, 20 g of kiwi and 20 g of carrot. Antioxidant activity of the 5 ingredients and its mixture was determined using two different methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and ferric reduction power (FRAP). Total phenolic and total flavonoids content was also assessed by spectrophotometric methodology. The determination of total vitamin C, L-ascorbic acid and dehydroascorbic acid was performed by HPLC with diode array detection. Predicted values were calculated through the mathematical sum of the total phenolic, total flavonoids, antioxidant capacity (DPPH$^{\bullet}$ and FRAP) and vitamin C values, obtained for the ingredients. Then, the analytical values of the mixture were compared with the respective predicted ones. Concerning ingredients, spinach was the sample with the highest total phenolics and total flavonoids contents, with 621.1 ± 40 mg of gallic acid equivalents/100 g and 590.2 ± 34 mg of epicatechin equivalents/100 g, respectively. The total vitamin C varied between 6.28 ± 0.05 and 48 mg/100 g, for apple and kiwi, respectively. With respect to the mixture for total phenolics, total flavonoids, FRAP, DPPH$^{\bullet}$ and L-ascorbic acid the observed values were higher than the expected values, therefore probably we are facing a potential synergistic effect, while for total vitamin C and dehydroascorbic acid the opposite was observed, meaning a potential antagonistic effect. According to the obtained results, the mixture of these ingredients can be important to increase the potential health benefits associated with the consumption of fruits and vegetables. In our opinion, it is of utmost importance to understand the effects associated with mixtures of fruits and vegetables, like it happens in detox juices, to clarify if these mixtures from a public health point of view have more or less beneficial effects than the ingredients alone. In the next steps of this research work, different proportions of these ingredients will be used to perform several mixtures, to evaluate which is the best option for consumers.

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Vitamin E profile of agri-food by-products: biological relevance and potential applications

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Plant compounds have an important role in food research due to nutraceutical properties that can prevent the development of diseases. Vitamin E is a combination of 8 liposoluble antioxidants (α-, β-, γ-, δ-tocopherols and tocotrienols), composed by the chromanol ring and the hydrophobic side chain. The latter is composed of saturated isoprenoid side chains in tocopherols and isoprenyl side chains with 3 double bonds in tocotrienols. These compounds act as free radical scavengers in membranes and lipoproteins, protecting against oxidative stress.[1]

Particularly, α-tocopherol can modulate genes associated with cell cycle regulation. β- and γ-Tocopherols strongly inhibit intracellular tyrosinase. γ-Tocopherol exhibit natriuretic, anti-inflammatory, and antitumoral activities. Several studies reported that the ingestion of α-tocopherol reduces the serum levels of γ-tocopherol, consequently reducing the anticancer actions of the latter. However, from all the isomers, only α-tocopherol has been shown to protect against ataxia, a disfunction related to vitamin E deficiency. δ-Tocopherol promotes a proinflammatory response against reactive oxygen species, reduces lipid accumulation, has antiangiogenic effects, and increases neuronal differentiation.[2]

In this work, the vitamin E profile of extracts from different leaves (agri-food by-products) were determined by HPLC-DAD-FLD according to Alves *et al*., 2009.[3] The analysed samples were: olive leaves (*Olea europaea,* from Guarda, Portugal) - top and "Mamão" leaves from Madural, Verdeal, and Cobrançosa varieties; quince leaves (*Cydonia oblonga*, from Guarda, Portugal) - yellow, green, and mix of yellow/green leaves; and black fig leaves (*Ficus carica,* from Torres Novas, Portugal) - top and "Ladrões" leaves. The aim of this work was to discuss the biological relevance of their vitamin E contents and highlight potential applications.

Results revealed significant differences (p<0.05) between the vitamin E profiles but all samples presented α-, β- and γ-tocopherols in varying amounts (3.8-70, 0.2-1.6 and 0.3-1.8 mg/100 g, respectively). Also, α-tocopherol was the major isomer in all samples. The highest total vitamin E content was obtained for top black fig leaves (88 mg/100 g). β-tocotrienol was only identified in black fig leaves, presenting differences between the top leaves in relation to "Ladrões" leaves (14.5>5 mg/100 g, respectively) what was also verified for the other isomers, so top leaves totalized a higher total vitamin E content than "Ladrões" leaves (88>49 mg/100 g, respectively).

Regarding olive leaves, Verdeal top leaves presented the highest total vitamin E content while Madural "Mamão" leaves presented the lowest (24>4 mg/100 g, respectively). δ-Tocopherol was only identified in olive leaves from Verdeal "Mamão" variety (0.4 mg/100 g). Regarding quince leaves, the yellow leaves showed a vitamin E decrease with time in comparison to the green leaves (15<33 mg/100 g, respectively), what could be a result of its use during leaf maturation, e.g., against lipid peroxidation, particularly, polyunsaturated fatty acids.

To conclude, these samples are interesting sources of vitamin E, presenting high potential for applications in the food and cosmetic industries, e.g., as a natural additive to fortify foodstuffs, as ingredients of dietary supplements and dermocosmetics.

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Green coffee from organic and conventional production: chlorogenic acids profile and safety assessment regarding pesticides and mycotoxins

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Coffee is one of the most popular beverages worldwide and its richness in chlorogenic acids seems to be behind the several health benefits reported for moderate coffee consumption.[1] Recently, the current raise of environmental concerns has also contributed to the increasing popularity of organic coffee. Although some studies report a higher content of bioactive compounds in organic coffee compared to conventionally produced one, data in literature is still controversial.[2]

In this work, green coffee from both types of cultivation (organic and conventional practices) were studied and compared regarding their chlorogenic acids (CGA) profile. Moreover, a safety assessment was also performed by screening residues of pesticides and mycotoxins.

Samples of green coffee beans (all *Coffea arabica*) from different geographical origins were kindly provided by a national coffee roaster. Organic coffees (n=3) were from Colombia, Honduras, and Peru and coffees from conventional agriculture (n=3) were from Colombia, Honduras, and Brazil.

CGA (3-, 4, and 5-caffeoylquinic acids and 4-, 5-feroluylquinic acids) were extracted using a hydroethanolic solvent and analysed by RP-HPLC-DAD at 320 nm. For pesticides and mycotoxins screening (153 compounds in total), simultaneous extraction (using a QuEChERs approach) was performed according to Reichert et al. [3] followed by LC-ESI-MS/MS analysis.

At a first sight, comparing both groups of samples (organic *vs.* conventional) no significant differences (p>0.05) were observed between the individual CGA contents, with the Brazilian conventional coffee presenting the highest contents for all the caffeoylquinic acids. However, when comparing only coffees from the same geographical origin, those from organic production showed significantly higher amounts (p<0.05) of CGA, except for 5-feruloylquinic acid in Colombian coffees. Among all the samples, a pesticide residue (imidacloprid: 17 µg/kg) was found in only one of the conventionally produced coffees, although in lower amounts than the Maximum Residue Level allowed for green coffee beans (1.0 mg/kg). No mycotoxins were detected in the samples.

These results show that organic and most of the conventional coffees were free of the analysed pesticides and mycotoxins. Regarding CGA profile, although the results within each group were variable, when comparing samples from the same geographical origin, a tendency to present higher CGA contents was noticed in organic coffees. However, a study with a higher number of samples would be needed to support this hypothesis.

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Chemistry characterization of Scottish grain and malt spirits aged in Sherry Casks® and Brandy casks.

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Malted cereals spirits and/or grains of unmalted cereals spirits are the unaged distillates used to make whisky. It belongs to the so-called spirit drinks, those whose ethyl alcohol comes from distillates of the fermentation of agricultural origin (wine, cereals, beets, cane sugar, etc.). In the production of some spirits, an ageing process in wooden vessels is necessary and/or mandatory. This ageing affects the quality of the product and is a legal requirement for some spirits. As is the case of malted cereal spirits or grain distillates, which must be aged for at least three years to be called whisky.

During the ageing process a series of physicochemical and sensory changes take place, which are manifested by colour, flavour or aroma variations of the initial distillate. These changes are mainly influenced by several factors: the type of ageing process and the characteristics of the wooden cask (botanical origin, size, manufacturing process, levels of toasting, previous use and pre-treatments like the wine-seasoning process). American whisky (like Bourbon) producers use new barrels with a high level of toasting, while Scotch whiskies are mainly aged in barrels used to contain Bourbon or Sherry wine.

These casks that have previously contained some type of Sherry (Fino, Oloroso, Pedro Ximénez, etc.), are known as Sherry Cask®. The characteristics of Sherry Casks® depends on the Sherry wine used. Therefore, during the ageing of the spirit, Sherry Casks® provide not only with the compounds inherent to the cask wood, but also with those from the wine they initially contained and that were retained in the wood's pores.

In this work, the physicochemical changes of two types of grain distillates aged in Sherry Casks® and Brandy casks have been compared. A Scottish malt spirit was distilled in a traditional way (pot still) at 68% ABV with a high concentration of congeners. The other one, a Scottish unmalted grain spirit was produced in a continuous distillation column, getting a softer distillate and lower concentrations of congeners at 94% ABV. The grain spirit was diluted with demineralized water to 68% ABV for ageing.

Both distillates were aged in American oak (*Quercus alba*) casks, medium toast, with a capacity of 500 L and seasoned by 18% ABV Oloroso Sherry wine for 3 years (Sherry Cask®) and "Brandy casks" were used for ageing brandy for 3 years too.

Each test was carried out in duplicate, two barrels for each distillate and type of cask, following a static ageing process for 1 year.

Oenological control parameters, chromatic characteristics and total polyphenol index (TPI) were carried out according to the official methodology described by the OIV. Organic acids were determined by ion chromatography and total aldehydes, methanol, esters and higher alcohols were determined by GC-FID.

Statistical analyses for the ANOVAs and PCA were carried out with Statgraphics-18 software package.

A substantial difference has been observed between the distillates aged in Sherry Cask® and Brandy cask in the parameters influenced by ageing (**Figure 1**). Distillates aged in Sherry Cask® have showed greater increase in TPI, colour, total and volatile acidity, as well as the appearance of some organic compounds from the wine-seasoning (2,3-butanediol, diethyl succinate, tartaric acid, malic acid, succinic acid, etc). Furthermore, comparing the volatiles composition of the malt distillate and the whole grain distillate have been observed, that despite the different initial profiles (with very different concentrations of higher alcohols and esters), both distillates have showed a similar evolution profile.

These results can be synthesized with a principal component analysis (PCA), where 3 components with eigenvalues greater than 1,0 have been obtained: Component 1 (58,9%), Component 2 (29,3%) and Component 3 (7,1%). The first two components describe almost 88,1% of the variability of the original data (**Figure 2**). Component 1 explains the type of cask, distillates aged in Sherry Cask® being farther than Brandy cask to their respective initial spirits. Component 1 is mainly made up for variables that change in the ageing process (pH, total acidity, TPI, diethyl succinate, 2,3-butanediol, diethyl malate, colour parameters, tartaric, malic, succinic, lactic and acetic acids). Component 2 separates the spirits by type of distillate, that means that it is mainly formed for congeneric compounds of the distillates (total aldehydes, methanol, higher alcohols, fatty acid ethyl esters, ethyl acetate and benzaldehyde).

	GRAIN SPIRIT			MALT SPIRIT		
	INITIAL	Sherry Cask®	Brandy cask	INITIAL	Sherry Cask®	Brandy cask
Alcoholic strength (% ABV)	67,32 ± 0,01	67,42 ± 0,18	67,50 ± 0,01	68,56 ± 0,02	67,41 ± 0,04	68,30 ± 0,01
pH	6,56 ± 0,01 ^{a}	4,41 ± 0,03 ^{b}	4,76 ± 0,01 ^{c}	5,21 ± 0,00 ^{d}	4,50 ± 0,01 ^{e}	4,81 ± 0,04 ^{f}
Total acidity (mg acetic acid/L)	n.d.	306 ± 7 ^{a}	111 ± 4 ^{b}	72 ± 0 ^{c}	372 ± 0 ^{d}	168 ± 0 ^{e}
TPI (mg eq. Gallic acid/L)	15,91 ± 0,02 ^{a}	249,54 ± 6,98 ^{b}	84,04 ± 0,12 ^{c}	64,52 ± 2,25 ^{d}	283,33 ± 0,26 ^{e}	117,57 ± 6,00 ^{f}
Ethyl acetate (mg/L)	90,57 ± 4,64 ^{a}	134,99 ± 3,14 ^{b}	130,17 ± 0,11 ^{b}	263,52 ± 0,03 ^{c}	290,09 ± 9,40 ^{d}	282,35 ± 5,74 ^{d}
Methanol (mg/L)	108,79 ± 1,39 ^{a}	112,41 ± 2,18 ^{a}	117,9 ± 0,2 ^{b}	47,9 ± 0,3 ^{c}	50,7 ± 0,8 ^{d}	64,4 ± 0,9 ^{e}
Benzaldehyde (mg/L)	0,11 ± 0,02 ^{a}	0,18 ± 0,02 ^{b}	0,12 ± 0,04 ^{a}	0,43 ± 0,02 ^{c}	0,36 ± 0,02 ^{d}	0,33 ± 0,04 ^{d}
Total aldehydes (mg/L)	34,98 ± 1,66 ^{a}	43,71 ± 1,46 ^{b}	38,43 ± 0,17 ^{a}	84,20 ± 0,58 ^{c}	97,47 ± 2,79 ^{d}	91,55 ± 3,77 ^{e}
Higher Alcohols (mg/L)	855,0 ± 0,9 ^{a}	854,8 ± 5,2 ^{a}	845,64 ± 0,91 ^{a}	2464,1 ± 0,4 ^{b}	2361,9 ± 4,6 ^{c}	2409,8 ± 4,6 ^{d}
Fatty acid ethyl esters (mg/L)	6,9 ± 1,1 ^{a}	4,1 ± 0,1 ^{a}	3,6 ± 0,1 ^{a}	164,7 ± 0,1 ^{b}	163,6 ± 0,1 ^{b}	156,50 ± 0,20 ^{c}
Diethyl Succinate (mg/L)	0,13 ± 0,01 ^{a}	4,19 ± 0,57 ^{b}	0,32 ± 0,07 ^{a}	0,45 ± 0,10 ^{a}	3,71 ± 0,13 ^{c}	0,66 ± 0,03 ^{a}
2,3-Butanediol (mg/L)	0,70 ± 0,03 ^{a}	20,62 ± 0,17 ^{b}	0,29 ± 0,06 ^{a}	0,79 ± 0,01 ^{a}	21,6 ± 0,50 ^{c}	0,59 ± 0,07 ^{a}
Diethyl malate (mg/L)	n.d.	1,04 ± 0,17 ^{a}	n.d.	n.d.	0,44 ± 0,03 ^{b}	n.d.
Tartaric acid (mg/L)	n.d.	174,72 ± 4,10 ^{a}	n.d.	n.d.	209,59 ± 16,57 ^{b}	n.d.
Malic acid (mg/L)	n.d.	4,94 ± 0,56 ^{a}	n.d.	n.d.	9,31 ± 0,11 ^{b}	n.d.
Succinic acid (mg/L)	n.d.	26,16 ± 2,45 ^{a}	n.d.	n.d.	24,44 ± 0,77 ^{b}	n.d.
Lactic acid (mg/L)	n.d.	9,19 ± 2,00 ^{a}	1,65 ± 1,91 ^{b}	n.d.	7,46 ± 0,56 ^{c}	2,16 ± 0,33 ^{d}
Acetic acid (mg/L)	n.d.	97,02 ± 2,00 ^{a}	41,87 ± 48,35 ^{b}	28,45 ± 10,59 ^{c}	138,99 ± 9,01 ^{d}	93,15 ± 3,92 ^{a}
Lightness (L*) %	100,1 ± 0,0 ^{a}	90,2 ± 0,4 ^{b}	98,8 ± 0,0 ^{a}	100,5 ± 0,0 ^{a}	90,8 ± 0,5 ^{b}	99,3 ± 0,2 ^{a}
Green-Red hues (a*)	-0,03 ± 0,00 ^{a}	0,42 ± 0,32 ^{b}	-1,09 ± 0,00 ^{c}	-0,09 ± 0,00 ^{a}	0,45 ± 0,38 ^{b}	-0,96 ± 0,13 ^{c}
Blue-Yellow hues (b*)	0,19 ± 0,04 ^{a}	36,80 ± 1,24 ^{b}	6,44 ± 0,01 ^{c}	0,14 ± 0,02 ^{a}	37,30 ± 1,29 ^{b}	5,39 ± 0,87 ^{c}
ΔE_{00}	--	20,5 ± 0,45 ^{a}	5,67 ± 0,01 ^{b}	--	21,00 ± 0,45 ^{c}	4,84 ± 0,71 ^{b}

Figure 1: Mean values ± standard deviation (n = 4) are shown; ANOVA: different letters (a, b, c, d, e, f) in the same row indicate significant individual parameter differences ($p<0,05$); ΔE_{00}, colour difference between aged spirit and INITIAL distillate. n.d., not detected.

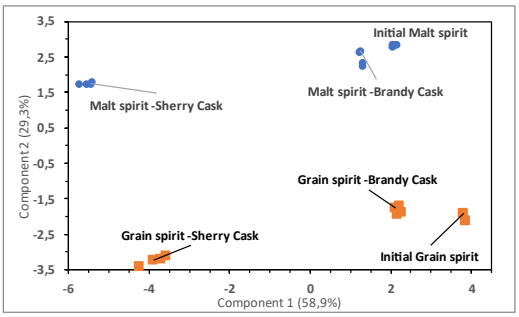

Figure 2: Dispersion plot of the 2 principal components obtained from the PCA, with type of distillate (malt or grain spirit) and type of cask (Sherry Cask® or Brandy cask) labels.

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Diaion™ HP20LX resin to improve antitumoral activity of *Gunnera tinctoria* extracts in a pancreatic cell line

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Gunnera tinctoria, also known as Nalca, belongs to the Gunneracea family and is used for culinary purposes in countries of South America. It can also be found in Azores, Portugal, where it is considered an invasive plant.[1] Gallic acid, ellagic acid, catechin, epicatechin, and quercetin have been described as major phenolics of *G. tinctoria* and are widely known for their antioxidant properties. [2]

In this work, adsorbent resin column chromatography was used to enrich *G. tinctoria* extracts, regarding their phenolic concentration, to consequently improve the extracts bioactivity.

A hydroethanolic extraction was performed on *G. tinctoria* adult leaves. For that, a freeze-dried ground sample was macerated, in triplicate, with different and subsequent ethanol: water mixtures (100:0, 50:50, 30:70, and 0:100; 2 L each). The extracts were combined, and ethanol was recovered. The concentrated aqueous extract was subjected to a subsequent enrichment by adsorbent resin column chromatography (Diaion™ HP20LX). The phenolic compounds were adsorbed on the resin surface, and uninteresting compounds were subsequently eliminated. The enriched fraction was then desorbed with ethanol, and the enriched phenolic fraction freeze-dried. Total flavonoids and phenolics of this fraction were analysed according to reported methodologies.[3]

An antitumoral bioassay was performed with an AsPC-1 pancreatic cell line. To test the effects of the extracts on cell viability, culture growth and proliferation, cells were exposed to the extracts (0.1 and 1 mg/mL, w/v) for 24h. After cellular leakage of lactate dehydrogenase to the extracellular culture medium was determined to assess cell viability. Culture growth was determined by the sulforhodamine B assay, which reports on intracellular protein content. Cell proliferation rates were determined by a 3H-thymidine incorporation assay; DNA synthesis rate was evaluated through quantification of 3H-thymidine incorporation; and intracellular radioactivity was measured by liquid scintillation counting (LKB Wallac 1209 Rackbeta, Turku, Finland). The results were normalized for total protein content.

The resin enrichment enhanced total flavonoids and total phenolics contents from 25 to 43 mg/L and from 35 to 83 mg/L, respectively. Both the original extract and the phenolic-enriched fraction showed a remarkable effect on cell viability at both concentrations, with a strong cytotoxic activity. Moreover, the antiproliferative effect was concentration dependent.

Antitumoral results show that both crude extracts and phenolics-enriched fractions could have substantial activities against cancer, particularly in inhibiting cancer cell proliferation and tumor growth.

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Determination of arsenic, cadmium, and lead in shellfish samples collected in Todos os Santos Bay - São Francisco do Conde City

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The Todos os Santos Bay, the second largest in Brazil, with an area of 1233 km^2, is located in Salvador City and close to the Camaçari Petrochemical Complex and Aratu Industrial Center. This region has many chemicals, petrochemical, metallurgical, pharmaceutical, automotive, fertilizer, and food industries. However, this ecosystem has an infinity of shellfish in many areas, constituting an important food source for local communities and fishing activities that revert to micro and macro commercial businesses.
This work reports the determination of arsenic, cadmium, and lead in shellfish samples collected in Todos os Santos Bay, São Francisco do Conde City region. The shellfish species sampled were lambreta (*Lucina pectinate*), camarão (*Pennaeus smithii*), ostra (*Crassostrea rhizophorae*), chumbinho (*Anomalocardia brasiliana*), and sururu (*Mytella guianensis*). After mineralization in a microwave oven, the chemical elements were determined by inductively coupled plasma optical emission spectrometry (ICP OES). The chemical element contents in the analyzed seafood samples varied from 0.062 to 0.094 mg kg^{-1} for arsenic, from 0.087 to 0.96 mg kg^{-1} for lead, and from 0.022 to 0.033 mg kg^{-1} for cadmium. Lead concentrations were higher than cadmium and arsenic in all shellfish investigated, suggesting the presence of an anthropogenic source in this region. A preliminary assessment of human health risk intake was performed using the Estimated weekly (EWI) index, the target hazard quotient (THQ) index, and the Maximum Safe Consuming Quantity (MSQC)[1,2]. The results indicated that lead could present a risk condition, but a systematic sampling should be performed to obtain data that can find a more consistent conclusion.

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Talinum paniculatum (Jacq.) Gaertn: evaluation of antioxidant and antimicrobial potential

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Unconventional Food Plants (UFP) are a considerable source of natural products still little known and applied. The food industry has given importance to the use of plant extracts as a possible alternative to the use of synthetic food preservatives (antimicrobial and antioxidant). *Talinum paniculatum* (Jacq.) Gaertn., popularly known as "major gomes" is a UFP widely used in medicine and as a food source, but still poorly studied[1]. Thus, the aim of this study was to evaluate the antimicrobial and antioxidant potential of aqueous and hydroalcoholic extracts of *T. paniculatum* leaves against the bacteria *Yersinia enterocolitica* CLIST 3438 and *Staphylococcus aureus* ATCC 29213, and to evaluate the antioxidant capacity of the extracts. Extracts were prepared from the plant leaves. For the preparation of the aqueous extract, 3 g of lyophilized leaves and 300 mL of ultrapure water were used. For the hydroalcoholic extract, 3 g of the lyophilized leaves were extracted with 90 mL of ethanol/water (80:20, v/v)[2]. Subsequently, the extracts were filtered and lyophilized. The minimum inhibitory concentration (MIC) was evaluated by the colorimetric method by micro-dilution with resazurin dye, applying the extracts at concentrations from 55.6 to 0.027 mg/mL in 96 wells microplates with Mueller-Hinton broth. Levofloxacin was used as a positive control at concentrations from 64,0 a 0,031 mg mL^{-1}. The determination of antioxidant activity was performed using the methods: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), and FRAP (Iron ion reducing activity using 2,4,6-tripyridyl-s-triazine). All results were expressed in mg Trolox equivalent per 100 g of sample. For the concentrations tested in MIC, the hydroalcoholic extract was more efficient than the aqueous extract. For the hydroalcoholic extract, the MIC were 0.86 and 13.9 mg/mL for *Yersinia enterocolitica* and *Staphylococcus aureus*, respectively. The aqueous extract did not show bactericidal activity for the microorganisms tested. In contrast, the aqueous extract had greater antioxidant potential for the DPPH and FRAP method, 7755.31 mg Trolox/100 g^{-} and 1.576 mg Trolox/100 g, while the hydroalcoholic extract was of 4108.33 mg Trolox/100 g and 0.977 mg Trolox/100 g, respectively. The hydroalcoholic extract showed better results by the method ABTS, 2097.28 84 mg Trolox/100 g as compared to 1544.52 mg Trolox/100 g for the aqueous extract. The results obtained for the antimicrobial activity of the hydroalcoholic extract of *T. paniculatum* leaves for *Y. enterocolitica* and *S. aureus* are relevant considering its importance for public health, and the use of a natural extract for their inhibition. Additionally, it is necessary to highlight the importance of further studies on *T. paniculatum* antimicrobial activity against other microorganisms. Overall, all extracts had antioxidant capacity, with the aqueous extract presenting the highest hydrogen ion donor activity to the ABTS+ radical, while the hydroalcoholic extract had the highest radical scavenging activity of $DPPH^{\bullet}$. The results obtained in the FRAP show that the aqueous extract of TP leaves has in its composition considerable amounts of substances capable of reducing Fe^{3+} to Fe^{2+}, that is, electron-donating antioxidants. The accomplishment of this study allowed us to present innovative results in relation to the biological properties of a little-studied plant.

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Thermal stability of organic *Spirulina (Arthrospira platensis)* biomass

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Introduction: *Spirulina (Arthrospira platensis)*, popularly known as a blue-greenish microalgae, is recognized as a filamentous cyanobacteria in spiral-shaped form with the ability to conduct photosynthesis. In the last 1000 years, *Spirulina* biomass has been used in Asia-Pacific region due to its nutritional value, that can produce not only proteins, from 53 to 72 % in its dry biomass, but also lipids, minerals, vitamins, carbohydrates, fibers and bioactive compounds, as the blue pigment C-phycocyanin (C-PC). Recent researches has shown that *Spirulina* biomass has been wide exploited, mainly because its antioxidant properties, in pharmaceutic, food, feed and beauty industries. Therefore, according to Credence Research Market Analysis in the period of 2017-2026 it is expected that the global market for algae products, will register an annual growth rate of 5.8%, reaching more than USD 53 billion. Besides economic potential, *Spirulina* biomass also represents an interesting sustainable raw material alternative, legally authorized as a food supplement with no toxic effects in the Japan, United States of America[1], Europe[2] and Brazil. Although *Spirulina* biomass has desirable beneficial effects, its antioxidant potential can easily degrade when exposed to heat and light, indicating the need for care to be taken during its storage[3]. Thus, this work aimed to evaluate the thermal stability of *Spirulina* biomass (SB) and *Spirulina* remaining biomass (RB), after cyanophyte-phycocyanin (C-PC) extraction, to enable food application. **Material and Methods:** To conduct the analyses, distilled water was added into SB and RB powder, after that the samples were filtered using 0.22 µm cellulose acetate membranes (Analítica®, São Paulo Brazil) and then, the absorbance of each sample was standardized at 0.80 using a UV-visible spectrophotometer (Varian Cary®50 Bio). Subsequently, the initial concentration of Chlorophyll-*a* (Cα) was calculated by the Lichtenthale (1987) equation as follow: C*a* (mg/mL) = $16.72*A_{665} - 9.16*A_{652}$, where A corresponded to Absorbance readings at the wavelengths of 665 nm (absolute absorption peak of C*a*) and 652 nm (absolute absorption peak of Chlorophyll *b*). The thermal stability was established by incubating the samples in a water bath at temperatures of 45, 60 and 80 °C for SB and 45, 80 and 90 °C for RB, protected from light. Samples were taken at predetermined time intervals and the thermal denaturation constants (Kd), half-life ($t_{1/2}$) and the activation energy of the denaturation reaction (Ed) were calculated based on linear regression analysis, determined by the Arrhenius method. The analyses were performed in triplicate. **Results and Discussion:** The C*a* concentration results (mean ± standard deviation) of SB and RB samples were respectively: (5.76 ± 0.34) mg/mL and (8.55 ± 0.21) mg/mL. The samples degradation followed a first-order kinetic model, obtained by constant kinetic determination. The half-lives ($t_{1/2}$) of SB at 45, 60 and 80 °C and RB at temperatures of 45, 80 and 90 °C corresponded to 193, 96 and 40 minutes and 165, 46 and 58 minutes, respectively. These results demonstrated that $t_{1/2}$ values of SB solution decreased as a function of increasing temperature. The closest recent results were described by Colla and collaborators (2016)[3] that evaluated thermal and photo stability of the antioxidant potential (AP) of the powdered *Spirulina* biomass, showing that the antioxidant potential decreased more than 50% after 30 days of exposition in all storage conditions. The mainly literature results have described the thermolability of C-PC but not *Spirulina* biomass instead. RB sample has demonstrated different characteristics, with an increase of 12 min in RB stability with more 10 °C (from 80 to 90 °C in temperature), it is important to emphasize that this is the first studying RB samples. Moreover, the activation energy (Ed) for SB and RB was 42.06 kJ/mol and 25.70 kJ/mol, consequently, this indicates that the higher the activation energy the slower the degradation reaction will occur, the color differences can be seen in Figure 1. Comparing SB and RB Ed results we can assume that RB will be degraded faster than SB. **Conclusion:** It was possible to examine that the temperature increment can cause the activation energy required to break the chemical bonds and contribute to SB and RB degradation. Further analysis needs to be done for better comprehension of thermal degradation behavior, as the nanoencapsulation analysis to verify the thermal protection and the maintenance of the bioactive properties after SB and RB entrapment in different conditions of *Spirulina* food application.

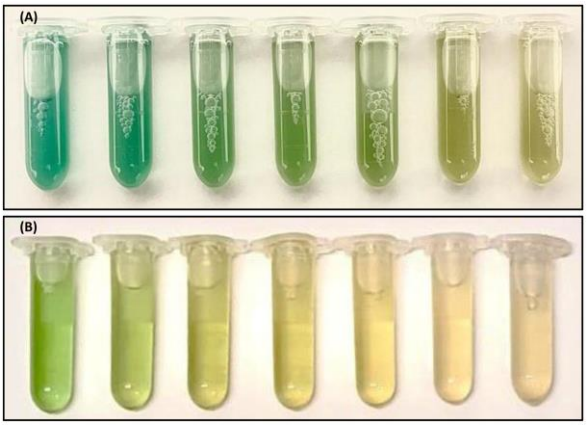

Figure 7 – Degradative color representation of *Spirulina* biomass (A) and Remaining biomass (B) after thermal stability analysis.

Keywords: *Spirulina*, thermodegradation, kinetics.

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Nutritional and chemical characterization of ivy gourd (*Coccinia grandis* (L.) Voigt)

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Recently, different unconventional food plants, also known as wild edible plants (WEP), have demonstrated great nutritional properties and important economic potential. They are found in nature growing spontaneously and therefore they are frequently perceived as being weeds. Owing to the lack of scientific and technical knowledge of their properties, their consumption is generally low, despite they are still traditional included in the diet of some rural communities around the world. *Coccinia grandis* (L.) Voigt, commonly known as ivy gourd, is a WEP consumed in Brazil where it is known as "pepininho silvestre". The fruits of this species have different colors in their maturation states, being green in its growth and development phase, and scarlet when ripe.[1] The green fruits are generally consumed in salads, resembling cucumbers, while in jams and sauces when mature. Besides its nutritional characteristics and use as food, different therapeutic and medicinal properties have been ascribed to different parts of this plant.[2] Nevertheless, there is still a scarcity of data and scientific research on the fruits of *C. grandis*, particularly in what regards their chemical composition along the different stages of the plant. Therefore, the present study aims to provide an evaluation of the nutritional and chemical profiles of different maturation stages, thus contributing to the valorization and increasing knowledge of this underexplored fruit.

C. grandis fruits were harvested in December 2021, in the southern region of Brazil (Paraná), in two stages of maturation, namely in the immature state (green) and ripe (scarlet). Samples were studied for their nutritional value following AOAC procedures and, free sugars, tocopherols and fatty acids were analyzed by chromatographic techniques[3].

Regarding the compositional results of the studied green and ripe samples, expressed in dry weight (dw), carbohydrates (98.2 and 92.1 g/100 g, respectively) were the major proximate constituents, followed by proteins (0.144 and 0.146 g/100g, respectively). The higher energy value (403.8 kcal/100 g) was presented by immature stage. Fructose was the most abundant soluble sugar, reaching 26.8 g/100 g in ripe stage and 18.2 g/100 g in immature stage. Unsaturated fatty acids predominated in immature samples, given the high contents of linoleic (34.7%) and oleic (24%) acids, immature stage had also the highest levels of tocopherols (21.4 mg/100 g).

Overall, the characterization accomplished in this work regarding the chemical and nutritional properties of *C. grandis* fruits, contributes to its recognition and valorization as mini-legume to be included in people's diets.

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Techno-functional and physicochemical properties of lentil flour with different particle sizes

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Bread and cookies are amongst the most consumed foods worldwide. Wheat and corn flours are traditionally used as their main components, providing nutritional and functional value. Legume flours are being increasingly used in industries and at home because they improve the nutritional profile of foods and help tackle waste production of uncommercialized grains. The particle size distribution of a given flour determines some of its techno-functional characteristics and can modify the final quality of products in which they are applied. The objective of this study was to evaluate the particle size distribution of flour obtained from thermally treated lentil and the impact of granulometry in different techno-functional and physicochemical characteristics.

Dry lentil (*Lens culinaris* Medikus var. variabilis) seeds (Don Elio, Argentina) were soaked in distilled water for 8 h (ca. 25 °C) and then were cooked in a fresh batch of water for 30 min at boiling temperature. Afterwards, the lentils were dried in a dehydrator (12 h - 60 °C), and ground using an electric domestic grinder. Lentil flour was fractionated using a set of sieves obtaining 5 fractions (F). Their particle sizes were in the following ranges, in mm: F1 ≥ 1.0; 1.0 > F2 ≥ 0.50; 0.50 > F3 ≥ 0.250; 0.250 > F4 ≥ 0.125; 0.125 > F5. The fractions' moisture content was evaluated by a moisture analyser, while the AACC 08-01 method[1] was used to assess ash contents. Also, the flours' Water Holding Capacity (WHC) and Oil Holding Capacity (OHC) were also evaluated. Fourier Transform Infrared spectroscopy with Attenuated Total Reflectance (ATR-FTIR) was used to evaluate the presence of macrocomponents. Besides, samples were observed using Scanning Electron Microscopy (SEM) in order to study the microstructure of the different fractions. Unfractionated flour (UF) was also submitted to all the previous analysis.

After fractionating, particle size distribution was: 17.8, 28.7, 17.8, 9.6, 26.1 %, from F1 to F5, meaning the major constituents of treated lentil's flour have between 1.0 and 0.250 mm of diameter. The obtained fractions were clearly physically different in terms of color and general appearance. F1 has a heterogenic aspect with particles of various shapes and light to dark tones, which turn to completely homogenous light-brownish particles in F5. Finer flours showed higher moisture contents with values going from 4.6 % in F1 to 7.6 % in F5, probably due to their greater surface areas. Ash content ranged between 1.2 and 2.0 %, observing the lowest content in F5, which can be related to the loss of the outer layers of the seed during grinding. Results of WHC were higher in the coarser fractions. OHC results were inconsistent with particle size, with F5 presenting the lowest value obtained. These differences may be due to the greater or lesser presence of lipophilic components in the different fractions. The ATR-FTIR analysis showed peaks related to carbohydrates, lipids, and protein in all spectra, with no noteworthy differences among the various samples. SEM images showed F1 is composed of F5 particles that with grinding detach from the main structure. F3 was shown to be an intermediate state, with the coarser and finer structures coexisting.

Thermally-treated lentil's flour is mainly composed of fractions with high granulometry. This is advantageous for preparing foods that require low enzymatic digestion. High WHC values are desirable when improving properties in baked goods, like their specific volume, while samples with high OHC values are useful for enhancing palatability and flavor retention. In this sense, F3 could be considered the best fraction, balancing functional properties with general aspect and an intermediate particle size. Overall, granulometry affected the physicochemical and techno-functional properties of the thermally-treated lentil's flour. The differences found between fractions would allow their use for the preparation of different foods according to different needs.

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Use of edible flowers as new potential antioxidant additives for food stability

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Butter lipid oxidation leads to depletion of the quality food products, resulting in shortening of shelf life, as well as in the reduction of their nutritional quality. Several studies using natural plants extracts have been carried out in order to minimize or limit the occurrence of lipid oxidation. Therefore, the addition of antioxidant substances is necessary from technological and nutritional point of view to control oxidation process.[1,2]

Edible flowers (EF) have been used since ancient times in traditional cuisine and alternative medicine, for their nutritional and health benefits. These benefits are attributed to their phenolic acids, organic acids, and flavonoids (anthocyanins), which are bioactive compounds that possess antioxidant capacity. [3]

The aim of the present study was to determine the phenolic composition and antioxidant capacity of five different edible flowers, namely *Viola tricolor*, *Rosa damascena* Mill., *Pelargonium graveolens,* and two different species of *Calendula officinalis* L.

High Performance Liquid Chromatography – Diode Array Detector (HPLC-DAD) was used for the identification and quantification of phenolic compounds. Total polyphenolics, *ortho*-diphenols, and flavonoids were determined by Folin-Ciocalteu and complexation with sodium molybdate and aluminum chloride, respectively. Antioxidant capacity was evaluated through ABTS, DPPH and FRAP methods.

These analyses allowed to select extracts with better antioxidant capacity, that will be further added to the butter. Furthermore, this study was designed to evaluate the effects of these natural antioxidants on the oxidative stability of butter under different storage times, temperature conditions, and in different concentrations.

The results showed that there were significant differences in the content of phenolic compounds and antioxidant capacity of the analyzed edible flowers. The total phenolic content, *ortho*-diphenols, and flavonoids ranged from 12.28 ± 0.29 to 82.06 ± 1.28 mg Gallic Acid/g; between 0.89 ± 0.00 and 222.67 ± 0.02 mg Gallic Acid/g and from 5.53 ± 0.38 to 12.97 ± 0.71 mg Catechin/g, respectively. For the antioxidant capacity, *Rosa damascena* Mill., and *Pelargonium graveolens* were the flowers that presented the highest antioxidant capacity for the three methods. Regarding the determination of phenolic compounds by HPLC-DAD, compounds belonging to flavonoids (flavonols and anthocyanins) and non-flavonoids (phenolic acids) classes were identified.

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Soil fertilization practices as a tool for enhancing wines characteristics – assessment of the amino acid and volatile content of Aragonez wines

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Due to their proximity to the Mediterranean Sea, Mediterranean vineyards, such as Alentejo vineyards, are placed in a climate hotspot. With the increasing aggravation of climate change effects, such regions are experiencing faster than average temperature rises and suffering significant rainfall losses, which translates into elevated risk for soil degradation.

Some risks associated with climate change mitigation and adaptation are declines in soil quality and fertility, plus reduced water infiltration and storage. As a result, many global organizations have been raising awareness of soil conservation and protecting soil from degradation. In addition, viticulture practices often aim to optimize wine grape quality rather than yield, as winemakers seek to bring out the flavors of a specific terroir. One impact of higher temperatures is grape ripeness too early before their aromas have a chance to develop fully.

This work aimed to understand the influence of several nutrient applications to soil vineyards on the amino acid and volatile content of the wines from Aragonez grapes. Most soils contain adequate micronutrients. However, principal macro-nutrients and secondary macro-nutrients are the ones that usually can limit grape production. For example, Magnesium (Mg), a secondary macro-nutrient, is required for metabolic processes and influences fruit formation and berry ripening as a component of chlorophyll molecules. The experiment was conducted in a randomized block design of Aragonez grapes, with three replications, in a split-plot arrangement, with the application of three principal macro-nutrients, Nitrogen (N), Phosphorus (P), and Potassium (K), as well as three secondary macro-nutrients, Magnesium (Mg), Calcium (Ca), and Sulphur (S). Two different doses of Mg were applied (D1 and D2). In addition, there were six other treatments for each amount of Mg used, varying in one of the macro-nutrients mentioned above missing. Furthermore, a plot with no Mg added was considered. Finally, the grapes were harvested, and the wines produced from each of the 13 plots above were analyzed. Oenological parameters of all wines were determined, HPLC-DAD quantified amino acid (AA) content, and the volatile composition of all wines produced was determined and evaluated by HS-SPME-GC/MS. One-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) test at $p<0.05$ and linear discriminant analysis (LDA) were performed. Results obtained from the wines under study showed an evident influence of soil fertilization on wine's amino acid and volatile content, meaning that vineyard fertilization is an essential factor for optimum grapevine growth.

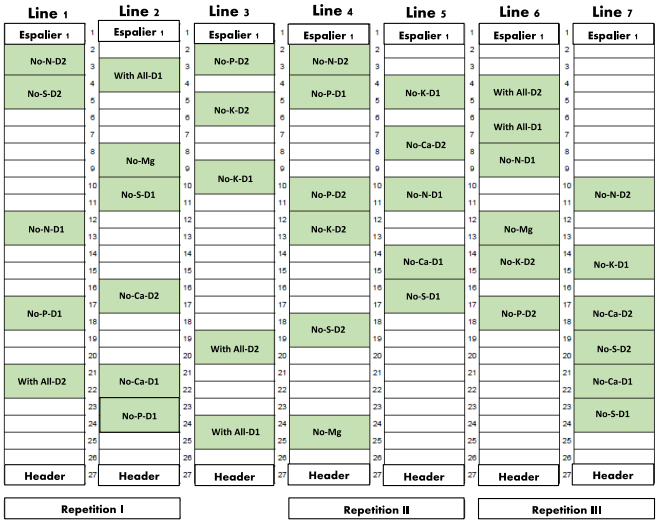

Figure 1: Experimental design for the soil fertilization assay with the different treatments.

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Inovação de Produtos e Tecnologias

Salt content control of tuna loins during processing: coccion operation

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There is an increasing concern to eat healthy. Nowadays consumers have a better idea of the nutritional quality of food and the effect of the presence of harmful substances to their health mainly if they are of frequent ingestion. This concern is followed by the food industry, which is constantly doing great effort to provide food of high nutritional quality and safety. Companies adopt measures and implement strict plans aiming at ensuring good manufacture practices. These strategies are applied not only in the evaluation of the product at the end of production, but also inline control during the processing stages. Routine analyses were introduced in the food industries, allowing to obtain quick and clear results, so that they can act, allowing to carry out a more rigorous control of their products quality. This work carried out in a tuna loins processing industry where routine analyses were implemented in order to control salt content during boiling operation and histamine detection, which are important indicators of fish quality. *Coccion* operations are quite difficult to control in terms of water salt content, because it evolves large volumes of brine bath used to cook the fish that comes directly from captured sea, introducing some salt into these tanks.[1,2] The main objective was to optimize the tuna cooking step, aiming at mitigating issues related to the variability of the salt content in final products.

Tuna fish from three capture FAO zones (Atlantic, Indic and Pacific Ocean) was processed to obtain cooked tuna loins than will be later supplied to canned tuna industry. Process was monitored in terms of tuna loins salt content determined by potentiometric titration. Sampling was performed at fish reception, after thawing (before cooking) and after being immersed in boiling water. Boiling water was also monitored in terms of salt content and pH, and water volume measured before and after each processing batch. Histamine was also determined by Biofish equipment enzymatic sensor.

In general, values in the final products were within the allowed limits, in terms of salt and histamine. Nevertheless, concerning salt, significant variation was found between different tuna species which was observed also after processing. Histamine was detected only once during this study due to unexpected high volume of tuna that had to be processed on that week.

The cooking step itself promotes some salt concentration mainly due to some evaporation, promoting higher mass transfer rates of salt into the fish resulting in higher values in final products. [3]

It was verified that this processing step is a critical in the control of salt levels foreseen for the final product. Undoubtedly the cooking step should be redefined, and some measures like the design of the equipment, monitoring of the water volume and monitoring the initial salt content of the raw material are the first to be implemented.

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Structure and performance of polysaccharides extracted from brown seaweeds of occidental Portuguese coast. A pilot study.

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The alginate was initially discovered by Stanford in 1881. This is a very abundant polysaccharide in brown seaweeds, comprising up to 40 % of the dry Weight (DW). It is located in the intercellular matrix as a gel containing sodium, calcium and other ions. In food technology the alginates are used for its rheological properties allowing an increase in viscosity, as a jellification agent, as a stabilizing agent of emulsions and aqueous solutions, as a thickener, and as a binding agent, between other possibilities. The alginate is listed in the Codex Alimentarium as generally recognized as safe (GRAS) additive. Alginate is traditionally obtained by extraction from brown seaweed in a six-step process involving: acid treatment, alkali extraction, whitening, precipitation and drying. The alkali extraction is the most important step, once it has direct influence in yields and in the physicochemical properties[1]. The actual extraction processes of sodium alginate gives low yields and a poor quality product being, therefore, unattractive for industrialization. To enhance productivities and quality, the optimization of an alkali extraction process is necessary[2]. In this study we used a brown seaweeds (*Laminaria hyperborea*) common in the shores of Viana do Castelo, Portugal, with the objective of evaluate the yields and quality of alginates using a variety of extraction methods with different solvents for precipitation and purification. Samples of the seaweed were collected in May and June, 2021 in the beaches of Castelo de Neiva, Viana do Castelo, Portugal. The collection took place in the intertidal area during the low tide. The extraction process followed the previously described six-steps: an initial pre-treatment with formaldehyde (CH_2O), an acid treatment with sulfuric acid (H_2SO_4) and then the other 4 steps. For the alkali extraction we varied the temperature, and the alkali. The alkali concentration was (1–10 % (w/v)), and the relation between alkali volume to dry seaweed was 10 mL of alkali: 1g of dry seaweeds. For the precipitation the agents also varied. Precipitation was followed by drying and grinding. The aim of this study is to evaluate the yields and quality of the different methods. This was a pilot study and a conventional study with a number of replications will follow. The different yields for the different methods are also given in **Table 1**.

Table 1: Experimental conditions and yields obtained in the pilot study.

Method	Extractor solution	Time (h)	Temperature (°C)	Precipitation agent	Yield (% w/w DW)
A1	$Ca(OH)_2$	40	90	ethanol	7.3
A2	$Ca(OH)_2$	40	90	isopropanol	6.0
B1	NaOH	40	90	ethanol	2.8
B2	NaOH	40	90	isopropanol	2.1
C1	NaOH	40	4	ethanol	2.3
C2	NaOH	40	4	isopropanol	4.4

NaOH sodium hydroxide; $Ca(OH)_2$ calcium hydroxide

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Integrated approaches for socio-economic boosting of the sustainable production and consumption of Montesinho mushrooms

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Montesinho Natural Park (MNP) represents a mountain area with a unique mycological heritage. Among the approximately two hundred edible mushrooms found in this protected area, most of them have been consumed since ancient times for their exquisite taste and nutritional value. [1] Nevertheless, the availability of these mountain products is limited by their seasonality and weather conditions, an issue intensified by climate change. On the other hand, unsustainable mushroom collection practices and illegal trade of high-value species have also been common practices with negative impacts in the ecosystem and regional and national economy. All these issues, together with the incapacity of local collectors to guarantee the authenticity/safety of the collected mushrooms, have led many restaurants to avoid their inclusion in their menus, safeguarding their business and consumer's health. Thus, it is proposed the production of appreciated edible mushrooms in controlled *ex-situ* environment. An extensive nutritional/chemical/biological characterization are being performed to ensure the high quality of the produced species and the preservation of their original characteristics. The development of a quality and safety seal, "Safe2Taste", that guarantee the traceability of the entire production chain, aims to increase consumers' confidence/loyalty on the products.

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Bio-based hybrid molecules for coloring and preservative purposes

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The increasing urgency to feed the growing world population, along with growing consumer awareness and expectations, have driven the evolution of food production systems and the processes and products applied in the food industry. Although substantial progress has been made in food additives, the controversy in which some of them are still embroiled has encouraged research into the next safer and healthier generations. These additives can come from natural sources and confer health benefits, in addition to serving to color or preserve, among others.[1] Limiting factors of these additives are often related to stability, sustainability, and cost-effectiveness issues, which justify the need for innovative solutions. Finding compounds that can have both capabilities (colorant and preservative) and additionally exert bioactive functions may be a promising solution. However, to obtain benefits such as antioxidant or antimicrobial activity, the concentrations of these compounds are often high, not meeting the acceptable daily intake (ADI) requirement. In addition, such compounds may take time to become part of the additives authorized for use by regulators, remembering that in addition to the research for these new molecules, they must undergo thorough toxicity and safety evaluation before their use is allowed for consumption.[2]

The research and development of new molecules through new chemical approaches, such as the modification of natural molecules already known and of accepted use worldwide, so that they can develop a better and double performance (colorant plus preservative), may be a path to be followed to circumvent the difficulties and monetize the use of these additive molecules in the food industry. Non-covalent complexation is a natural process and an important mechanism responsible for stabilizing and enhancing the blue, violet, and red colors in flowers, vegetables, and fruits, as well as in food products derived from them. The increased interest in copigmentation has been remarkable, especially by the food industry, in order to enhance the color palette. In view of its mastery and use through the selection of the better copigments to be added to food products, precise (computer-aided) control of the supramolecular assemblies of non-covalent supramolecular copigments is essential. In this regard, copigmentation with antioxidant/antimicrobial molecules can be explored, and the use of new cheminformatics tools and models can support the development of unique hybrid compounds with dual function (coloring and preserving), based on the screening of numerous biomolecules so as to spawn new bio-based molecules as the next generation of food additives.[3]

In this regard, and with the observed advances in computers and computational methodologies for *in silico* experimental aid, their exploitation for the research and development of these safer and more efficient bio-based hybrid molecules with dual functionality by predicting and verifying the experimental results, allow the study of certain physical characteristics that are not easily examined in the laboratory and are very promising, which can help and accelerate research on a topic that is now fundamental.

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Attalea speciosa mesocarp flour in-depth characterization and its application for the development of new bakery products

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Non-conventional food plants (NFPs) present themselves as a viable and efficient alternative for replacing the food products we consume today. Presenting a huge nutritional, chemical, physical, and biological potential, these plants are usually abundant and not competitors with other vegetable matrices used for human consumption.[1] *Attalea speciosa* (Mart. Ex Spreng - Babassu) is a palm tree from the botanical family Arecaceae found in Brazil, and its mesocarp (BM) represents about 20.4% of the fruit and most of its application it's in the manufacture of animal feed.[2] Thus, the present work aimed to deepen the study the nutritional characterization of BM by AOAC methods; the determination, by chromatographic methods, of free sugars (HPLC-RI), fatty acids (GC-FID), and organic acids content (UPLC-DAD); and the study of the phenolic profile (HPLC-DAD/ESI(MS) and bioactivities (antioxidant, antimicrobial, anti-inflammatory and cytotoxicity activities) of the hydroethanolic extracts. Furthermore, given the possible industrial application, bakery products (bread) were developed with 12, 18, and 24% substitutions of wheat flour by BM flour.

BM flour presented low moisture, fat, ash levels, being carbohydrates the main macronutrients, and palmitic (C16:0) and stearic (C18:0) acids were the main fatty acids found in the sample. Regarding phenolic composition, nine phenolic compounds were tentatively identified, six flavan-3-ols (catechin and epicatechin derivatives) and three *O*-glycosylated flavonoids (quercetin derivatives). Specifically, the flavan-3-ols group represented 99% of total amount of phenolic compounds mainly due to the presence of β-type (Epi)catechin dimer. The hydroethanolic extract of BM showed a high antioxidant capacity to inhibit lipid peroxidation and high anti-hemolytic capacity, showing an IC_{50} 99% and 75% more effective than the positive control used (Trolox) in these assays, respectively. Moreover, it was able to inhibit all the tumor cell lines tested (MCF-7, NCIH460, CaCo, and AGS), however, it showed some toxicity towards healthy cells of PLP2 and VERO lines. The results obtained for the antibacterial activity of the BM extract were lower when compared to the positive controls used (E211 and E224). Comparing the results obtained with 100% wheat flour products, it was noted that the formulations with substitutions by BM presented remarkable results, providing a reasonable increase in the PUFAs content (despite decreasing protein content), maintaining the antioxidant capacity with lower IC_{50} values than the positive control used, and not presenting hepatotoxic activity (first validation of this flour for the incorporation in food matrices). In general, the formulation B24 was the one that presented greater similarity to the control bread (B0), relatively to the nutritional aspects, however, concerning the physical parameters it was the one that presented bigger discrepancy, especially regarding the specific volume, texture, and the color parameter *L** (luminosity), tending for a darker and opaque coloration. All the formulations presented higher percentages of loss of rheological characteristics in the first three days of elaboration, however, formulation B24 was the one that presented the lowest percentage. This study presented innovative results regarding the nutritional, chemical and bioactive characterization of both the babassu mesocarp itself and the bread made from it, showing great potential to be applied in the food industry, however, there is still much to explore, especially in preparations that do not yet use non-conventional ingredients.

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Microbiological evaluation of vacuum-packed low sodium sliced cold-smoked rainbow trout (*Oncorhynchus mykiss*) stored under refrigeration

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The use of rainbow trout in the production of smoked products as an alternative to salmon has increased in recent years. On the other hand, salt (NaCl) reduction is one of the priorities of public health authorities worldwide, so the World Health Organization (WHO) has adopted a strategy of a 30% of salt (sodium) intake reduction by 2025. However, salt is indispensable for taste, functionality, processability, microbial stability, and shelf life of the final product. The aim of this study was to optimize the development of smoked trout with reduced NaCl (sodium) contents without compromising safety. Fresh farmed rainbow trout (*Oncorhynchus mykiss*) was purchased in April 2022. The fish was gutted, filleted, dry salted and cold smoked (2 trials). Based on previous experiments the following conditions were used: F1 – 2% NaCl; F2 - 3% NaCl; F3 - 2% mix (50% NaCl + 50% potassium chloride (KCl)) and F4 -3% mix (50% NaCl + 50% KCl). In all experiments, 1% of yellow sugar was added. Cold smoking was performed by using a traditional protocol. Smoked trout was sliced, vacuum packed and stored under refrigeration (5±1 °C). Microbiological evaluation (including total viable counts (TVC), *Enterobacteriaceae*, Lactic acid bacteria (LAB) and *Listeria monocytogenes*) was performed by using ISO methodologies, at 0, 10, 20 and 30 days under refrigerated conditions. A shelf life challenge test for the sliced smoked trout with *L. monocytogenes* 4a CECT 934, with inoculation at a level of 5 log cfu/g, was performed. The initial TVC counts in the sliced smoked trout were approximately 5 log cfu/g. The product was considered satisfactory according to the Health Protection Guidelines 1. The LAB group was responsible for these counts, since the product results from a maturation process by cold smoking. The counts of *L. monocytogenes* were satisfactory. The *Enterobacteriaceae* counts in the raw material (1-3 log cfu/g) are mainly responsible for the initial counts in the smoked trout (2-3 log cfu/g). Only salting condition F2 had a significant influence on TVC counts, which were significantly different ($p<0.05$) from the others under study. However, no significant effect of salting conditions on the microbial content of smoked trout was observed during storage time. After 10 and 20 days of storage, all products (F1, F2, F3 and F4) showed TVC≤ 8 log cfu/g, being considered acceptable. Since the *Enterobacteriaceae* counts were higher than 4 log cfu/g, the application of enhanced handling and preservation practices are strongly recommended to ensure better hygiene of the raw material. According to the results of the challenge test for *L. monocytogenes*, the increase in counts observed in the first 10 days was lower than 1.5 log cfu/g, and the salting process did not influence its growth. After 20 days of storage, the increase in *L. monocytogenes* was 2.2 log cfu/g, independently of the percentage of salt added. Microbiological assessment indicates that no more than 2-weeks shelf-life would be appropriate and safe in terms of accomplishment of the EU regulations and WHO recommendation, taking into account foreseeable storage temperatures (up to 5°C). Thus, it is possible to achieve a reduction of 20–40% of NaCl (sodium) in smoked trout through replacing NaCl by KCl, considering this product as “low sodium” and a “source” of K.

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Advantages and disadvantages of flavouring olive oils

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Flavoured and fortified olive oils have been a growing trend, meeting consumer preferences and offering differentiated and innovative products. However, these preparations can positively or negatively affect the physicochemical and sensory characteristics. The most noticeable changes occur in sensory terms, which can lead to the appearance of desired sensations, depending on the flavouring agent used. On the other hand, it can mask the presence of any sensory defect present in the olive oil. For the physicochemical characteristics and stability, flavouring can influence the levels of antioxidants and increase the olive oils' shelf-life, increasing the incorporation of antioxidant compounds, promoting oxidative stability and reducing oxidation. However, some studies report that incorporating flavouring agents has pro-oxidant effects.

Generally, three techniques are used for flavouring olive oils: i) through permanent or temporary contact with the flavouring agent, ii) by co-extraction, and iii) the the addition of essential oils[1]. From them, direct contact between the olive oil and the flavouring agent is the most usual and traditional technique. Currently, natural extracts rich in a certain compound, such as lycopene or lutein, in the olive oils are used to fortify olive oils[2]. These extracts are incorporated to enrich it in a certain compound or set of compounds, with nutritional and healthy properties but without sensory objectives. Nevertheless, fortification can cause turbidity in the olive oil and/or can promote the appearance of unpleasant sensory sensations. For the present work, a vast bibliographic review[3] was done devoted to the flavouring of olive oils, pointing out the interest in this ancient Mediterranean practice, as well as the diversity of techniques, agents and effects of the flavouring process. Based on the available data, it can be stated that the use of flavouring by direct contact is the most studied technique, which could be attributed to the easily of implementation of this technique, not requiring any change in the usual oil extraction process. Furthermore, considering that flavouring is performed directly on the extracted oils, the desirable sensory properties of the flavouring agent can migrate to the olive oil. Even so, a considerable number of studies still report the production of flavoured olive oils by co-extraction or by adding essential oils. However, a lower but significant number of studies have focused on the enrichment of olive oil with specific bioactive compounds, mainly antioxidants, to enhance the nutritional and healthy properties of olive oils.

Regarding the used flavouring agents and based on the compiled literature (**Figure 1**), different classes can be grouped, namely aromatic plants, which represent more than 42% of the references compiled in this work, followed by spices, with around 24% of the references, fruits, with 14%, and the other agents representing around 19%. Within the aromatic plants, oregano and rosemary were the most common agents (14 references), followed by thyme (12 references) and basil (9 references). The lemon dominates with eight references in fruit, followed by tomato (with 7) and orange (3 references). Regarding spices, three flavouring agents should be highlighted, namely garlic (11 references), pepper (7 references) and chilli (4 references). In the other flavouring agents, the olive leaves had been frequently used (11 references), which is justified as a by-product of olive growing. The huge use of aromatic plants could be related to different factors, such as the wide range of flavours and aromas, the great availability, low price and easiness of use.

It is also possible to infer that different, sometimes contradictory, effects were obtained by different studies when using the same flavouring agent or the same flavouring technique. These differences may be due to or attributed to different factors, which must be considered when comparing similar studies, such as the conditions under which the flavouring is carried out (time, temperature), the amount of flavouring agent used and its state (dry, fresh, powdered), the chemical composition of the flavouring and its storage conditions before use.

Hence, the world of flavoured olive oils still needs to be further studied, being clear the need to go through a path that would allow the standardization of this Mediterranean tradition, aiming to limit and/or overcome the intrinsic variabilities of the flavouring/fortification commercial strategies.

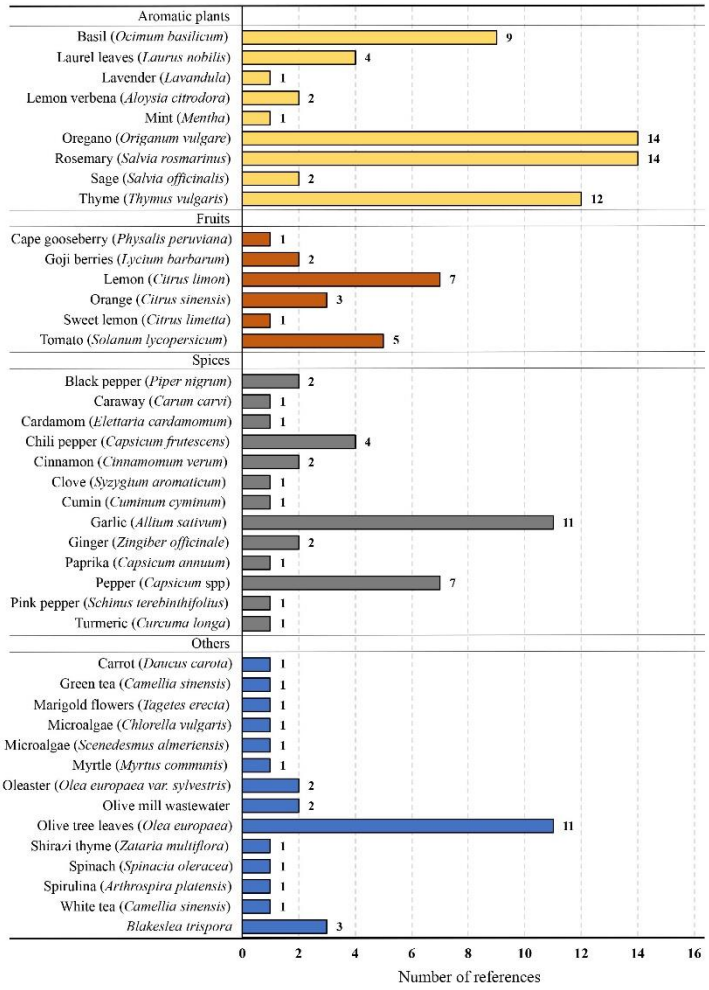

Figure 1: Number of references, compiled from the literature (1996–2022), of each flavouring and fortification agent used for obtaining flavoured olive oils[3].

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Influence of winemaking on the quality of Vinhão wines

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The Vinhão wines are traditional red wines from the Vinho Verde winegrowing region of Portugal. Usually these wines are low-alcohol, acid, and presented a deep purplish red colour, an excessive astringency [1]. Pre-fermentative and extended macerations are two of the various approaches in wine production that have been investigated in order to improve wine' quality. Extended skin contact is often applied to enhance the extraction of skin constituents into the must. During pre-fermentative maceration, phenolic compounds are transferred from berry solids into an alcohol-free environment of grape must under low temperatures to prevent the start of fermentation [2]. Phenolic compounds impact wine body, mouthfeel, astringency or color of wines. Moreover, the information on the content and composition of phenolic compounds in monovarietal wines may be fundamental for wine quality management [3].

In this study, one wine (TW) was traditionally vinified, made by fermentation the must with skin contact for 1 week at 25 °C. The second wine (P-FW), a different vinification technique was applied. It was performed a pre-fermentative maceration during 3 days at 10oC, followed by a fermentation without grape solids. The wines were produced in 2020 at Winery of Ponte da Barca and Arcos de Valdevez. The color intensity, phenolic composition, and antioxidant activity were analysed during an year. Results obtained for colour parameters, phenolic compounds, and antioxidant activity for 1 and 12 months after wine vinifications are shown (**Figure 1**)

	TW		P-FW	
	1 month	**12 months**	**1 month**	**12 months**
Color Intensity	37.06	21.40	13.67	11.28
Hue	0.43	0.56	0.49	0.59
Total Anthocyanins (mg/L)	1626.2	520.6	576.2	335.1
Total Tannins (g/L)	2.1	1.4	0.6	0.6
Antioxidant Activity (mM trolox)	6.7	4.5	2.5	2.8

Figure 1: Results obtained for colour parameters, phenolic compounds, and antioxidant activity for 1 and 12 months after wine vinifications.

The results showed that traditional vinifications leaded to wines with higher color intensity, total anthocyanins, total tannins, and antioxidant activity. For both wines, a decreased of values was observed at end of 12 months, however in a more pronounced way in cause of traditional wines. The alternative vinification can lead to an stabilization of phenolic compounds along the time, allowing to maintain wine quality of wines.

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Production of isoamyl butyrate by bioimprinting lipase-catalyzed esterification of isoamyl alcohol and butyric acid

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Isoamyl butyrate is an ester used as flavoring agent. It is a high-demand flavor and fragrance compound widely used in the preparation of a variety of fruit juice flavor, such as apricot, banana, pear, apple, and other fruit flavors. This study aimed to follow the time course of the esterification reaction of isoamyl alcohol and butyric acid for isoamyl butyrate production, using nonimprinted (free lipase) and bioimprinted heterologous *Candida antarctica* lipase B (CALB) produced in the cell factory *Komogataella phaffii* as biocatalyst. Isoamyl butyrate was produced, following the method of Brandão *et al.* (2021) with modifications [1]. Butyric acid and isoamyl alcohol were diluted in cyclohexane according to López-Fernández et al (2022) [2] and the reaction time was up to 24 h, at 30 °C. After 30 min reaction time, isoamyl butyrate conversion attained around 71 % using CALB nonimprinted and 78 % using CALB bioimprinted. The highest isoamyl butyrate conversion of 94 % was achieved either in the presence of CALB nonimprinted (free lipase) or bioimprinted, after 3h esterification. Thus, bioimprinting heterologous *Candida antarctica* lipase B (CALB) may have potential reuse in the production of isoamyl butyrate. These results suggest that isoamyl butyrate can be obtained by esterification catalysed by bioimprinted CALB, to be used as a flavoring agent in the food, beverage, cosmetic, and pharmaceutical industries.

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Molecular mechanism of lipase-mediated synthesis of flavoring compounds: The impact of enzyme active site hydrophobicity

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Isoamyl fragrance compounds are widely used in the preparation of a variety of food flavors. A rational approach is needed to choose the best route for isoamyl butyrate production by lipase-catalyzed reactions, using molecular docking simulations. In this work, a computational and experimental study was carried out to (i) characterize the structural framework obtained for isoamyl butyrate synthesis, using *Candida antarctica* lipase B (CALB) or *Rhizopus oryzae* lipase (proROL) as biocatalysts, and (ii) to choose the best lipase for esterification reaction route using molecular docking. Isoamyl butyrate synthesis using CALB or native proROL was performed according to the method of López-Fernández *et al*. [1]. Butyric acid and isoamyl alcohol were diluted in cyclohexane and the reaction was performed for 24 h, with 35 UA of lipase, at 30 °C. Similar results, in terms of maximum yield, were found with both biocatalysts: yield of 94% at 3h using CALB *vs*. 91% at 24h with proROL. However, faster reaction was observed with CALB. Then, molecular docking analysis was carried out for both enzymes (CALB and proROL) with reaction substrate (butyric acid). The following results were obtained for butyric acid docking affinity in the lipases structures: -4.0 kcal.mol^{-1} for CALB and -4.3 kcal.mol^{-1} for proROL. In addition, butyric acid showed favorable interaction with CALB amino acids: VAL154, through hydrophobic interactions, and with SER105, through hydrogen bond. Moreover, butyric acid interacts with the following proROL amino acids: ILE120, ILE123, PRO208, VAL236 and VAL239 through hydrophobic interactions and with LEU176 through hydrogen bonds. The obtained results showed the role of hydrophobic interactions at docking pose of butyric acid at both enzymes. The lowest hydrophobic interactions identified at docking pose of butyric acid in CALB structure could possibly lead to higher yields since the short alkyl chain of butyric acid is easily bound at lipase active site. The hydrophobic pocket near to proROL active site increases the repulsion of butyric acid and thus results in a slower esterification reaction. Therefore, the faster reaction time displayed for CALB, in comparison with proROL, for butyric acid conversion, is ruled by modulation of hydrophobicity in lipase active site. In summary, it is here demonstrated that the application of molecular docking analysis to identify the reaction mechanism of lipases to obtain isoamyl butyrate, is a helpful and interesting advance in the synthesis of novel flavoring compounds.

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Bread Waste into Beer: Optimizing bread incorporation in beer production

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Globally, bread is a cultural basic component of every diet in the whole geographic and social spectrum of humanity. Beer itself is one of the most consumed beverages in the world, quite known, distributed and appreciated in each society.

Food industry defines solid solutions to fight food waste. Reprocessing bread waste to breadcrumbs or animal feed are among the most common strategies to reduce the waste. Obviously, it only occurs if all hygiene and food safety parameters are guaranteed. Developed countries are studying new alternatives for the use of surplus bread and beer production from bread waste turns out to be a valid choice. Some companies along the United Kingdom, Singapore, New Zealand and Portugal keep an active and continuous search to improve sustainable production and commercial methods. Besides the efforts in these countries to achieve a successful business, only the first two countries can present proven efficiency in both fields, for what the use of staling bread in beer production is concerned. Their incorporation stands in replacing 25% of the necessary malt to produce beer. The studies conducted in this work are eager to achieve a substantial malt replacement by bread waste, overcoming all the scientific and technological barriers of the process, pointed out in previous works on this subject.

Annual bread production exceeds 100M tones, splitting through Europe (53.6%), USA (28.6%), Asia (10.9%), being the rest produced in Africa and Middle East (6.9%). It is estimated a waste/loss of 10% of the total production, as it stands for 10M tones of bread. This mainly occurs at the supply chain level, due to quality control and unforeseen failures. At the level of the consumer, it is due to a short shelf life[1]. Bread waste/loss is alarming at different levels. First, it is an obvious waste of essential nutrients, considering a world problem of cereals deficiency, aggravated by the present war in Europe. Secondly, there is a chain of events in which it is possible to count a whole range of losses regarding human and energetic resources. All efforts applied in the cultivation, harvesting, processing, packaging and transport of both ingredients and final product must be taken in consideration. An alarm echoes higher and higher as we increase the carbon footprint of our industry, each time our final product ends up as waste or loss.

Starch is a generic fermentation source, with a huge interest in the industry for alcoholic beverages. Since bread is rich in starch, there is some viability in recirculate its components for the production of fermented beverages. The chemical composition of bread is similar to the composition of flour, however, hydrolysis requires more attention because of the whole processing of baking. Starch is partially protected from the major enzymatic activity by the gluten network. Maillard reactions from the baking may react some sugars with amino acids. Also, starch gelatinization creates different resistance to hydrolysis inducing higher temperatures[2]. Usually, food waste is rich in polysaccharides, reachable by appropriate hydrolysis techniques. Finding ideal hydrolysis condition allows an higher yield of fermentable saccharides, meaning that an optimized hydrolysis grants an increased access to starch depolymerization to produce final ethanol[3].

The conducted experiments in this work required different trials of wheat and rye bread incorporation, in order to produce two types of beer - Witbier and Pale Ale. The final goal is defined in a successful 50% incorporation, meaning a replacement of 50% of bread waste/loss instead of malt in the production of beer. We choose water with a pH of 4.7, a value close enough to the ideal production conditions. The beer remains with a similar pH throughout the whole process. We have been studying the best strategies to reach a higher percentage of fermentable sugars from bread, adjusting times and temperatures on each phase of the process with important enzymatic activity. To define the best conditions, we have been evaluating the ºBrix evolution of the wort in each phase. This way, we can optimize hydrolysis and achieve the required ethanol yield necessary in the final product.

Standing in the edge of a serious cereal crisis, consequently conducting to an aggravation of world hunger, a relentless search to new alternatives must be on the agenda. Sustainable production sustained by a philosophy of circular economy, may just be the fastest and vivid solution to this threat.

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Salt and sugar-reduced strategies: An approach to mustard formulation

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Over the years, food product innovation and diversification have been the food industry's focus, including the condiments and seasonings sector. Higher intakes of salt and sugar represent a risk factor for cardiovascular diseases and hypertension. Therefore, the reformulation of food products is currently very topical. This study aimed to bring comprehensive information about the possibility of reducing the salt and sugar content in mustard formulation with identical consumer acceptability and similar stability, by simply reducing these constituents or by adding clean-label substitutes. Thus, a series of trials were developed: 1st) Mustard prototypes with reduced salt and sugar content (25, 50, 75 and 100% less) and Control (prepared according to the industry recipe) were developed to find out the possible levels of reduction in this product. The samples were stored at refrigerated temperature until evaluation. Sensory analysis (9-point hedonic scale; colour, flavour, taste, consistency, global acceptability; 13 trained panelists), pH, and soluble solids content (SSC; °Brix) were evaluated. The results made it possible to conclude that the formulation with a reduction of 25% salt and 25% sugar obtained similar sensory acceptance compared to the control sample, and the pH value was within the range required for the original product (Control); 2nd) To determine whether or not a sensory difference exists between the control sample and the formulation with 25% salt and 25% sugar reduction, a duo-trio test (32 panelists) was performed. It was concluded that there were no significant differences between the two types of formulations; 3rd) Finally, the evaluation of new formulations with salt (KCl) or sugar substitutes (liquorice and sweet potato puree) was tested, considering three levels of reduction (25, 50 and 75%). The samples were characterized in pH, SSC, CIELab colour parameters, and sensory analysis (9-point hedonic scale; 16 trained panellists). The results of this third trial demonstrated that when KCl was added, the panel negatively perceived this substitute, regarding the flavour and the global appreciation, compared to the control product. Overall, the mustard formulations with added sugar substitutes (liquorice and sweet potato puree) were sensory accepted, with scores very similar to those obtained in the original formulation in all the attributes considered. The colourimetric parameters, pH, and SSC values did not show significant differences between the constituted samples and the control sample, indicating the absence of effect of the tested replacements. These findings provide an insight into the possibility of reducing the salt and sugar content in mustard products, providing alternative "clean label" products without compromising the organoleptic perception of the product, and enabling people to adopt healthy behaviours. More work should be developed to evaluate the shelf-life of the reformulated optimized product.

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Development of high-quality sauces with probiotic potential based on fermented green tomatoes

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Using green tomatoes to produce high-quality fermented ingredients has proven a viable strategy to support circular economy-oriented innovation for immature fruit rejected by the tomato paste industry and left in the field in high quantities (can reach 25 tons/ha)[1,2]. Fermented green tomato can be used as an ingredient to develop food formulations with high nutritional and functional value, mainly when using probiotic potential lactic acid bacteria (LAB) as starter cultures[3]. The present study aimed to formulate a sensory appealing, nutritious, and probiotic potential sauce based on the blend of fermented green tomato with other valuable ingredients (avocado, parsley, and honey). The first trial was conducted to confirm the probiotic character of *Lactiplantibacillus plantarum* (LAB97, isolated from the microbiota of green tomato fruits). The second trial aimed to establish the formulation and operation technology of the sauce as well as to evaluate its storage capacity under refrigeration. For the tolerance of LAB97 to gastrointestinal conditions assessment (1st assay), mouth (sterile electrolyte solution (SES), pH 6.9, lysozyme, 5-min at 37 °C), stomach (SES, pH 2.5–5, pepsin, 1-h at 37 °C) and small intestine (SES, pH 8, bile salts, pancreatin, 2-h at 37 °C) conditions were established. The strain was sequentially diluted in these simulated juices, and its probiotic potential was assessed by bacterial viability throughout the process (LAB plate counts). The results indicated that LAB97 counts reached *ca*. 6 log UFC/mL after the *in vitro* gastrointestinal digestion simulation, meeting the viability criterion for potential probiotic capacity. The green tomato (*cv*. H1015) fermentation was prepared by inoculating LAB97 (10^8 CFU/ml, 100 rpm, 20°C) on homogenised immature fruits. After 72 h, the fermentate maintained LAB counts > 8 log CFU/ml and was used in the sauce formulation. Additional ingredients were trialled to design the sauce (2nd assay) to obtain a healthier product offering sensory acceptance. The selected formula comprised fermented green tomato (65%) and a mixture of ingredients (avocado, honey, and parsley, in a proportion of 4:2:1). Three technological strategies were tested to prevent microbial contamination by the additional ingredients and promote the sauce shelf-life. For this purpose, three sauce samples (triplicates) were prepared as follows: partial decontamination (immersion of avocado and parsley in a 100 °C water bath for 15 s and 30 s, respectively; Id: PD), mixed ingredients sterilisation (110 °C for 2 min; Id: S) and no treatment applied (raw ingredients; Id: F). The samples were kept in glass jars at 4 °C for 21 days, and aliquots were taken at regular intervals (0, 7, 14, and 21 days) for the following analysis: LAB counts (CFU/mL), pH, soluble solids content (SSC, °Brix), CIELab parameters (h^*, WI index), total phenolic content (TPC, Folin-Ciocalteu method, mg GAE/100 g FW), and antioxidant activity (AOx, DPPH method, µmol ET/100 g FW) and sensory analysis (9-point hedonic scale, 13 panellists). LAB counts in all sauce formulations maintained viability during the 21 days (> 8 log CFU/mL), pointing to the product's probiotic potential. The pH and SSC values for all samples were within the range of 3.35–3.55 and 13–14 °Brix, respectively, during the tested period. No significant variation in SSC was observed, except for the PD sample, which showed a consistently higher value (14 °Brix) over the assessed period. The TPC and AOx mean values were similar between sample types (51.4 ± 3.4 mg EAG/100 g FW and 2304.2 ± 224.2 µmol ET/100 g FW, respectively), and no significant changes throughout the storage were observed. The sensory panel's evaluation of the samples' colour showed no variations over time. However, mean scores for the PD sample were lower than the remaining samples (loss of the green hue). The consistency attribute showed a tendency to decrease as the storage period progressed without influencing the overall acceptance of the samples. The aroma and taste attribute scores were higher for the F and PD than the S samples. These results concluded that the formulated sauces presented overall acceptance levels above the acceptance threshold, nutritional and functional value (based on the blended ingredients), and probiotic potential. The tested decontaminated treatments did not contribute to improving the sauce stability during the refrigerated storage (5 °C, 21 days). However, further validation is required to confirm the probiotic potential of the formulated sauce.

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Assessment of alternatives to salt and sugar in healthy Ketchup formulations

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Ketchup is a vegetable sauce produced from tomato concentrate and sugar, vinegar, salt, and different spices, used to modify the flavour and aroma of certain foods and culinary preparations[1]. Salt and sugar are widely used in sauces such as ketchup, contributing significantly to the product's sensory acceptance by influencing its taste and aroma[2]. Currently, the intake of salt and sugar in the human diet is considered above the recommended values in Europe. Their consumption is associated with an increased risk of disease development, significantly impacting public health. Today's consumer, increasingly informed and concerned about their well-being, is looking for more natural and healthy alternatives. The food industry is responding to these expectations by reformulating and innovating food products with reduced levels of salt and sugar[3]. This work aims to evaluate the replacement of salt and sugar with alternative ingredients (KCl, liquorice, and sweet potato puree) in ketchup formulations to produce acceptable sensorial products with similar storage capacity. The evaluation of the ingredients (KCL versus salt, liquorice and sweet potato as an alternative to sugar) under consideration also included different testing levels of substitution. For KCL and sweet potato puree, three levels of reduction were considered (25, 50, and 75%), and liquorice was assessed at two concentrations (2,59 and 7,00 g/L). The experimental design included a total of 9 samples (triplicate) identified by KCL (25, 50, and 75%); LIQ (2,59 and 7,00), SP (25, 50, and 75%), and CTR (as the original recipe). Ketchup samples were prepared at a laboratory scale based on information provided by Mendes Gonçalves (formulation ingredients and procedures) and stored in glass jars until analysis (5°C,< 2 h). Sample evaluation included pH, soluble solids content (SSC, ºBrix), CIELab colour parameters, and a sensory panel assessment (nine-point hedonic scale). The pH levels of the tested formulations (regardless of the concentration) showed significant variations from the control samples, except for the replacement of salt with KCL. The pH values of the SP samples showed significant decreases about the control, of about 0.5 units, enough to fall outside the range of values required for this product (3.55-3.75). However, the pH variation in the LIQ samples was close to this range. Similar behaviour was observed regarding the effect of the substitutes on the formulation's SSC. In the SP and LIQ samples, a significant decrease in SSC was observed (*ca.* 20 °Brix), while no variations were recorded towards the CTR in the KCL samples. The instrumental colour assessment (a*, Chroma, h°, and WI) revealed significant variations between CTR and the remaining samples, particularly for sugar substitutes, with CTR showing lower values for all parameters. The tested formulations' sensory effect depended on the substitute type. The replacement of salt with KCL (regardless of the concentrations tested) did not influence the attributes evaluated (colour, flavour, taste, consistency), with the KCL samples obtaining similar sensory ratings and overall acceptance relative to the original product (CTR samples). In contrast, sugar substitution (SP and LIQ samples) harmed product appreciation, with lower ratings ($p < 0.05$) than the CTR samples and below the threshold level of acceptance. We concluded that only the substitution of salt by KCL was successful in terms of sensory and preservation attributes (pH and SSC). These outcomes suggest that sugar substitution is more challenging given the negative impact on the expected sensory characteristics of ketchup and on the SSC values, which can compromise the stability of the product during storage.

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Development of Cobrançosa "functional olive oils" by co-processing techniques

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Virgin olive oil is a food product that is part of the Mediterranean diet, representing one of the most important products included in this diet, since it is in the center of the pyramid, and should be the main source of fat.
"Flavored olive oils" appear on the market as a new food product, whose main objective is to improve the nutritional characteristics, sensorial and shelf life of olive oil, and to avoid or even disguise problems of oxidative degradation reactions and sensorial defects. In addition, the use of olive oil among non-traditional consumers increases, consequently adding value to this important Mediterranean agricultural product.
The aim of this work was to develop "functional olive oils" by co-processing techniques of 'Cobrançosa' olives with the addition of *Thymus citriodorus* (TL, lemon thyme) and *T. mastichina* L. (TM) from organic agriculture. The "functional olive oils" were prepared by: (i) thyme addition to the olives during the unit operations of crushing or malaxation, and (ii) implementation of ultrasound before the malaxation of the olive paste.
The trials were performed using the ripest fruits that remained on the trees, which contain a low aromatic potential. Several parameters were evaluated in the "functional olive oil" and in the virgin olive oil obtained without co-processing, namely: quality criteria parameters, total phenols, fatty acid composition, chlorophyllin pigments, and phenolic profile. Subsequently, the effect of storage (temperature 22-23 °C) on the chemical and sensory characteristics of the oils was studied.
The results obtained showed an important sensorial improvement of all the "functional olive oils" obtained by co-processing. In what concerns the phenol content, all the co-processed oils with TM showed a significant ($p < 0.05$) improvement of phenol compounds, while with TL no significant differences from the control were observed. In turn, in the trials with TL, the extraction of chlorophyllin pigments were higher, particularly in crushing co-processing. The main fatty acids were within the following intervals (%): oleic acid (71.1-72), palmitic acid (12.1-12.9), linoleic acid (8.8-9.8) and stearic acid (3.1-3.5). After the first four months, all the oils showed significant increase of oxidation, measured by peroxide value and UV absorbances, which was not observed in previous studies[1]; this could be explained by the fatty acid composition of 'Cobrançosa' cultivar, more prone to oxidation due to its higher polyunsaturated fatty acids content.

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Bread development with partial replacement of wheat flour by sorghum flour (*Sorghum bicolor* (L.) Moench) germinated and *in natura*

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Sorghum (*Sorghum bicolor (L.) Moench*) is an Unconventional Food Plant (PANC) that has nutritional and chemical qualities with potential applications in the development of new products. Even with all the abundance of plants that have food potential, more than half of the global energy need is currently met by just four crops: rice, potato, wheat, and corn. Therefore, there is a gap in food biodiversity for human consumption. Bread products are the most consumed and easily accepted by the consumer, however, the application of sorghum for bread formulation requires additional technological practices, since PANC do not have the gliadin and glutenin proteins that form gluten, which is responsible for the structure and softness, essential and appreciation characteristics derived from wheat flour. Thus, the use of sorghum in bakery is a more complex process that requires association with other types of flour and technologies to give better results to the final product[1]. Sorghum germination is a technological alternative for nutritional and chemical improvement, since the germination process can increase starch and protein digestibility in addition to reducing anti-nutritional compounds such as phytate inhibitors, tannins and enzyme inhibitors and increase the concentration of enzymes, proteins and phenolic compounds, favors the release of bioactive peptides, which can exert a wide range of biological functions, beneficially affecting antioxidant, anti-inflammatory and antimicrobial activity, being of great value to the bakery industry[2]. The present work aims to develop four baking formulations with replacement of 15 and 30%, respectively, of wheat flour by germinated and *in natura* sorghum flour. The germination process was carried out with maceration of the grains for 24 hours, then drained, followed by another 24 hours at 30°C in an incubator for germination and then dried and crushed. The following physical parameters were evaluated: texture at different storage times (24, 72 and 120 hours) according to the AACC 74-094 method, using a texturometer (TA HD plus model, Stable Micro System, Godalming, United Kingdom), specific volume by seed displacement technique, colorimeter staining (model CR400, Konica Minolta, New Jersey, USA) and water activity of the loaves were determined using AquaLabDew Point water activity meter. The formulations with substitutions of 15% and 30% of *in natura* sorghum flour showed lower crumb firmness after 24h of storage (237.16 ± 8.72 and 789.17 ± 14.62 g/force, respectively) and higher volumes specific, namely, 2.95 ± 0.11 and 3.89 ± 0.17g/mL. The bread formulations with the replacement of 15% and 30% of germinated sorghum showed greater crumb firmness (1388.35 ± 43.66 and 2998.26 ± 137.79 g/force, respectively) and lower specific volumes (2 .29 ± 0.04 and 2.43 ± 0.11 g/mL, respectively). The formulation with 30% of germinated sorghum flour showed greater firmness after 120 h of storage with 3525.55 ± 19.58 g/force, whereas the formulation with 15% of *in natura* sorghum flour showed less firmness after 120h of storage (335.02±4.92 g/force). The formulations with percentages of sorghum flour had no significant differences in terms of water activity after 120 h of storage. The control formulation showed significant differences in firmness on the day of preparation. The color of germinated and *in natura* sorghum flours only showed significant differences for the a* parameter. Regarding the coloring of the baked bread formulations, all formulations showed significant differences. The formulations with germinated sorghum flour replacement showed higher averages for the parameters L*, a* and b*, therefore, they presented a darker color in relation to the formulations of *in natura* breads. Breads with partial replacement of wheat flour by *in natura* and germinated sorghum flour obtained crumb structure, specific volume and color similar to other formulations of breads rich in fiber and whole grain[3], demonstrating the technological potential for use of germinated sorghum flour in bakery products development.

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Influence of Germination Process in Sorghum Grains (*Sorghum Bicolor* L. Moench) on the Starch Gel Technological Characteristics

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Sorghum (Sorghum bicolor (L.) Moench) is the fifth most cultivated cereal around the world, of African origin. Interest in the use of this cereal for human consumption has grown considerably, due to its nutritional potential and possibly being an alternative product to wheat and other cereals[1].Despite being a complex phenomenon, germination is defined as a process in which the embryonic axis of the seed continues the development, which has already been modified by the biological structure. The composition of sorghum grains varies according to the genotype, and in all of them, starch is the main macronutrient, which can be used in several special applications, mainly as a food ingredient[2]. In their native form, starches do not always have the desired properties for processing, so starches are often modified to be used in food manufacturing. The modifications make it possible to obtain starches with greater use in the industry, mainly in the food sector, to help control texture, appearance, humidity, shelf-life, and others. The objective of this work is to analyze the influence of different germination times and temperatures of sorghum (Sorghum bicolor L. Moench) on the technological properties of starch. The germination process was carried out in an incubator following a factorial experimental design, considering the independent variables Time (24, 48 and 72 h) and Temperature (20, 25 and 30 °C). The effects of germination were observed in relation to the aging rate (24, 48 and 72 hours) of the gel of twinned sorghum flours according to the method of AACC 74-09, using a texturometer (model TAHDplus, Stable Micro System, Godalming, UK). The swelling power and solubility were determined at a temperature of 90°C. The solubility was calculated by the ratio of the soluble mass and the initial mass of flour, expressed in percentage, while the swelling power was obtained by the ratio of the final mass swollen by the initial mass of flour. It was observed that the firmness of the sorghum flour gels obtained by the different germination treatments (time and temperature) was significantly lower than that of the in natura sorghum flour gel. It was observed that increasing the treatment time negatively affected the firmness of the gels. The longer the samples were kept in germination processes, the lower rates of firmness were recorded, indicating that time has a significant influence on retrogradation and syneresis of germinated sorghum starch gels. The treatment with shorter germination time and temperature (Time 24 hours, Temperature 20°C) resulted in higher starch gel firmness (104.733 ± 8.405 N) among the treatments with grain germination. When the longer time and temperature were applied (Time 72 hours, Temperature 30°C), it resulted in lower starch gel firmness (6.036 ± 0.016 N). For *in natura* sorghum grain starch gel, the highest firmness index (204.346 ± 6.629N) was observed. The aging rate of the gels and the consequent increase in gel firmness as a result of retrogradation and syneresis were more evident in the experiment with lower germination time and temperature (Time 24 hours, Temperature 20°C). The germination of the grains under the conditions of the treatment with longer time and lower temperature (Time 72 hours, Temperature 20°C) and also the treatment with longer time and temperature (Time 72 hours, Temperature 30°C) resulted in less retrogradation along storage, an interesting feature for the bakery area. It was observed that the treatments with longer time and temperature (Time 72 hours, Temperature 30°C) influenced in order to increase the solubility (2.617 ± 0.013%). The swelling power decreased significantly in the condition of lower time and temperature (Time 24 hours, Temperature 20°C), being similar to the behavior observed by the starch gel of in natura sorghum flour. The swelling power decreased with increasing grain germination time, as well as for higher germination temperature, a behavior also observed in other studies with starch gels[3]. The swelling power significantly decreased when compared to the control, while the solubility increased. It is concluded that germination is an efficient and low-cost alternative method for starch modification with improved technological properties without chemical modification or genetic engineering. The results observed in this study of the behavior of sorghum flour gels, under the applied conditions, allow us to indicate the products for foods that require a lower degree of swelling and greater solubility of starch gels, such as for sauces and instant drinks.

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Effect of protein fortification and hydrocolloids coatings and on shelf life Sarrajão fillets (*Sarda sarda*)

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Portugal is one of the major consumers of seafood in Europe and in the World. According to the FAO and the WHO, fish consumption should be increasingly recommended in a healthy and sustainable diet, not only for the diversity of species, but also for its health benefits to the consumers. Fish is an important source of nutrients, as it is low in fat content and high level in protein, vitamins and minerals. In recent decades, the consumption of this food group has increased and has become available to consumers far from coast areas. However, fish is highly perishable due to several intrinsic factors, such as the high post-mortem pH, the presence of high contents of non-protein nitrogen, unsaturated fatty acids, autolytic enzymes and natural microbial contamination. The use of edible coatings and the optimization of food preservation techniques, is a promising way that allows to protect the quality of fishery products, increasing shelf time, without compromising their freshness. The need to store and safely transport fish to the consumers are factors that enhance the importance of this issue specially to the industry, and make fish preservation imperative in order to maintain its nutritional properties, organoleptic characteristics and extend its shelf life. The use of proteins to enrich food has been used. Whey protein is an animal protein and can be an important additive because it contains high nutritional value and good characteristics for the formation of edible coatings. Pea protein, a vegetable protein, has several advantages such as the presence of essential amino acids, which are essential for muscle mass, and provides good digestibility and low allergenicity for the body.

The objective of this work was the fortification of fillets of Sarrajão (*Sarda sarda*) through the application of hydrocolloids and protein coatings, sodium alginate and agar-agar (7:3), whey protein (WP) and pea protein (PP), with a final concentration of 10% (p/p). The effect of the concentration of 2%, 3% and 4% of hydrocolloids, immersion time of 0, 5, 10 20 and 30 minutes and the effect of cooking on the protein content of fillets were studied.

After filleting process, the fillets were immersed in the coating solution and packed into polyethylene bags. Samples were storage at 4°C during 0, 2, 4 and 8 days. After that the fillets were cooked in a convector oven at 180°C during 15 min. A control experiment without the immersion step was also performed, and an experiment without cooking step was also made. Protein content (AOAC method 955:04, 1995), moisture content and water activity were studied. The analysis of variance (ANOVA) and the Tukey test were used to determine statistically different values at a significant level of ($p<0.05$).

Regarding hydrocolloids concentration results showed that the concentration of 3% in the coating solution presented the highest protein content when comparing to the samples without coating, regardless of the type of the protein used. The maximum protein content in the fillets was obtained with a 10 min immersion WP coating solution and after cooking process. There were no significant differences in protein concentration for immersion times greater than 10 min. No significant differences in water activity and moisture content during storage time were found, regardless of the protein used. Concerning storage time effect on the protein content, results showed that protein content remained constant until the end of storage time.

With this work, it was possible to conclude that it is possible to increase 1.5 times the protein content in Sarrajão fillets, after cooking, through the use of coating solutions with hydrocolloids and protein, maintaining the stability of this fortification over a shelf life of 8 days.

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How *Cynara cardunculus* ecotypes affect the production of Castelo Branco PDO cheese - a case study.

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Cheese manufacture is a way of preserving a very perishable food, milk. This product is a versatile food that offers various flavours and textures, making it a convenient food and a source of nutrients[1]. Last available data from *Instituto Nacional de Estatística* (INE) indicates that the annual dairy consumption reaches 1218 thousand tons in Portugal and the third more consumed dairy product is cheese, with 137 thousand tons per year[2]. In 2021, the world cheese trade was forecast to rise 4 % to hit the record of 3.6 million tonnes[3].
There are eleven Protected Designation of Origin (PDO) cheeses in Portugal, and for the designation PDO Beira Baixa there are three types Amarelo, Castelo Branco and Picante. The Castelo Branco sub-category is the only one that uses *Cynara cardunculus* for coagulation. These flowers contain an enzyme (cardosins) that allows milk to clot and has unique proteolytic properties. The Beira Baixa PDO cheese results from the slow depletion of the raw milk curd of the sheep breeds adapted to the delimited region of the Beira Baixa PDO Cheeses.
To understand the differences between the ecotypes (with unique cardosin profiles) of *Cynara cardunculus*, cheese manufacture test trial was made. Only sheep's milk, salt and thistle were used in the production.
The cheeses were maintained for ten days with a temperature of 7±1 °C and 90±5% of humidity, followed by thirty-two days with 12±1 °C and slowly reduction of the humidity to 70±5 %.
The evolution of colour and weight was recorded on a weekly basis. At the end of the maturation, the different cheeses were evaluated for pH, fat, protein, salt, texture and sensory analyses.

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Technological characteristics of pineapple jellies produced with psyllium

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Extending the shelf life of food products is ideal for any food industry. This can be achieved using many operations, such as jelly manufacture. In this technique heat and high sugar and acid concentrations contribute to increasing the osmotic pressure of the medium and decreasing the water activity, resulting in an inappropriate environment for the development of most microorganisms, enhancing preservation. Jelly is a food spread consumed all over the world and made with fruit juice, sugar, acid, and pectin. Pectin is responsible for the firmness and spreading of the jellies due to the capacity of gell formation. This carbohydrate interacts with acid and sugar, forming a fibrillar network that traps water molecules, enhancing the viscosity. Besides being a natural cheap compound, pectin could be replaced by other gelling agents, such as psyllium, which is rich in dietary fiber that increases satiety, reducing caloric intake. The dietary fibers found in psyllium rusk are able to swell several times forming a gel in the presence of water (Bhat et al., 2018). Therefore, the objective of the present study was to produce pineapple jellies with psyllium as a gelling agent, comparing its technological characteristics with a control formulation produced with pectin. After washing, sanitizing, and extracting the pulp of the pineapples, the jellies were prepared using 50% (w/w) of fruit pulp, 50% (w/w) of sugar, and 0.65% of citric acid (related to the sugar mass). The control jelly (C) was prepared using 1% of pectin (related to the sugar mass) while 0.25% (related to the sugar mass) of psyllium was used in the new formulation (P). Rheological behavior, pH, titrable acidity, total soluble solids, water activity, and color of the jellies were evaluated to compare their technological characteristics. The data obtained were submitted to analysis of variance (ANOVA) and the means were compared by Tukey's test at 5% significance. No significant differences between the formulations C and P were found for the titrable acidity and total soluble solids. The total soluble solids of the jellies were higher tem 65%, as demanded in Brazilian legislation for jellies of extra type (at least 50% sugar). The water activity of C jelly was significantly higher than the P sample which indicates that psyllium presents a higher capacity than pectin to bind with water molecules, forming strong gells. In addition, the water activity of both samples was high, around 0.85, which does not guarantee food security. The pH of P jelly was significantly higher than the control, however, the titratable acidity did not differ within the samples. This fact is related to the buffering action of dietary fibers (Bhat et al., 2018) and to the lack of complete dissolution of the psyllium gel in the jelly (Wärnberg et al., 2009), which could cause inaccuracy in pH determination. The CIELAB color parameters did not significantly differ between C and P formulations, and the jellies were light (L* around 38), and yellow-greenish (a* around -3, and b* approximately 18), as expected for pineapple jellies. The apparent viscosity of P formulations was higher than the control, confirming its gelling property, as expected, and reported by Bhat et al. (2018) and Wärnberg et al. (2010). The apparent viscosity of the control jelly varies from 95.604 ± 48.285 cP at 0,5 rpm to 39.491 ± 5.585 cP at 5 rpm; for the P formulations, its values vary from 248.121 ± 32.008 cP at 0,5 rpm to 73.027 ± 3.274 cP at 5 rpm. Increasing the angular speed of the spindle resulted in a decrease in the apparent viscosity of both formulations, indicating non-Newtonian behavior, as reported by Hojjatoleslamyi et al. (2013). The results pointed out that psyllium presents the potential to be used as a gelling agent for jelly production, leading to innovation and possibly fiber-enriched food products.

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Pressurized solvent extraction for the production of fish protein hydrolysates from industry by-products.

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Fish by-products produced from the fishery processing industry represents up to 75% (by weight) of total biomass.[1] These by-products contain a wide range of high valuable compounds, including proteins and oils. [1-3] Hydrolysis has been used to produce fish protein hydrolysates (FPHs) containing bioactive peptides, which can be included in functional food and feed formulations. [2,3]

The present work aimed at the preparation of FPHs by sub-critical water hydrolysis treatment using multispecies fish by-products as raw material.

The raw material was deoiled by centrifugation and the oil phase was collected and preserved for further studies. Sub-critical water hydrolysis used deoiled biomass at 5 % (dw/v). Different temperatures were tested (from 150 °C to 210 °C). Reaction time was 30 minutes. Sodium hydroxide was also used as additive at 0.02 M, to promote protein solubilization. After the treatment two end products were obtained: liquid hydrolysates and insoluble solids (**Figure 1A**). Biomass solubilization yields were determined gravimetrically and all end products were analyzed for their ash and protein contents. Peptides' profile in the hydrolysates was evaluated by high performance liquid chromatography (HPLC). Bioactivity of hydrolysates were also assessed *in vitro* considering antioxidant and antihypertensive activities using colorimetric assays.

Results showed that the oil phase collected from the centrifugation step represented 26.4 ± 2.6 % of the total biomass weight. Maximum biomass solubilization yield of the remaining biomass reached after treatment was approximately 90 % (dry weight). Biomass solubilization yields increased with increasing temperature when using distilled water (**Figure 1B**). NaOH addition also increased biomass solubilization yields for all temperatures tested when comparing with the same conditions using only distilled water as solvent (10 to 40% increment; **Figure 1B**). The protein solubilization and hydrolysis patterns are in accordance with the peptides' profiles obtained. All hydrolysates presented interesting bioactive features.

Sub-critical water hydrolysis of fish by-products resulted in the production of promising bioactive protein hydrolysates with potential applications in human or animal nutrition, using an environmentally friendly and easily scalable process.

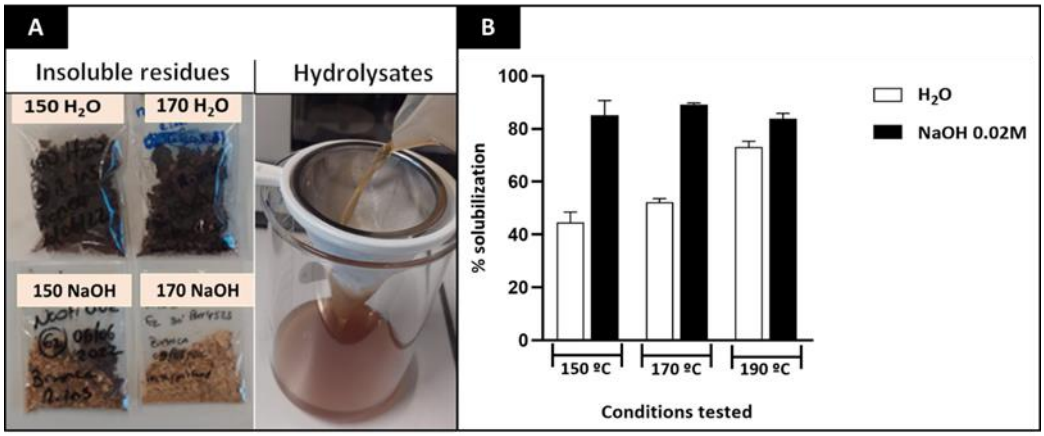

Figure 8 - End-products (A) and biomass solubilization yields (B) of hydrolysis

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Antioxidant activity of alginate edible films containing plant extracts

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Nowadays, food packaging has been the target of increasing attention. Traditional food packaging is usually made of plastic and accounts for 36.9% of the plastics' demand, being considered the largest market for plastic industry. Additionally, plastics are mostly produced with polymers from non-renewable sources and so, they contribute to environmental pollution. A way to circumvent this problem is the development of edible films. Edible films can act as complements to traditional plastics because their functional properties are able to extend food's shelf-life[1].
Plant extracts are obtained by plants and possess antioxidant activity because of their high concentrations of phenolic compounds. They can be effective at low concentrations, are cost-effective and easy to apply, presenting low toxicity levels and high stability during processing and may not affect the sensory characteristics of food products[2].
The addition of plant extracts to edible films confers them the ability to act as food preservatives because they present antimicrobial activity against a broad spectrum of food poisoning microorganisms and antioxidant properties that helps to avoid the deterioration of fats and other food constituents [3].
So, the main objective of this work was to evaluate the antioxidant activity of alginate edible films containing extracts of licorice (*Glycyrrhiza glabra* L.), eucalyptus (*Eucalyptus globulus* Labill.) and sage (*Salvia officinalis* L.). The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-difenil-1-picrilhidrazil (DPPH) scavenging tests were performed on the films and the results show that the films incorporated with plant extracts present antioxidant activity when compared to the control (alginate film without plant extract).
The film incorporated with sage extract was the one that presented the best antioxidant activity by both ABTS (4024,169 Trolox equivalents (μM)/mg film) and DPPH (3954,813 Trolox equivalents (μM)/mg film), followed by the film with eucalyptus extract (ABTS: 3706,291 Trolox equivalents (μM)/mg film; DPPH: 3706,291 Trolox equivalents (μM)/mg film). On the other hand, the film incorporated with licorice extract (ABTS: 942,5195 Trolox equivalents (μM)/mg film; DPPH: 1040,669 Trolox equivalents (μM)/mg film) was the one with least antioxidant activity.

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VEGarum: Innovation of an ancestral gastronomic delicacy through the fermentation of Portuguese macroalgae

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With the rising interest in following plant-based diets, new ranges of opportunities arise in the development of innovative products. These products represent a clean and healthy food intake. Macroalgae have a huge potential as a substitute to animal products due to its rich nutritional content in fat acids, proteins, vitamins, fibers, minerals, and bioactive elements[1].

The present work aims to develop and characterize an innovative sauce based on the famous ancestor Roman sauce known as *Garum*. Original *Garum* is a fermented sauce, produced at the time with the fish waste, by adding salt (20-30%) and herbs. The addition of salt and herbs were a common technique to preserve food. Roman gastronomy applied this sauce mainly in stews, as a substitute to salt. *Garum* enhances food flavours since it adds Umami[2] to the final dish, making it an desired component in the new gastronomical trends. This study looks forward to innovate *Garum*, by producing this product from algae instead of the viscera and less noble parts of the fish, taking advantage of the macroalgae potential and fermentation benefits.

Indigenous macroalgae from the Portuguese coast - *Palmaria palmata* (dulse) and *Ulva rigida* (sea lettuce) were used. Two fermenting trials were performed using the autochthonous microbiota, divided in four steps: 1) macroalgae hydration; 2) 5% NaCl salt and 10% inactivated koji addition, 3) grind and 4) fermentation. Koji has an important role as a source of hydrolytic enzymes, since *Aspergillus oryzae* fungus holds a great capacity to produce enzymes (amylases, proteases, and lipases) to break macromolecules[3]. The enzymatic activity allows the indigenous microbiota to access the necessary nutrients and begin the fermentation process. In order to control fermentation and microbiota performance daily samples were taken along 14 days. From this samples we could evaluate pH and °Brix. We also studied its physical and chemical properties, as protein content, acids production, secondary metabolites, phenolic compounds, and antioxidant activity.

Along the fermentation, for both macroalgae, lactic acid bacteria species were dominant in the first days of fermentation, followed by a dominance of yeast and acetic acid bacteria up to the end. The fermentation process of *Palmaria palmata* tends to reduce pH, between the 2nd and 4th day, going from 5.85 to 4.36 and ending in 4.07 (**Figure 1**). As for *Ulva rigida*, pH reduction is more gradual, going from 5.47 to 4.07 after 11 days, and stabilizing at 4.51. These values are in accordance with the expected presence of lactic acid bacteria, homofermentatives and heterofermentatives, responsible of weak acids production (lactic and acetic). The initial values of titratable acidity were similar for both species - 88.6mg/100g for *P. palmata* and 86.7mg/100g for *U. rigida*. After the fermentation process titratable acidity reached higher levels - 343.9mg/100g in P. palmata and 210.7mg/100g in *U. rigida*, most probably due to lactic acid produced during the process. These final values are in accordance with the final pH values for both fermentations. In terms of °Brix, *P. palmata* evolved gradually until the 11th day reaching a limit of 12.2. Lower values were observed for *U. rigida*, since it reached its limit at the 7th day, with a 9.6 value for °Brix. Afterwards, the value decreased to 8.2 and stabilized. This reduction may be justified by the presence of indigenous halophilic yeast that consumed fermentable sugars.

Sugars content have a direct impact in the fermentation process and different macroalgae species may have different available sugars content. With the present results, it is possible to conclude that both species are easy fermentable and able to produce innovative and tasty sauces in line with the most current food trends. Standing over the sea with scope on innovation, this project strives to achieve sustainable sources and introduce alternatives in the food chain. Processing sea vegetables to develop and recreate healthy and viable products may be valid strategy to a more sustainable food production.

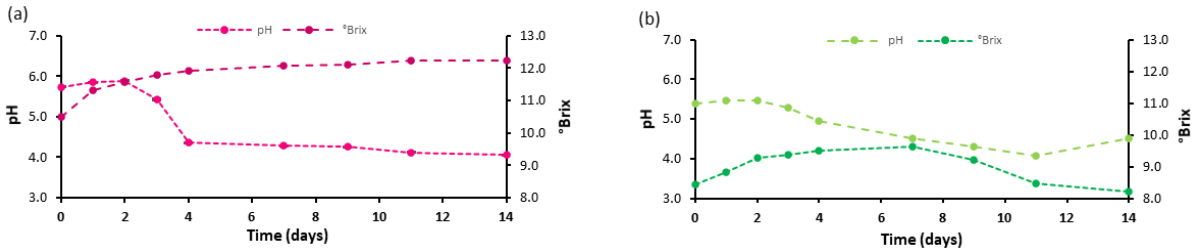

Figure 1: ºBrix and pH evolution of VEGarum from *Palmaria palmata* (a) e *Ulva rigida* (b) macroalgae over 14 days.

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Biodegradable films produced with arabinoxylan extracted from corn fiber

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The corn starch industry produces various by-products in vast amounts, including corn fiber, which is mainly used in animal feed applications. This raw material is rich in arabinoxylan, a hydrophilic polysaccharide with film-forming properties. In this work, arabinoxylan was extracted from corn fiber with a mild alkaline solution[1] and further purified with membranes processes, more precisely, a pre-concentration step by ultrafiltration followed by purification with ultrafiltration in diafiltration mode[2]. The purified arabinoxylan extract was used as the basis for the production of films for packaging applications[3].

The crude and purified extracts were characterized in terms of bioactivity (cytotoxicity and antiproliferative activity), showing no toxicity to Caco-2 cells (IC50>10mg/mL), which potentiates their application in the production of edible films, and some antiproliferative activity to HT29 cells (the lowest being EC50=0.12 ± 0.02mg/mL), related to a potential anticancer effect.

The resulting purified aqueous extract presented an intense brown color. Therefore, decolorization was attempted, for a more appealing appearance, employing activated charcoal or hydrogen peroxide. Decolorization with activated charcoal was not successful, however, partial decolorization was achieved with hydrogen peroxide, resulting in a light-yellow solution[3].

Films were formulated with 30% (w/w dry basis) glycerol as plasticizer. Though presenting a high solubility in water, they showed promising properties to be used as wrapping materials. Decolorized films still presented significant antioxidant activity ($(3.21 \pm 0.40)\cdot10^{-5}$ mmol Trolox/mg film), and water vapor permeability values ($(2.94 \pm 0.49)\cdot10^{-11}$ mol·m/m^2·s·Pa) similar to that of non-decolorized films and other polysaccharides. In addition, they showed good mechanical properties under perforation (Tension of Perforation = (1.22 ± 0.41) MPa and Deformation = (53.0 ± 1.7) %), meaning that the decolorization process did not significantly alter the properties of the films. These results show that these films have promising properties for food packaging applications, especially for lower moisture content food products[3].

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Sequential extractions of red seaweed biomass: a cascading biorefinery approach to the recovery of multiple value-added products from *Gracilaria vermiculophylla*

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Industrially, red seaweeds have long been explored for their hydrocolloids, mainly agar and carrageenans. These texturizing fractions are extracted by the tons, in a multibillion-dollar market, that has still been expanding in recent years[1]. Commercially, the recovery of these hydrocolloids is preceded by an alkaline pretreatment, aimed at removing sulphate, improving their gelling capacity and inherent market value. Nonetheless, the liquid effluent produced, and the remaining spent seaweed biomass, are discarded or used in low-value applications (e.g. as fertilizers).

Since the alkali conditions traditionally used for hydrocolloid extraction pretreatment (1.5 M NaOH, 85ºC)[2] are substantially more severe than the ones used for protein recovery (0.1M to 0.5M NaOH, room temperature to 65 ºC)(3) from these biomasses, the aim of this work was to assess if an intermediate condition allowed for both protein recovery and increase in agar texturizing behaviour, from the seaweed *Gracilaria vermiculophylla*. Thus, a series of alkaline pretreatments (with NaOH concentrations ranging from 0.1 M to 0.5 M, hydrolysis times from 1h to 2h, and temperatures from 30 ºC to 60 ºC) were assessed, followed by a conventional agar extraction (distilled water, 85 ºC, 2h). For control purposes, a conventional alkaline pretreatment and an agar extraction without any pretreatment (native agar) were performed in the same conditions. Agar and protein yield, total seaweed solubilization, texturizing properties (gelling strength) and structural composition (FTIR, 3,6-anydrogalactose and sulphate content) of the hydrocolloid fraction were evaluated for each condition.

The protein recovery yield (measured by the Bradford method, using a bovine serum albumin standard) increased as treatments became more severe, reaching 10 mg per g of seaweed, as opposed to 2 mg per g of seaweed in the non-pretreated condition. Moreover, in this condition, there was no significant effect on the agar gelling capacity when compared to the non-pretreated sample (remaining at 200 g/cm^2). Despite resulting in a small decrease in the agar extraction yield (from 12 % to 10 %), the result is far superior to the ones obtained using the traditional alkaline conditions (averaging only 3%).

To further increase protein recovery, a three-step extraction approach was also assessed. In this procedure, the seaweed samples were subjected to a cold-water extraction (targeting phycobiliprotein recovery), followed by mild alkaline pretreatment and hot-water extraction. In this case, it was possible to observe that the recovery of phycobiliproteins had no negative impact on the gelling capability of the extracted agar and, at the same time provided another value-added fraction from the same biomass (with a yield of 300 µg per g of seaweed).

Overall, a sequential extraction protocol using i) a cold-water extraction; ii) a moderate alkali extraction; iii) a hot-water extraction, proved to be a compromising condition between the conventional alkali pretreatment and native agar extraction. This proposed protocol provides two-additional highly desirable side protein-rich products from the red seaweed, in a cascading biorefinery approach, without compromising the texturizing behaviour and market value of its principal product.

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Valorization of Acorn Starch and Polyphenols by Pulsed Electric Fields

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Acorns are a novel food very rich in carbohydrates, namely gluten-free starch. Yet, more than 50% of the total Portuguese production is underused. In addition, acorns are also rich in phenolic compounds, namely tannins, which are valuable in leather production as a more sustainable alternative to the toxic chromium salts, despite being anti-nutritional compounds. Thus, the exploitation of acorns as a starch and tannin source can be profitability from an industrial standpoint if the extraction is carried out together, knowing that starch must be isolated without significant changes to the granules to have economical value [1,2].

So far, the low alkaline extraction with three sieves is the preferred method to preserve the structural integrity of acorn starch with the highest yields, purity, and most relevant properties. But the usage of such starches as food additives has not yet been approved by the European Union on the Regulation (EC) nº1333/2008. Thus, it is important to create clean-label starches and extract both starch and polyphenols using environmentally friendlier solvents. Pulsed Electric Field is a non-thermal emerging technique that is governed by the electro-pulsation phenomenon, i.e., the exposure of cells to electric pulses. It has also been widely used to assist extraction processes from several matrices, with the general outcomes showing increases in extraction yields, improved selectivity, and ability to preserve thermolabile compounds, but no literature was found regarding its applicability to extract starch besides its modification [3].

Hence, this research aims to (1) study the impact of the electric field intensity and extraction time on the recovery of starch and phenolics from Q. robur acorn cotyledons, (2) perform an individual and multi-response optimization to archive maximum yields using the response surface methodology, and (3) to analyze the morphology of starch granules obtained under multi-response optimization and obtained by alkaline extraction. The electric field intensity did not significantly affect the phenolic content but had a negative quadratic effect on tannin extraction and antioxidant activity. Time had a negative quadratic effect on phenolics, tannins, and antioxidant activity. The field intensity had a positive quadratic effect on starch yields, but time had no effect.

The optimum extraction condition for starch, phenolics, tannins, and antioxidant activity was 0.1 kV/cm during 63.3 µs (87 % desirability), whose contents were 5-8% higher than at 0 kV/cm. The extracts under optimal conditions showed increases in phenolics, tannins, and antioxidant activity by 24, 1012, and 15%, respectively, regarding the alkaline extract. In addition to these extracts being aqueous extracts and with a higher tannin content than alkaline extracts, the usage of PEF makes it possible to value the extract obtained during extraction and reduce waste disposal costs associated with the use of NaOH. Although the starch yield was 30% lower than the alkaline method, the pulsed electric field isolated starches are clean label starches, since only water was used as extraction solvent, and thus more secure for human consumption, unlike the alkaline starches. In addition, the granular morphology of the starch granules obtained under multifactorial optimum conditions was also not affected when compared to the control and the alkaline isolated starches. Thus, these starches can be economically valuable.

Hence, pulsed electric field technology is a more environmental and greener alternative to classic extractions.

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Combination of hydrothermal treatments and enzymes for the valorization of chestnut shell residues

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Approximately one-third of food meant for human consumption is globally wasted, and the by-products created in agri-food processing industry constitute a notable part of the waste that gets landfilled.[1] The chestnut had a big impact on the European diet, due to its carbohydrate content, being the most used starchy product before potatoes were introduced in Europe in the XVIII century. This impact is still noticeable in all traditional food applications due to its nutritional value, and, throughout the years, the chestnut farming and processing industry have expanded, causing an increase in chestnut by-products.[2] The shells constitute between 10 and 20% of the fruit's weight and have no current commercial applications, despite their phenolic and carbohydrate-rich contents. Thus, there is an opportunity for the development of new applications for these undervalued by-products, contributing to the valorization of its constituents, in a sustainable circular economy approach.[3]

The present work aimed to produce value-added fractions from the discarded chestnut shells using a two-step approach. Firstly, non-isothermal hydrothermal pre-treatments (in the range of 160 °C to 200 °C, corresponding to severities (S_0) between 2.26 and 3.46), were performed using a solid to solvent (water) ratio of 1kg to 8kg. After the hydrothermal pre-treatment, the liquid and solid fractions were separated and enzymatic hydrolyses were performed in both fractions using the enzymatic cocktail Cellic CTec 2 (using 15 FPU of enzyme per g of substrate, pH 5, 50 °C, for 96h). For oligosaccharide quantification, aliquots from the extractions and residues were subjected to hydrolysis with 4% H_2SO_4 (121 °C, 20 min). The resulting post-hydrolysed liquid and the directly extracted liquid were filtered through 0.22 µm membranes and analysed by high-performance liquid chromatography (HPLC). In addition, phenolic composition and antioxidant activity of the liquid fractions were assessed using standard colourimetric assays.

Chestnut shells' solubilization increased with the rise in the treatment's severity, surpassing 50% in the most severe condition. The monosaccharide content in the liquor was maximum in the 180 °C to 200 °C range, totalling 10 g/L. Glucose and xylose contribute each with a third of the sugar content, with arabinose, glucuronic acid, fucose and rhamnose being present in minor concentrations. As for the fermentation inhibitors, acetic and levulinic acids, furfural and hydroxymethylfurfural presented in relevant concentrations only in the treatment performed at 200° C. Furthermore, the oligosaccharide content (composed of glucooligosaccharides and xylooligosaccharides), proved to be highest at 200° C, nearing 20 g/L. These more complex solubilized sugars proved to be highly susceptible to enzymatic hydrolysis, with saccharification yields surpassing 85% in all treatments performed.

Moreover, the samples extracted at higher severities proved to have substantial phenolic content, resulting in significant bioactivity. This emphasises the multiple possible uses of these fractions as fermentation media, or as a source of antioxidant ingredients to be included in food formulation as technological agents or bioactive fractions. The remaining hydrothermally pre-treated chestnut residues were still composed of 40 % of carbohydrates (mainly glucans), which, when subjected to enzymatic hydrolysis, resulted in glucose concentrations surpassing 20 g/L. This saccharification yield (reaching 60% at the optimal condition) presented itself as more than double the one obtained in the samples without pre-treatment (direct hydrolysis of chestnut shells). This reinforces the potential of these by-products to be explored in a biorefinery approach, recovering technological/bioactive agents and using the residual fraction as substrate in biotechnological processes to produce different commodities (e.g. bioethanol, bioplastics or prebiotics).

Taken together, the combination of autohydrolysis with enzymatic hydrolysis has proved to be an efficient and sustainable method to recover fermentable sugars and bioactive compounds, like phenolics and flavonoids from chestnut residues.

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Linking abiotic stress treatments to shelf-life extension: fresh-cut carrot as a case study

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The rapid quality deterioration and reduced shelf-life of fresh-cut shredded carrot (FCC) are primarily due to microbial proliferation during storage, quickly surpassing the well-defined microbiological thresholds, and high respiration rate promoted by cut operations, rapidly reaching inadequate modified atmosphere packaging (MAP) conditions. The excessive microbial growth contributing to the development of off-putting odours and flavours are difficult to perceive commercially until package overture. Consequently, repeat buying by consumers could be affected and compromise product's market continuity. Further, changes in fresh-cut carrot sensory quality, such as losses in orange intensity and fresh-like flavour, are caused by minimal processing standard technology that uses hypochlorite solutions (HIPO) as the sanitising agent. Besides compound leaching phenomena (phenolics and carotenoids), compound oxidation is also promoted by HIPO, further compromising the bioactive and sensorial quality of the product. Integrating heat shock treatments and MAP packaging solutions with micro-perforated films (PM-MAP)[1] has demonstrated significant effects on the retention of fresh-cut carrot quality (control of microbial growth, increased bioactivity and preservation of fresh-like quality) as an alternative technology to standard processing. The novel strategy targets the improvement and maintenance of the product's fresh-like quality through the use of abiotic stresses while responding to the non-chemical usage by the consumers and environmental sustainability. This study aimed to compare the behaviour of FCC when processed by the two technological approaches (heat shock, 100 °C/45 s, and HIPO, 200 ppm/1 min, using PM-MAP) by modulating the quality changes during storage (14 days, 5 °C). For standard processing samples (Id.: HIPO), carrots were peeled, shredded and decontaminated by immersion in chlorinated-water (200 ppm free chlorine/1 min, 5 °C) and subsequently rinsed (ice-cold water for 2 min). For heat-treated samples (Id.: HS), carrots were peeled, immersed in a thermostatically controlled pilot-scale steriliser set to 100 °C for 45 s, cooled ice-cold water (5 min), dried, and shredded. Both sample types were packaged in portions of 125 g using PPlus® - 35PA120 bags (200 x 110 mm) (Amcor Flexibles Neocel – Embalagens Lda., Lisboa, Portugal) and stored at 5 °C and analysed at days 0, 3, 5, 7, 10, 12 and 14. Analytical procedures included total aerobic plate count (TAPC), total phenolic content (TPC) and sensory evaluation (5-point numeric rating scale for Colour, Fresh-like appearance and Fresh-like aroma; 5-point hedonic scale for Rejection index). The TAPC response was fitted to the Gompertz equation and the TPC to the equation proposed by Amodio et al.[2]. The sensorial data were compared by ANOVA. From the Gompertz model fitted to the TAPC experimental data (**Figure 1**), the microbiological threshold limit (7.5 Log_{10} cfu.g^{-1})[3] in HS samples is reached after ≈19 days of storage, 12 days more than in HIPO samples. The model proposed by Amodio et al. describes the two distinct phenomena related to phenolic changes during storage: the *de novo* synthesis of phenolic compounds as a stress response and the respective oxidation. The projection of phenolic changes during storage (**Figure 2**) showed significant differences in the phenolic accumulation pattern between HS and HIPO samples: a more prolonged synthesis phase was observed in HS samples, contributing to significant phenolic accumulation, surpassing the raw material TPC level as early as day 5, a situation never achieved in HIPO samples. Sensorial scores of HS samples regarding colour, fresh-like appearance and aroma, and rejection index were always significantly lower than those of HIPO samples during the 14-day storage. HIPO samples were rejected after 7 days, while HS samples were consistently scored below the cut-off level. The heat shock and PM-MAP allowed to meet three key FCC quality criteria: microbiological, sensorial and bioactive. The proposed technology emphasises fresh-cut shredded carrot freshness, bioactive composition, and the possibility of at least doubling the shelf-life of the commercially (HIPO) available product.

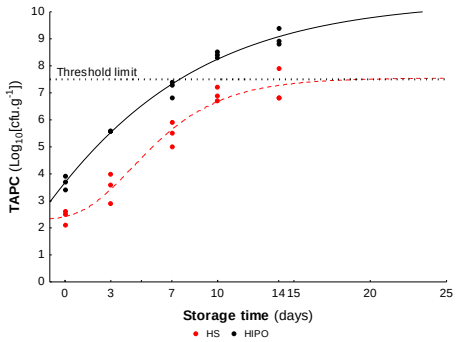

Figure 1: Growth curve of TAPC fitted with the Gompertz equation during storage (5 °C) of heat- (HS, 100 °C/45 s) and chlorine-treated (HIPO; 200 ppm/1 min) fresh-cut shredded carrot samples packaged with a micro-perforated film (PM-MAP).

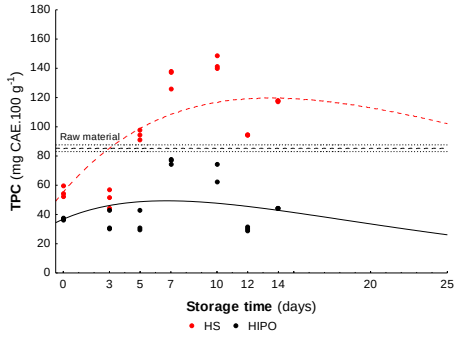

Figure 2: Changes in TPC and data fitting to the proposed equation by Amodio et al.[2] applied to heat- HS, 100 °C/45 s) and chlorine-treated (HIPO; 200 ppm/1 min) fresh-cut shredded carrot samples packaged with a micro-perforated film (PM-MAP) during storage (5 °C).

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Acorn Starch and Polyphenol Extraction by High Hydrostatic Pressure

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More than 50% of the total acorn production in Portugal is underused. Considered to be a novel food rich in resistant starch, the exploration of acorns as a starch source can be profitable for the food industry while attending to its demands for new starch sources [1]. Hence, starch granules must be isolated without significant changes to be economically valuable. So far, the low alkaline extraction with three sieves is the best extraction method to preserve the structural integrity of acorn starch with the highest yields, purity, and most relevant functional properties. However, the usage of bleached or alkali-treated starches as a food additive has not yet been approved by the European Union. Therefore, it is important to produce clean-label starches. High-pressure assisted extraction is an emerging nonthermal technology used for food pasteurization carried out at room temperature (or below) and from 100 to 1000 MPa. Besides food pasteurization, it has also been widely used to assist extraction processes from several matrices, with the general outcomes showing increases in extraction yields, improved selectivity, and ability to preserve thermolabile compounds, but no literature was found regarding its applicability to extract starch [2]. Besides being rich in starch, acorns are also rich in tannins, which are anti-nutritional compounds [3]. Thus, it is important to remove such compounds for acorn starch to be used with greater safety.

Hence, the high hydrostatic pressure technology was used to (1) optimize the extraction of starch and phenolics from Q. pyrenaica and Q. robur cotyledons at room temperature with the aid of a full box-Behnken design using 3 different pressure levels (0.1, 250, and 500 MPa) and 3 different processing times (5, 12.5, and 20 min); and (2) compare results with those obtained by alkaline extraction.

For Q. pyrenaica, the pressure level had a negative quadratic effect on the phenolics, hydrolyzable tannins, and antioxidant activity, while the processing time had a positive quadratic effect. Pressure had a positive quadratic effect on the extraction of starch, but time had a negative quadratic effect. For Q. robur, the pressure level had a negative quadratic effect on the phenolics, hydrolyzable tannins, and antioxidant activity. The processing time had a negative quadratic effect on the extraction of phenolics, but the effect was positive linear for the extraction of hydrolyzable tannins and antioxidant activity values. Neither pressure nor time had a significant effect on starch extraction from Q. robur. The optimum multifactorial extraction condition of starch, phenolics, hydrolyzable tannins, and antioxidant activity from Q. pyrenaica acorn was 460 MPa/20 min (86 % desirability) and 333 MPa/17.4 min for Q. robur (80 % desirability), whose contents were higher than those at 0.1 MPa. Even though the starch, phenolic, and antioxidant activity yields were lower than the alkaline method, these are clean label starches since only water was used during extraction and are safer for human consumption, unlike the alkaline isolated starches. The SEM micrographs show no relevant changes in the morphology of the starch granule extracted via alkaline, control, or under optimum multifactorial conditions for Q. pyrenaica and Q. robur. Thus, the high-pressure isolated starches can also be economically viable. The aqueous extracts obtained under optimal multifactorial conditions for both Q. pyrenaica and Q. robur had a superior hydrolyzable tannin content than the alkaline ones, making the former extract more attractive for leader production as a more sustainable alternative.

Besides isolating starches more sustainably, it is possible to valorize the extracts and reduce waste disposal costs. Hence, high-pressure technology is a greener alternative to alkaline extraction.

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Influence of the storage in bottle on the antioxidant activity of wine spirit aged by sustainable technology of micro-oxygenation with Limousin oak staves

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The traditional ageing technology (TAT) has always been associated with oak barrels ageing and involves staging the wine distillate (WD) in wooden barrels with a continuous innate diffusion of oxygen through the wood and space between staves, under which the beverage spirit undergoes positive changes that contribute to the enhancement of its chemical composition and sensory properties. It is worth mentioning, that these physicochemical and sensory characteristics of aged wine spirit (WS) are prompted by direct extraction of wood components, decomposition of wood macromolecules and subsequent extraction, reactions between wood and wine distillate constituents, and reaction involving wood extractable compounds and distillate components, among other events.[1]

Furthermore, due to its good permeability, oak wood has been most commonly used in ageing process of beverage spirits, allowing trace oxygen to enter the vascular bundle of oak and cracks between the boards slowly and steadily, causing slow and continuous oxidation of WD, and thus extracting the target compounds. However, TAT is costly and time consuming, which seriously affect the production capacity and economic benefits of wineries. For these reasons, our research team has studied the use of the alternative ageing technology (AAT) for the ageing of WS,[2] using oak wood staves combined with micro-oxygenation (MOX) applied to wine spirit (WS) stored in tanks, with the goal of simulating the ageing process that occurs in wooden barrel (TAT), but in a more sustainable way: lower environmental impact, less time and lower cost.

In this context, despite the interesting results attained on some physicochemical and sensory properties of the WSs during the ageing process using AAT, it is also essential to assess the overall quality of the aged WSs during the storage in bottle to select the best MOX strategy. Some factors, including the closure, temperature, exposure to light, bottle position and the availability of oxygen in headspace height, may change the characteristics of the aged WS during this stage.[3] Thus, this study aimed to analysed, for the first time, the influence of the storage in bottle over 12 months on the evolution of antioxidant activities (DPPH and FRAP assays) and total phenolic content (TPI) of the WSs aged through three modalities (MOX levels: O15, O30 and O60) and one control (N) from AAT. The work was conducted as part of the Oxyrebrand project (https://projects.iniav.pt/oxyrebrand), which includes a detailed explanation about the experimental design. Briefly, the samples were aged with Limousin oak wood by an alternative technology, using 50 L glass demijohns with wood staves with the total of 48 samples (4 modalities × 2 replicates × 2 sampling bottles × 3 storage times).

Figure 1 depicts average values of total phenolic index (TPI) and the antioxidant activity of the aged WSs from the four ageing modalities (O15, O30, O60, N) during storage time (0, 6, 12 months) in bottle. The results show that TPI values of WS from four ageing modalities were not significantly different in the beginning of storage (t0), however the antioxidant activity of ageing modalities (O60 and N) were higher than O15 and O30 (**Figure 1B**). The differences in antioxidant activity between the ageing modalities at t0 could be ascribed to the MOX level, the wood variability (even with the same surface to volume ratio used) and variability of the phenolic profile extracted. After six months

(t6), an increase significant in the antioxidant activities by DPPH and FRAP assays for the WSs in all modalities was observed. These changes in the antioxidant activities are presumably due to differential transformation of phenolic

compounds into polymeric and condensed forms, under different oxygen content resulting from the MOX levels combined with the action of factors ruling the storage in bottle (light, temperature, oxygen transfer from the cork, among others), being possible showed by little reduction of TPI values **(Figure 1A**). Polymeric phenolic compounds exhibit different chemical properties and reactivity towards the organic radical reagent (DPPH assay) and with a Fe(III) complex (FRAP assay), according to their degree of hydroxylation and extent of conjugation.[3] After 12 months (t12), the antioxidant activity by FRAP assays decreased significantly compared to t6, however were not significantly different in the beginning of storage (t0) for ageing modalities (O30, O60, N), except for O15 WS. Thus, O60 modality resulted in higher preservation of the phenolic contents and antioxidant activity of aged WS, assuring the aged WS quality. According to this study, this technological alternative (AAT) appears to be the most suitable for wine spirit quality and ageing sustainability.

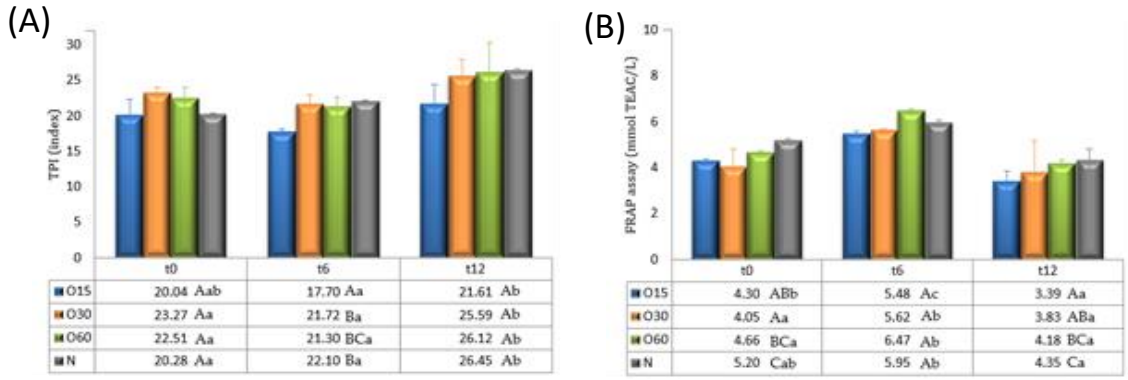

Figure 1: Average values of TPI and antioxidant activity of aged WS in each storage time (0, 6, 12 months) according to ageing modalities (O15, O30, O60 and N). (A) Total phenolic index (TPI); (B) Antioxidant activity using FRAP assay. For each analytical determination: different uppercase letters (A, B, C) in the same column denote significant differences between ageing modalities in each storage time by Tukey's test ($p < 0.05$); different lowercase letters (a, b, c) in the same row denote significant differences between storage times for each ageing modality by Tukey's test ($p < 0.05$).

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Ketchup processing under ohmic heating: effects on physical-chemical properties

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Ohmic heating (OH) or Joule heating consists in using an alternating flow of electric current through a semi-conductive material allowing generation of internal heat. This heating process allows high-heating rate. [1] OH has also the advantage of volumetric and uniform heating, since it is not under the dependency of conduction and convection heat transfer, being thus appropriate for the treatment of viscous products. [2] Further, the use of OH in opposition to the conventional heating may lead to better energy and heating efficiencies which is more sustainable for the environment and the industry. [2]

In this context, the main objective of this work was to make a preliminary assessment of the impact of OH on physical and chemical properties of a tomato sauce (i.e., ketchup formulation) aiming at replacing conventional heating, as a more efficient and sustainable alternative.

For both conventional and ohmic heating, the ketchup formulation was processed in a kitchen robot. However, for OH, the heating step was performed in small glass reactor equipped with two stainless steel electrodes. The same thermal profile was kept in both experiments (heating ramp until 88-93 °C and temperature maintained for 9-12 minutes with a cooling ramp). The voltage was manually controlled to reach and stay at the desire temperature during holding time. Assessed parameters included: pH, water content, soluble solids and water activity. Further, viscosity was measured at room temperature 24 hours after processing. Color was measured immediately after processing (while the ketchup is still hot), 2 hours after (at room temperature) and 24 hours (after storage at 4 °C overnight, at room temperature) with a colorimeter, using CIELAB coordinates and the ΔE* (difference of color) calculation. Lycopene was estimated through a colorimetric method.

The main differences occurred in terms of viscosity and color. The ketchup made with OH presented lower viscosity than the conventionally heated one, being the difference approximately of 15 to 20 cP. This difference was perceptible even without measurements since it was easier to transfer the sauce between recipients. In terms of color, the ketchup was evaluated considering the total color difference, and more specifically considering differences in the red color (the characteristic color of this sauce). Overall, it was possible to see the differences of color from the conventionally and ohmic heated, where OH presented lower differences of color between the finished ketchup and the mixture of all ingredients before heating. The ΔE* values at 24 h ranged from 18.88 ±0.47 for the conventional ketchup, while for the OH ketchup varied from 15.04 ± 0.48. This means that the OH processing was more efficient in maintaining the characteristic color after the heating process. With a closer look at the coordinate a* (higher value, more red color) it was possible to notice also that the OH ketchup presented higher values for a* and closer to the unprocessed sample in every assay, when compared to the conventional ketchup. This difference was also observable at naked high, knowing that redder is a desirable characteristic.

Besides the known advantages such as low maintenance costs and energetic efficiency, positive effects in some quality indicators such as color were observed. Further studies are needed to allow the process of scale-up, and to understand how OH can be designed to improve and tune not only the physical-chemical aspects but also organoleptic properties of processed tomato sauce, but these results suggest that OH can be an interesting alternative technology to conventional heating in the ketchup processing.

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Ohmic heating as an innovative green technology for the boiling stage of the brewing process

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The brewing industry is regarded as a fiercely competitive and insatiable sector of activity, driven by the significant technological improvements observed over the last years and the most recent consumer trends pointing at a sharp demand for sensory enhanced beers. In turn, brewing is a complex process which involves the harmonization of water, malt, hops and yeast, resulting in a unique product. Throughout the several stages of this process, wort boiling is indisputably one of the most important steps, since it is responsible for many chemical reactions that contribute to the sensory properties of beer. Traditionally, wort boiling is performed for 60-90 min and important goals are achieved, namely the extraction of hop (Humulus lupulus) compounds which contribute to beer´s bitterness, flavour and aroma[1].

Ohmic heating (OH) has been showing promising potential for thermal food processing over the years. This technology consists on the delivery of an electric field into a given (semi-) conductive medium, generating internal heat due to the intrinsic electric resistance towards the flow of the electric current. OH allows a very high energy efficiency (>95%) and heating rates, uniform heat distribution and absence of hot surfaces in the system[2].

In this work, an assessment of the conductivity profiles of beer´s wort and beer´s wort in the presence of hop pellets from the Saaz variety (2.7 mg of hop pellet/mL of beer´s wort) in a temperature range from 27 °C to 102 °C has been performed. The results of these experiments, revealed a conductivity range from 4.510 ± 0.093 to 11,509 ± 0.019 mS/cm and from 4.698 ± 0.004 to 12.712 ± 0.101 mS/cm, for beer´s wort and for beer´s wort in the presence of hops, respectively. The obtained results showed that there is a tendency of the conductivity values being increased while the temperature is also increased, for both beer´s wort and beer´s wort in the presence of hops. At the same time, it is possible to conclude that beer´s wort consists on an adequate matrix for OH processing, taking into account the conductivity values reached throughout the entire temperature range tested.

Lastly, OH technology is also viewed as an interesting tool for the electrothermal permeabilization of cellular tissues and cellular membranes, and has been shown to enhance the extraction of bioactive compounds from vegetable tissues[3]. Due to the strong evidence pointing at an efficient extraction of compounds present in vegetable tissues through OH technology, and the adequacy of the matrix of beer´s wort for OH processing shown in this work, the focus of our future work is being aligned with the extraction of a set of compounds which are considered to be of major interest for the brewing process (e.g. α-acids, essential oils and polyphenols), from hop pellets and hop cones.

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Production of fibrillar protein aggregates under the effects of electric fields

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Proteins are the main "building blocks" used for the formation of biomaterials´ in aqueous environments as the case of hydrogels. Protein hydrogels can retain large amounts of biological fluids within their 3D network being currently considered as one most attractive material for the development of food supplements, controlled release of bioactive compounds or drugs, pharmaceuticals and structures for cell communication such as scaffolds[1,2], thus covering food and biomedical fields. Fibrillar hydrogels are now considered as an outstanding protein-based biomaterial with a great potential to functionalize foods through the production of high elastic and transparent gels using protein sources such as the ones from whey at low gelation concentrations – e.g. typically < 2 % w/v. In this work β-lactoglobulin (BLG) fibrillar-aggregates were produced under the influence of moderate electric fields (< 20 V/cm at electrical frequency ranging up to 20 kHz), at 90 °C for 15 h using whey protein isolate (WPI) aqueous solutions of 1 % (m/v) at pH 2. Kinetics of fibrillar aggregates were established and advanced fluorescence spectroscopy techniques such as Thioflavin T (ThT) fluorescence and circular dichroism were used to follow BLG fibril formation and to characterize the produced structures. Conventional heating (without application of electric fields) was performed under identical treatment conditions, which includes reactor geometry/material, stirring and heating conditions, to benchmark obtained results. A sigmoidal growth Hill curve was well fitted to describe fibrillar aggregation kinetics with a correlation coefficient ranging from 0.93 to 0.99. The half-time of fibril formation was of approximately of 2.5 h and not significant different (p < 0.05) under the influence of electric fields. However, results have shown that cooperativity level of fibril formation can be enhanced under moderate electric fields, suggesting the occurrence of structural modifications. Electrochemical reactions can also give rise to different levels of protein aggregation during protein hydrolysis.

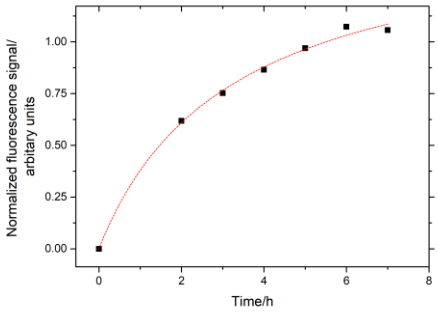

Figure 1: ThT normalized fluorescence and correspondent fitted model (dashed line).

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Effects of ohmic heating on cellular morphology of *Chlorella vulgaris* – effects on proteins extraction

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The world population is increasing and it is necessary to find new and alternative food sources, such as the case of microalgae. The microalgae are a rich source of proteins and valuable chemical compounds but also present a strong cell wall which limits the access to their intracellular compounds. *Chlorella vulgaris* is a widely known microalgae approved for human consumption presenting a cell wall composed by strong polysaccharides, which affects the digestibility of *C. vulgaris*, and consequently the bioaccessibility of their proteins. It can be of great interest to ensure that the cells can be fragilized and/or partially disrupted before being consumed[1]. The application of electric fields on the processing of food products is an emergent technology with potential to improve the digestibility of microalgae biomass. The electric fields can induce electrical breakdown of microalgae cell membrane, known as electroporation effects[2]. Beside these effects, this technology can also change the cells morphology of certain microorganisms, opening the possibility to have similar effects on microalgae[3]. This work aims at understanding how the application of electric field- combined with ohmic heating acts on the cell morphology and viability of *C. vulgaris*, and consequently on extraction yield of protein-rich fractions. Several treatments can be applied, by varying electric fields intensity, the maximum temperature achieved, the number of treatment cycles and total thermal load. From preliminary results, is possible to conclude that higher temperatures and number of treatment cycles can induce morphological changes on the cells, according to flow cytometry analysis. These changes are correlated with yield of compounds released from during aqueous extraction procedures. The treatments where higher temperatures were applied have shown chlorophyll release during extraction suggesting partial disruption of the cellular structure. These results could hint the possibility of using electric fields approach to process microalgae biomass to improve bioaccessibility of nutrients during gastrointestinal digestion.

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Extraction bioactive molecules from *Coelastrella* sp. LFR1 biomass using ohmic heating

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Coelastrella sp. is a terrestrial unicellular microalgae highly adaptable to extreme conditions, that typically display coccoid, elliptical until citriform and double-layered cell wall. These microalgae are a novel source of pigments (such as carotenoids) and fatty acids, which are attracting attention for diverse biotechnological and potential food and feed applications. The presence of the resistant cell wall contributes for a lower bioaccessibility of the intracellular bioactive compounds. The synergistic combination of thermal and electrical effects through Ohmic Heating (OH), contributes as a fine-tune strategy to extract biocompounds. Through the evaluation of the impact that the application of OH treatments have in the pigment extraction there is a positive indication that, at equal extraction temperatures and under identical holding treatment temperature (i.e., 10 min), the presence of moderate electric fields may positively influence the extraction of chlorophyll a. OH extraction allowed to recover 16.3% of chlorophyll a, about 2× higher than the percentage observed for a conventional extraction treatment under a treatment temperature of 70 °C. In terms of protein extraction, OH showed a better performance than conventional heating at temperatures <70 °C; at 70 °C the performance was the same in both methodologies. In addition to the better extraction yields when thermal and electrical effects were combined, the energetic performance was also more promising. In this work energy input to perform OH at 70 °C was of 99.9 ± 8.5 kWh/KgDW, which was much lower than the 1678 kWh/kgDW needed to heat up water to 70 °C, through indirect heating.

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Whey-gelatine film combined with lactic acid bacteria to prevent cheese fungal contamination

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Cheese whey is one of the largest by-products of the dairy industry[1]. This biodegradable and renewable source of proteins with high biological value can be used to produce edible films. The functionality of these films can be improved by adding active ingredients such as antimicrobial compounds or live lactic acid bacteria (LAB) to protect cheese from microbial contamination. In this work, the incorporation of LAB with antifungal properties (*Lactococcus lactis* L3B1M7, *Latilactobacillus curvatus* SJC53, *Lacticaseibacillus paracasei* SJC87, *Lacticaseibacillus casei* SJC87 and *Levilactobacillus brevis* SJC120) into gelatine whey films was tested to inhibit the growth of *Aspergillus chevalieri* MUM 00.07, *Aspergillus flavus* MUM 16.106, *Penicillium brevicompactum* MUM 9906, *Penicillium commune* MUM 16.56, *Penicillium nordicum* MUM 16.93 and *Mucor racemosus* ATCC 42647. LAB were cultured in pasteurised cheese whey for 48 hours at 30°C and added to a film-forming solution containing gelatine (6% w/v) and glycerol (2% w/v). The film-forming solutions were poured into Petri dishes (10 ml), and the films were dried in an oven at 20°C with ventilation for 48 hours. These films were able to maintain the viability of LAB within 10^8 CFU/g for 30 days at 12°C. The antifungal activity of the films against the target fungi was determined by the ability of the fungi (10^4 spores/mL) to grow on the film surface for 15 days at 12°C and 20°C. The whey gelatine film to which all five LAB were added showed inhibition against the tested fungi. Of the five isolates, Lb brevis SJC120 showed the highest inhibitory effect on all six fungi tested at 20°C. Addition of this strain to the film prevented the growth of all *Penicilium* species and *A. chevalieri* for 15 days and showed a reduction in colony size of *M. racemosus* for 10 days. At 12°C, the film with Lb brevis SJ120 was also able to reduce the growth of *Penincilium* species and *M. racemosus*, but the elimination of fungi was not achieved as at 20°C.

The results suggest that the addition of *L. brevis* SJC120 to edible whey/gelatine films can reduce or eliminate the growth of fungal species isolated from cheese and the cheese-making environment.

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Macroalgae-based nanoparticles: current status and potentiallyemerging applications in the food industry

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Nanotechnology has opened a new frontier in recent years, capable of providingnew ways of controlling and structuring products with greater market value and also offeringimportant opportunities for the development of innovative products and applications in food processing, preservation and packaging. It is also of interest for the development of productive and sustainable activities. On the other hand, algae are the largest photoautotrophic group of microorganisms as a potential source of secondary metabolites, pigments and proteins, which can serve as nanobiofactories for nanoparticles. The main focus of using algae for nanoparticle synthesis is based on the high potential to take over from chemical counterparts, the low cost of cultivation, the reduction of production time andthe eco-friendly and commercially viable synthesisofcompetent compounds. Consequently,the aim of this review will be to summarize the available information on the use of algae forthe development and synthesis of nanomaterials focused on the food industry, as well as possible and innovative applications such as the development of active packaging based onthe bioactive properties attributed to algal nanoparticles (antioxidant, antimicrobial, oxygenabsorption capacity, UV impermeability, ...), which means that this new technology appliedto the food industry and correctly regulated can continue to expand in a promising and profitable way for consumers, industry and research.

Key words: Nanoparticles, Algae, Food, Synthesis.

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Influence of pulp preparation in the sensorial, nutritional and antioxidant properties of a mixed "Pera Rocha do Oeste" and strawberry structured product

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Structured Fruits (SF) are innovative products formulated with pulp fruit and small concentration of hydrocolloids. The use of high contents of fruit pulp requires improved technology to produce SF with good sensorial acceptance and nutritional quality [1]. For instance, the formulation of SF with dried pulp (high solids contents) is advantageous to the firmness, pH, and water activity attributes of the final product. Also, the addition of glycerol, a short chain low molecular weight (94.04 g/mol) polyol, could positively contribute to the organoleptic quality (texture) of pectin gel, decrease of water activity, and plasticity of the formulated product [2].

This study aimed to evaluate the influence of pulp preparation in the elaboration of mixed "Pera Rocha do Oeste" and strawberry SF. Experiments were performed to evaluate the *(i)* addition of glycerol to the mixed pulp and *(ii)* the effect of dehydration of strawberries for a period of 6 or 12 hours.

Pear (pH 4.8, 11.6 °Brix, 79.6% humidity) and strawberry (pH 3.2, 8.5 °Brix, 78.2% humidity) were acquired in the local market. After dehydration (Excalibur 9 Tray Dehydrator, Model 4926T, USA) for 6 and 12 hours, the strawberry samples presented 49.0° Brix, 14.9% humidity and 55.2 °Brix, 78.2% humidity, respectively. Four SF (**Figure 1**) were prepared using 100 g of fruit (80 g of pear pulp + 20 g of strawberry) and a hydrocolloid composition of 2.25% agar and 2.4% locust bean gum in relation to agar. The pear pulp used in all formulations was obtained after cooking the whole (peel + pulp) fruit at 90 °C for 15 min. For each formulation, 100 g of mixture puree were transferred to a food processor (Thermomix®, model TM5) and mixed with the hydrocolloids. The mixtures were kept at 90 ± 2 °C for 8 minutes and under stirring (rotation speed 6) and then poured into spheric silicone molds (W x H x L = 25 × 15 × 30 mm), where they remained at room temperature (± 25 °C) for 30 min. After remove from the molds, SF were placed in a hermetically sealed container and stored under refrigeration at 5 ± 1 °C for 12 h to complete the gel maturation of the SF. Samples A-D were evaluated regarding some physicochemical (pH, moisture, water activity), nutritional (carbohydrates, reducing sugars, dietary fiber, and ash) and antioxidant (DPPH, FRAP and ABTS assays and total phenolic contents) parameters and were sensorially evaluated by a group of 13 untrained panelist of both genders, using a five-point hedonic scale ("1-dislike extremely" – "5-like extremely").

The results of the sensory analysis (**Figure 1**) indicated that all samples were scored with "homogeneous texture". The SF prepared with glycerol (sample B) and 6h-dehydrated strawberry (sample C) were scored with higher "global acceptance" and "general flavor". Samples A and B were the preferred products concerning the "pear flavor" attribute (mean score of 3.2), while the SF prepared with dried strawberry (samples C and D) were scored with higher "strawberry flavor" and "sour taste" (mean score of 4.2 for both attributes). In fact, 40% of the panelists made the following positive comments to sample C: "good strawberry and pear aroma" and "the product is the sweeter of all". Samples D received the negative comment "product too acidic taste" by 10% of the panelist and 20% of the panelist referred that sample A "has to little pear and strawberry taste". Concerning the antioxidant capacity ($ABTS^{\bullet+}$ results) of the formulated products, the mean values found were 168, 282, 328 and 442 mg Trolox Equivalents/ 100 g fw for samples A, B, C and D, respectively. The ABTS results were positively correlated with the results of DPPH, FRAP and total phenolic content assays. These results suggest that dehydration of strawberry for a period of 6 or 12 h contribute to the formulation of SF with higher antioxidant activity. Overall, this study provides a promising perspective to produce a mixed "Pera Rocha do Oeste" and strawberry structured product with pear pulp and dried (around 49.0° Brix) strawberry, with good sensorial acceptance and antioxidant properties.

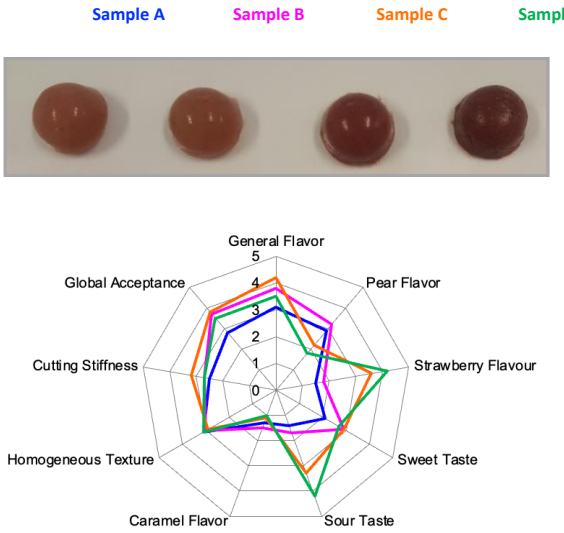

Figure 1: Sensory profiles of mixed "Pera Rocha do Oeste" and strawberry SF based on averages of ratings by n= 13 panelists.
Scale: 1-dislike extremely; 2-dislike slightly; 3-neither like nor dislike; 4-like slightly; 5-like extremely.
Sample A - SF prepared with pear pulp (90 °C, 15 min) + strawberry pulp (90 °C, 3 min)
Sample B - SF prepared with pear pulp (90 °C, 15 min) + strawberry pulp (90 °C, 3 min) + 10% glycerol
Sample C - SF prepared with pear pulp (90 °C, 15 min) + (6 h) dehydrated strawberry
Sample D - SF prepared with pear pulp (90 °C, 15 min) + (12 h) dehydrated strawberry.

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Pressurized liquid extraction for the recovery of bioactive compounds from seaweeds for the food industry application

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Seaweeds are an under-utilized food in Western World but commonly consumed in Asia, being China the principal producer in the world. Seaweeds have increased the attention of the food industry in the past years because of its composition, which includes polysaccharides, a good quality profile of lipids, proteins, dietary fibre and different bioactive compounds such as vitamins, essential minerals, phenols and carotenoids. To obtain these components, an extraction technique is needed, where the pressurized liquid extraction may be a good option. Pressurized liquid extraction (PLE) is considered a green technique in which high temperature and pressure in combination with a solvent is needed to extract components of a solid matrix. It is important to notice that critical point is not reached in both pressured and temperature. To increase the efficiency of this technique the optimization of different parameters such as the solvent extract used, temperature, pressure, time of extraction and number of cycles influence considerately. It is significant to emphasize that PLE conditions allows to extract the target analyte in a short-time period and using reduced quantities of solvent but keeping a good recovery percentage. Moreover, the combination of PLE with other techniques was already led to extracts compounds from different matrices in which edible seaweeds was not included. In this way, the combination of PLE-SFE-CO_2 seems to be the best option taking into account both the higher yields obtained and the economic feasibility of a scaling-up approximation. In addition to this, data shows that the incorporation of the compounds extracted from the edible seaweeds into food packaging (including edible coating, bioplastics and bio-nanocomposites incorporated into bioplastics), and into food products and animal feed to improve their nutritional profile and technological properties is an interesting future trend. Finally, this review tempts to compile and analyse the current data available of PLE and edible seaweeds in order to determine the application of this extraction technique to obtain compounds of interest for the food industry application.

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Dynamic sensory characterization of functional chocolate ice creams using Temporal Check-All-That-Apply (TCATA) methodologies

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Sensory perception of a food product is considered a dynamic process, changing across consumption experiences. Additionally, food-evoked emotions constitute relevant information for predicting food choices and can aid industry players in improving marketing and sales. Consumers' tendency toward healthier but sensory-pleasing food alternatives is accompanied by the development of several innovative sensory analysis techniques, such as Temporal Check-All-That-Apply (TCATA). This methodology allows for a multi-attribute simultaneous analysis during a given time, using sensory or emotional parameters[1].

This work aimed to evaluate the dynamic emotional and sensory profiles of chocolate ice creams with incorporated vitamins (D and B12) and *whey* protein in comparison with a control sample (no-added bioactive compounds). Two commercial products of protein ice cream were also evaluated (CP1 and CP2). A TCATA with emotions (TCATA-E) ballot was used[2], in which 95 consumers were asked to select all the applicable attributes for 30 seconds. Attribute fading was set to 8 seconds. Finally, consumers were asked to rate their overall liking, using a 9-point hedonic scale[3] (ranging from 1 - "dislike extremely" and 9 - "like extremely"). Two TCATA ballots using texture and taste attributes were also performed using a trained panel of 9 tasters.

The prototype samples showed no significant differences in terms of overall liking, or whether the commercial references were significantly less appreciated by the consumers. Following this, the most cited emotional attributes were *Pleased*, *Satisfied*, *Happy* and *Interested* in the prototype samples; and *Unsatisfied*, *Unpleased* and *Disappointed* for the commercial products. The differences between the prototypes and the commercial products were notable, with higher citations of positive emotional attributes (e.g., *Satisfied*, *Pleased*) in the prototype samples, while a higher elicitation rate of negative attributes (e.g., *Disappointed, Unpleased*) being registered for the commercial products.

The trained panel of judges perceived significantly more texture attributes such as *Spongy* or *Adhesive* for the commercial samples, while more typical and desirable ice cream attributes (e.g., *Creamy, Homogeneous*) were noted in the prototypes. An identical tendency was verified along the taste ballot, with significantly more citations for *Atypical* or *Caramel* tastes being detected in the commercial samples, in comparison with the *Chocolate, Cocoa* or *Sweet* attributes, more cited in the prototype samples (**Figure 1**).

Within the whole duration of the trial, slight citation increments could be notified for *Homogeneous* (in the vitamin-enriched products), *Creamy* (for the B12-enriched sample) or *Velvety* (all the functional samples). The inclusion of *whey* protein also increased the perceived *hardness* and *adhesiveness* of the ice cream. Furthermore, in the taste curves, it is notable the increase in the perception of *Chocolate* (*whey* protein and vitamin D-added samples), which also occurred for the attributes *Typical* and *Intense* taste. Finally, a slight increase in the perception of *Cocoa* taste was followed by an increment in the citations of *Bitter* (vitamin-enriched samples).

The products' emotional trajectories accompanied this tendency, with the prototype samples' curves being centred among emotional attributes such as *Interested* or *Enthusiastic*, and CP1/CP2 approaching negative parameters, such as *Disgusted* or *Disappointed*. This trend was kept for the trajectories respecting the TCATA ballots, however, CP1 always approached the positive attributes to a higher extent than CP2, which also justifies the significant differences encountered between these samples.

The incorporation of bioactive components in a traditional chocolate ice cream formulation resulted in no major changes on the sensory profile of the product, nor its overall liking, resulting in emotionally and sensory well-connected products, giving space to introduce more functional foods in the market. However, the increasing proportion of *whey* protein in this product category leads to a decrease in sensory quality, which must be approached carefully.

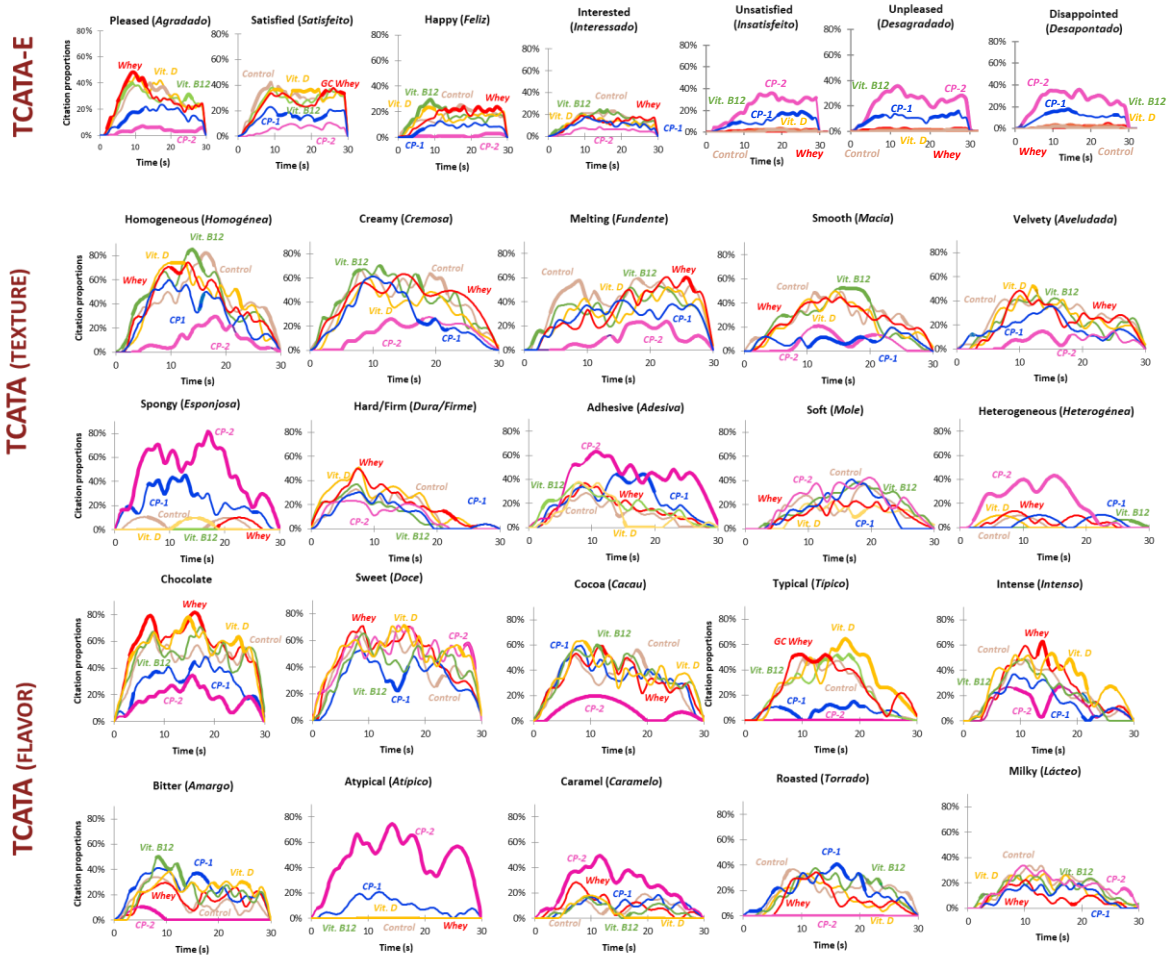

Figure 1: Resulting curves from the application of the TCATA methodologies. A 20 % citation proportion filter was applied and the most cited attributes were overlapped for the 6 analyzed chocolate ice cream samples (Control: No bioactive compound incorporated; Vit. D: Vitamin D-enriched ice cream; Vit. B12: Vitamin B12-added ice cream; Whey: *Whey* protein-enriched ice cream; CP-1: Commercial sample; CP-2: Commercial sample), for better visualization of the variations registered.

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Effect of pulsed electric fields and mild heating combination on physicochemical properties of goat milk

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Pulsed electric fields (PEF) is a nonthermal processing technology that consists of exposing food to pulses of high-voltage electric fields during a short period of time (from nanoseconds to milliseconds) at moderate temperatures. As other non-thermal processing technologies, PEF is potentially interesting to dairy industries as it allows the maintenance of the original characteristics of the products without compromising food safety aspects. In addition to ensuring the obtention of a safe product, it also allows to maintain the nutritional and organoleptic characteristics of the unprocessed product. Although several studies support the use of PEF technology to process milk at low temperatures, some of them indicate a limited impact of low intensity PEF in microbial inactivation. Combining low intensity PEF with mild heating (below usual heat pasteurization conditions) can be explored as an alternative method to enhance the safety and preserve the quality of fresh goat milk and goat milk products. There are several published reports on PEF-treated milk and its influence on physicochemical properties. However, the different experimental conditions used by different authors and the diversity of available equipment limits the comparison of results. Also, most of these studies focus on bovine milk, with very few studies on goat milk, which has some particular characteristics in comparison to its bovine counterpart.

This study was carried out to evaluate and compare the physicochemical properties (pH, conductivity, total soluble solids, titratable acidity and viscosity) of goat milk treated by pasteurization (heat only) and with a combination of low intensity PEF and mild heating. Raw goat milk (from a traditional cheese dairy – Prados de Melgaço) was pasteurized in a Armfield FT74XTA HTST/UHT system applying the temperatures of 63, 66, 69, 72 and 75ºC for 2 s combined with and without a continuous flow system with a colinear treatment chamber design of PEF (EPULSUS®-LPM1A-10, EnergyPulse Systems, Lda) treatment, with a flow rate of 2,92 L/h and an electric field strength, pulse width and frequency of 10 kV/cm, 50 µs and 3 Hz, respectively.

pH and conductivity values of treated and untreated samples were determined using a pH/Conductivity Multiparameter (Meter Orion Star A215) according with AOAC (981.12:2016). Total soluble solids were measured using a refractometer (Abbe, WYA-IS) following the AOAC (932.12:2016). Titratable acidity was determined according to the AOAC (947.05:2016). Viscosity measurements were carried out using a rotational viscosimeter (VT 550 (Thermo Haake), with concentric cylinders (NV ST 807-0713 CE and NV 807-0702), and the following analysis conditions: a shear rate between 10.82 s^{-1} to 221.80 s^{-1} for 2 minutes. All measurements were performed in triplicate at room temperature (20 ± 2ºC). Data collected using Haake RheoWin (version 2.93).

The average values of pH (6.76±0.03) and total soluble solids (10.70±0.26) in raw milk did not change after the treatment. The titratable acidity decreased from a maximum of 0.150±0.003% (% in lactic acid) in raw milk to values below 0.136% for both pasteurized and combined treatment samples. Conductivity decreased as a function of the increase in temperature for both treatments. The conductivity of raw milk changed from 5.47±0.06 mS to a minimum of 3.34±0.02 mS when the milk was pasteurized at 75ºC. These changes may be due to a decrease in calcium ion (Ca^{2+}), which is one of the main constituents of the casein micelle's structure, together with phosphorus. The decrease in Ca^{2+} causes a decrease in conductivity and leads to phosphate protonation, which decreases titratable acidity. Regarding the viscosity values, they did not differ between treated and untreated samples, with goat's milk showing a non-Newtonian fluid behavior. The viscosity decreases with increasing shear rate, showing a pseudoplastics behavior in all the samples.

The results showed that for all physicochemical parameters analyzed, the samples subjected to pasteurization and to PEF-mild heating treatment did not differ from each other. Only for titratable acidity and electrical conductivity differences were observed between treated and untreated samples, and these differences were caused by the different temperatures tested and not by the treatments themselves. However, further investigations into physicochemical alterations are needed in complementarity with PEF microbial inactivation studies to ensure that PEF-treated samples are safe and minimally modified from their original characteristics.

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Sunflower oil enriched with bioactive compounds from Sea Fennel (*Crithmum maritimum* L.) flowers by ultrasound-assisted extraction

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Edible oils, especially those rich in polyunsaturated fatty acids, are prone to lipid oxidation that causes a decrease in their nutritional value and shelf life. To overcome this constraint, antioxidants may be added to the oils. Nowadays, there is an increasing interest in the use of natural sources of antioxidants, mostly from plant origin. In this context, halophytes have drawn some attention in the food markets.
Sea fennel (*Crithmum maritimum* L.) is a halophyte plant widespread on the rocky coasts of the Black Sea and the Atlantic Ocean, mainly in the UK and Portugal. This plant, which is very rich in flavour, both spicy and salty, can be used as an aromatic herb in salads or as a side dish with seafood. Sea Fennel also has many biological properties, such as antioxidant and antimicrobial properties.
In this study, lyophilized flowers of sea fennel were incorporated into sunflower oil and an ultrasound assisted extraction was performed, aiming at improving its biological and economic values and oxidation stability. Additionally, the effect of ultrasound operation time, in the sunflower oil properties was assessed.
This technique shows several advantages in favour of a greener process such as a decrease in energy consumption, a decrease in process time and an improvement in the extraction of phenolic compounds. In this study, sunflower oil was enriched with 12.5% m/v of lyophilized sea fennel flower and the extraction was performed in ultrasound water bath working at a frequency of 35 kHz, for different periods of time, from 5 to 20 min. These conditions come from previous studies [1,2]. In parallel, non-supplemented sunflower oil was submitted to same periods of US.
The extraction of pigments (chlorophylls and carotenoids) and phenolic compounds of supplemented oils was maximum after 15 min of extraction, decreasing thereafter. A higher antioxidant capacity of the supplemented samples was observed, and the sample subjected to 15 minutes of ultrasound had the highest antioxidant capacity according to the results of DPPH (9.17 mg Trolox equivalent/kg oil) and FRAP (131.75 mg Trolox equivalent/kg oil) analyses and the quantification of phenolic compounds (49.95 mg gallic acid/kg oil). The results for the supplemented sample subjected to 20 minutes of US treatment showed a deterioration in the properties of the oil, notable by the decrease in the antioxidant activity of supplemented oils subjected to more than 15 min of extraction. The results showed that ultrasound time had no clear effect on the oxidative state of supplemented and non-supplemented oil.
Sea fennel flowers showed to be a good source of phenolic compounds with antioxidant activity, and ultrasound-assisted extraction is an efficient technique for direct supplementation of sunflower oil.

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Sub-lethal pressure pre-treatments for subsequent shorter and improved egg yolk thermal pasteurization

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Salmonellosis is the second most common zoonosis in humans in the European Union, with eggs and egg products being the food vehicles most frequently associated to this infection [1]. The use of liquid egg products, in the food industry, requires processing, but thermal pasteurization (TP) causes changes in eggs properties [2]. Thus, a possibility to minimize these limitations is the use of pressure pre-treatments, to cause sub-lethal damages in microorganisms, thus decreasing their thermal resistance, allowing a subsequent less intense TP.

Thus, this study aimed to evaluate the performance of pressure pre-treatments (50 – 400 MPa/5 min) before a shorter TP (60 ºC/3 min) on egg yolk (EY), compared with the commercial TP procedure (60 ºC/6.2 min), to assess the effect on *Salmonella* Senftenberg 775/W inactivation and on egg quality properties.

The results showed that EY TP preceded by pressure pre-treatments decreased by 1.8 to at least 6.1 $\log_{10}$ cycles of *S.* Senftenberg 775/W counts, reducing the thermal resistance of this microorganism (synergistic lethal effect), while commercial TP obtained lower microbial inactivation (3.3 $\log_{10}$ cycles). However, for combined treatments at pressures ≥ 125 MPa, considerable increments in viscosity were observed, and so, for quality properties assessments, only moderate pressures (MP, 50 and 90 MPa) were studied (MP-TP). Concerning EY quality, in general, the treatments reduced soluble protein and total carotenoids of raw EY, whereas increased the viscosity, secondary lipid oxidation and improved emulsifying properties. Otherwise, compared to commercial TP, the MP-TP-treated EY exhibited higher soluble protein, total carotenoids content and emulsifying capacity, being less viscous and oxidized. In addition, the EY volatile profile suffered changes with treatments, especially with MP-TP treatments, presenting higher amounts of volatile compounds. A traditional dessert, "Doce de Ovos", was prepared based on the treated EYs, with MP-TP (90 MPa)-treated EY showing a greater sensory acceptability by panellists.

Therefore, the results open the possibility of using a MP pre-treatment before a shorter TP, as an alternative to commercial TP, offering a similar lethality against *S.* Senftenberg 775/W and with lower impact on global EY quality.

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Influence of electrical stimulation on the final pH of bovine carcasses and evolution of cooling temperature in washed and unwashed carcasses

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The role of the meat, slaughter and processing industry is of utmost importance, and has become even more important with the creation of new standards proposed by the EU to respond to health crises such as the epidemic of Transmissible Spongiform Encephalopathy of Cattle and Foot-and-Mouth Disease in the early 19th century. 21ST.
The pH of meat plays a determining role in its quality, influencing characteristics such as colour, water hold capacity, texture, juiciness and microbiological stability, so it should be considered an important criteria, especially in the case of meats for specific designations and classifications, differentiating quality identifiers [1]. Since the optimum pH value of the meat, 24 h after slaughter, should be 5.5 or lower, in cases where this value exceeds 6, the meat is considered to be of lower quality.
Electrical stimulation of carcasses during slaughter results in the improvement of meat tenderness. The mechanism of this action lies, first, in structural damage (ruptures) caused in the tissues as a result of violent contractions of the muscles, when they are subjected to an electrical stimulus. In addition, electrical stimulation has significant effects on proteolytic enzymatic activity in tissues, particularly in the calpains system. Electrical stimulation produces a rapid drop in pH, even when the carcass temperature remains high, shortening the period of rigor mortis and preventing muscle stiffness caused by cold (*cold-shortening*) [2].
The washing of carcass can influence the cooling speed of the carcass, i.e. the washing of these increases the temperature drop, at the surface or in depth, and can be a credible method when it is intended to reduce the temperature of carcasses in the shortest possible time.
A total of 100 carcasses were analyzed, of which 60 were in the carcass washing test and 40 in the electrical stimulation test. In the performance of the carcass wash test, after washing the carcass, the temperature was controlled at three places of the carcass, hourly, during the first 24 h after slaughter, and after 24h, the control was carried out every 2 hours until the carcass was removed from the chamber (for dispatch or for the cutting). Three tests were performed at different stages, each with 20 carcasses, 10 washed and 10 unwashed. The tests allowed to assess the evolution of pH and temperature of the carcasses. The air speed inside the chambers and in cooling tunnel was also analyzed and its influence on the cooling speed of the carcasses.
In all tests where temperature was controlled over 24-30 hours after slaughter, it was found that the evolution of the temperature on the surface of washed carcasses was more pronounced compared to that of unwashed carcasses. Washing carcasses with water lowers the temperature on the surface of the carcasses but does not influence the temperature drop in depth in the first 25-30 hours after slaughter, [3] they performed microbiological counting on carcasses washed with water and other microbiological count in carcasses that were not washed (control) and it was observed that the washed carcasses had considerably less total coliforms and thermotolerant coliforms compared to unwashed carcasses, about one fifth less. Therefore, it is safe to admit that washing carcasses can prevent microbial contamination much more efficiently in contrast to unwashed carcases.
Regarding electrical stimulation, there was a direct influence on the pH of the meat in the carcasses to which it was applied, when compared with carcasses not subject to stimulation.
The ventilation speed of the chambers before the chamber begins to be filled with carcasses, i.e. at the beginning of slaughter, is higher in the places closest to the condensers when compared to the ventilation speed while the chambers are partially or fully filled, and this is due to the presence of the carcass inside the chamber partially cutting off the ventilation.
The results obtained allow us to verify that the washing of the carcasses influences the temperature on the surface of the carcass. The ventilation speed inside the chambers and in the tunnel is higher in the absence of carcasses. Electrical stimulation has a direct influence on pH descent.

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Optimization and characterization of cultivation substrates for edible mushroom species – the *MicoCoating* initiative

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The food industry has evolved throughout the years to answer consumers' necessities, namely the search for healthy foods with extended shelf-lives and less added preservatives. Furthermore, we have observed a tendency to avoid the excessive use of single use plastic in food packaging. A novel alternative to single use plastics consists of edible coatings films of biological origin, that represent an innovative solution to consumers' environmental concerns and which can be developed under a circular economy approach. Highly perishable foods, such as mushrooms, reveal themselves to be a challenge in this context due to their very short shelf life. The *MicoCoating* initiative intends to develop and apply a preservative coating for fresh mushrooms, developed from mycelium and mushrooms that do not meet the market standards, thus ensuring an edible and natural alternative that maintains the physicochemical characteristics of the foods, while minimizing perishability. Accordingly, wild mushroom species from the Centre and North regions of Portugal with bioactive and functional compounds (antimicrobial, antioxidant, antitumor, among others) have been selected to be cultivated in closed systems, and their potential for the development of natural and edible coatings tested. A main component of this initiative is to produce mushrooms from species of interest to the region Beira Serra.

Thus, it was necessary to create, optimise and characterise growth substrates,[1] in order to ensure that the cultivated mushrooms follow the same quality standards and nutritional profiles as the ones collected from Nature. It is widely known that the contents of the substrate can directly affect not only the quality of the cultivated mushrooms,[2] but also the bioactive compounds they may be able to produce. From the species collected in the Centre and North regions of Portugal, the species *Pleurotus ostreatus*, *P. eryngii*, and *Agrocybe cylindracea* (among others) were selected.[2] For these species, two different cultivation substrates, specifically designed to allow the production of bioactive compounds in the mushrooms, were proposed and tested. These substrates were optimized according to their chemical composition and the bioavailability of nutrients for mycelial growth and fructification. The contents in proteins, lipids, fibres, humidity and ash[3] were evaluated in this study, and their influence on the quality of the products will be discussed. Both substrates will be compared and evaluated to access if the quality of the mushrooms produced meet the standard, and the adequate conditions to allow for bioactive compound production. This initiative will also discuss the influence of the cultivation time and production yield for the studied species of mushrooms.

The next step of this initiative aims at the optimization of the substrates and of the cultivation conditions for other mushroom species of fine chemical market interest, to ensure a diverse catalogue of species with biotechnological potential.

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Characterization of waste biomass generated in mushroom production and their potential for the extraction of bioactive compounds for food coatings

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Mushroom production involves the generation of a considerable amount of biomass waste, such as stems and mushrooms not conforming to the stipulated commercial standards and other by-products such as surplus production (≤5%) or mycelium retained in the substrate (>20% of production weight). However, these residues contain compounds that help in their preservation.[1,2]
It is important to optimise the conservation methods of edible products such as fresh mushrooms, increasing their shelf-life, in order to reach international markets with a high level of quality. Conservation is a fundamental and extremely important factor in guaranteeing the quality of the product for the longest shelf life possible, while simultaneously ensuring its optimal flow to the different distribution channels, towards a Circular and Sustainable Bioeconomy.
The MicoBioExtract project proposes the use of mushroom residues and by-products as a source of bioactive compounds to produce preservative coatings for food, in particular fresh mushrooms.
Throughout the production cycle, material was collected from the *Pleurotus ostreatus*, *Pleurotus eryngii and Agrocybe cylindracea* species and analysed for general characteristics, such as proteins, lipids, fats, ash, fibre, moisture and energy. The extracts rich on polysaccharides were prepared from the abovementioned residues following a hot water extraction technique.[3]
Herein we will disclose the preliminary results on the application of these extracts on fresh mushrooms and their impact in preservation, considering the colour, texture, weight, moisture properties and preliminary anti-microbial tests (estimated total bacterial count at 30°C). We studied the shelf-life for t = 0 and t = 12 days on uncoated mushroom samples covered with a conventional cellophane film (*wocoat_ce*) and mushrooms coated with a biofilm (*coat_woce*). We observed a higher dehydration and weight loss on *coat_woce* than on the currently commercial system wocoat_ce, indicating that the biofilm does not prevent dehydration on the mushroom, perhaps due to the lower exchange of gases between the sample and the air. While in the colour study, a slightly darker shade is verified in the *coat_woce* sample, due to the coating itself having a brownish hue. Despite the results of the previous analyses, visually, it can be seen that *coat_woce* is less affected by moulds over time, which results in less degradation. The coating does not affect the pH value, that is around 6 in all samples, ideal value for the growth of plants and fungi. In terms of texture, there is a lower hardness and elasticity in *coat_woce*, possibly the result of a greater degree of dehydration of the sample. Through microbiological analysis, the growth of some bacterial genera was identified, but, according to the literature, were all found as bacterial colonies, already identified in food, as a result of their natural decomposition.

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Innovative edible coatings to increase the shelf life of smoked sausages

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Feed and Food waste is a growing problem worldwide, which fueled by the increase in world population, calls for the need of finding solutions for the sustainable development of a greater amount of feed and food. Largely reliant on the use of plastic, namely in packaging, food is one of the most polluting industries in the world. It Is thus crucial to combat this issue, with circular economy emerging as a solution. Smoked sausages products contain high amounts of plastic and preservatives additives. Accordingly, the reduction of these compounds is an important measure towards a more environmentally friendly feed and food industry, while improving consumer health.

Consumers are increasingly more demanding and seek safer and more natural products, including feed and foods free of synthetic preservatives [1]. Traditionality, smoked sausages products are high nitrate and nitrites levels which serve as preservatives, preventing development *Clostridium botulinum* spores. However, in organism these products react with amines to form nitrosamines, which are carcinogenic chemical compounds, increasing the probability of brain and stomach cancer and others diseases[2].

The development of edible coatings with bioactive compounds to protect food this demand for safer, plastic-free and overall healthier foods. These coatings are applied on the surface of a food and can be consumed without risk to the consumer, as these are composed of safe, bioactive and biodegradable ingredients.[3]

The BeirInov project is an important and innovate Portuguese initiative, in the field of circular and sustainable bioeconomy, aiming at the ecologic conception and development of a strategic approach for the reduction of food waste and of plastic and chemicals in food traditional products. Indeed, BeirInov intends to contribute to the sustainable production of smoked sausages products of Serra da Estrela, without the loss of their natural characteristics. The BeirInov project aims to create edible coatings, reducing waste, and provide natural alternatives to synthetic preservatives, in order to improve the shelf life of products, while reducing toxicity. In the first phase, edible coatings were developed and tested with chitosan, pectins and alginate. The coatings formed a biofilm, while either maintaining or improving the characteristics of the tested products. Compounds such as citric and ascorbic acid and plant extracts with improved biological activity obtained from endogenous raw materials incorporated into the coatings were also tested in order to preserve the products, without the need for the addition of synthetic preservatives.

Herein we will show the aim of the BeirInov project, disclosed and discussed preliminary results of the project in the coating of smoked traditional food products of Serra da Estrela.

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Development of bioactive food products and ingredients using endogenous Portuguese agricultural resources for healthy nutrition

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Consumers are increasingly demanding custom, clean label and nutritious feed and food products (without synthetics and fossil-based additives), with identifiable bioingredients and/or origin towards a sustainable and bio society.
In response to consumers demand, products with natural plant-based ingredients and their bioextracts have been increasingly introduced on the market, mainly as snacks and drinks. However, plant-based ingredients and their extracts have been mainly obtained from non-indigenous species (e.g., ginseng and hibiscus), with disregard for endogenous Portuguese plant sources such as traditional fruits and aromatic plants. Accordingly, this work aimed at developing natural concentrates for healthy consumption patterns based on extracts, rich in bioactive properties and new sensorial profiles, through the valorisation of agro-forestry resources based on low calibre or scrap fruit ('Bravo de Esmolfe' apple[1], quince, 'Cova da Beira' cherry[2] and aromatic plants[2] such as *Lavandula pedunculata* and *Mentha suaveolens*).
An optimization of the extraction process to obtain bioextracts with enhanced biological activity from endogenous plant resources for food application was performed. In this sense, the best extraction yields were obtained for the following methods: (i) conventional extraction with a mixture of EtOH:H_2O (50:50%) v/v for 'Bravo de Esmolfe' apple extracts (12,66% yield); (ii) ultrasound-assisted extraction with a mixture of EtOH:H_2O (70:30%) v/v for quince extracts (16,34% yield); (iii) microwave-assisted extraction with a mixture of EtOH:H_2O (50:50%) v/v acidified with 0,1% HCL for 'Cova da Beira' cherry extracts (19,03% yield); (iv) conventional extraction with a mixture of EtOH:H_2O (50:50%) v/v for *Lavandula pedunculata* (23,91% yield); (v) microwave -assisted extraction with a mixture of EtOH:H_2O (80:20%) v/v for *Mentha suaveolens* (23,58% yield). The identification and characterisation of bioactive compounds from each obtained extract was performed using chromatographic techniques (namely HPLC and GC-MS) and the evaluation of bioactive activities was determined by chemical, enzymatic and cellular methods[1,2].
Based on these extracts and their characteristics, functional concentrate formulations were developed to obtain the final product. The product's appearance, taste and texture will then be evaluated by an external specialised panel through sensory analysis. Thus, a strategy was devised to create a new innovative product based on a by-product generated in cheese-making (in particular, second cheese whey). Considering the cheese industry of the Interior Centro Region struggles to valorise its by-products, which have a dangerous impact on the environment when not properly treated, the second cheese whey was used to produce the new functional concentrate. An innovative product with high added value was obtained, which is based on the principles of circular and sustainable bioeconomy and enhances the valorisation of by-products, minimizing the impact on the environment, improving the quality of life of the affected populations, water resources and soils. The functional concentrate is essentially made up of sorghum, extracts of improved biological activity and dehydrated endogenous fruit. This food product is a healthier, circular and nature-based alternative to most products already on the food market.

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Natural food ingredients from quince peel: Towards a "zero-waste" production system

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Quince is the yellow fruit of the deciduous tree *Cydonia oblonga* Mill. Its taste is sour and astringent when eaten raw, so it is usually processed into marmalade and many other food products, mostly sweets, through processes that discard the peel as a by-product. Therefore, this work was carried out to promote the upcycling of quince peel into valuable food ingredients following a "zero waste" approach. A response surface methodology (RSM)-coupled experimental design with 20 runs, combining five levels of time (1–120 min), temperature (25–95 °C) and ethanol concentration (0–100%), was implemented for maximizing the extraction of soluble solids and malic acid while simultaneously obtaining fiber concentrate extracts. The yields of soluble solids and fiber concentrate extracts were determined gravimetrically. Malic acid, which has been used as a food preservative, was analyzed by ultra-fast liquid chromatography with photodiode array detection.[1] The dietary fiber content of the fiber concentrate extracts was determined by an enzymatic-gravimetric method (AOAC Official Method 985.29) and their color was measured with a portable colorimeter.[2,3] The three independent variables affected significantly the extraction process and the predictive model equations were validated based on different statistical criteria, which allowed to determine the optimal extraction conditions. In general, the soluble solids yield was promoted by lower temperatures and ethanol concentrations, while malic acid was better extracted at higher temperatures for longer processing times. In turn, the fiber concentrate extracts remaining after extraction had opposite yields to the soluble solids. However, the highest yields of these fiber concentrates were not in agreement with their dietary fiber contents. The highest fiber values were recorded in the concentrate extracts obtained at high temperatures and medium-low ethanol concentrations. In fact, only these two independent variables significantly impacted its extraction, through linear, quadratic, and also interactive effects. Furthermore, the lighter fiber concentrate extracts were those with the highest yields. Overall, quince peel can be totally upcycled into natural food ingredients rich in malic acid and dietary fiber.

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Nutritional quality of mealworm (*Tenebrio molitor*) oil obtained by extrusion

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The world is rapidly changing, obviously at an environmental level, always at an economic level, and unfortunately at a social level, leading all of this to significant cultural changes, namely what people eat. One of the main issues of concern is precisely the availability of food resources in parallel with its nutritional and bioactive potential, which is why the industry and academia have been looking for alternative sources of essential molecules for the well-being and health of the consumers, such as: proteins, bioactives, essential fatty acids, and fibres. Edible insects have gained some prominence for their protein content and fatty acid content. However, there is still a great aversion on the part of consumers to insect-based food products, especially at the organoleptic level (texture, taste and appearance). Oil-rich components are just one of the answers to maximize the use of these new sources of fatty acids, mainly omega-3 and -6, such as the oil extracted from *Tenebrio molitor* (mealworm).[1] The larvae are traditionally used for animal feed, pets and fish, but in some countries like China and Netherlands, they have been already consumed for food purposes. Its legal acceptance for incorporation and development of new food products was already stablished and, since it is tolerant to various environmental conditions and does not require a large area for growth, it is perfectly suitable for mass production.[2] In this context, the present work aimed to characterize the mealworm oil, obtained by extrusion techniques, regarding its content in fatty acids by gas-chromatography coupled to a flame ionization (GC-FID) detector, as also its salt content (NaCl) by the Mohr titration method. The results regarding the fatty acids profile in terms of total saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) are described in **Table 1**. Twenty-two individual fatty acids were identified, having been found, in percentages higher than 0.1%, the following compounds: elaidic acid (C18:1n9, 44.7±0.1%), linoleic acid (C18:2n6, 31.4±0.5%), palmitic acid (C16:0, 14.2±0.4%), octadecanoic acid (C18:0, 2.9±0.2%), α-linoleic acid (C18:3n3, 1.293±0.001%), and heptadecanoic acid (C17:1, 0.123±0.002%), which are in accordance with previous bibliography.[3] Regarding salt content, 2.3±0.1 mg/mL was within the accepted limits. This is one of the attributes that most affects the organoleptic acceptance of mealworms for food purposes, besides the particle size; as such, future studies are needed to further investigate the physicochemical characteristics of the studied oil sample. The results obtained revealed the huge potentiality of mealworm larva for the development of new food products, representing one of the greatest answers to the increasing unavailability of sustainable natural sources rich in molecules beneficial for human health.

Table 1. Fatty acid profile (relative percentage, %) and salt content (mg/mL) of mealworm oil (Mean±SD).

Saturated fatty acids (%)	17.4±0.4
Monounsaturated fatty acids (%)	49.9±0.1
Polyunsaturated fatty acids (%)	32.7±0.5
Salt (mg/mL)	2.3±0.1

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Application of autochthonous lactic acid bacteria as starter cultures for ewe's milk cheese production

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In cheese making the use of a standardized inoculum composed of representative autochthonous strains can drive the fermentation process in a desired direction. The obtained product will present peculiar characteristics of uniqueness and quality due to the inhibition of undesired pathogenic and spoilage microorganisms involved in hygienic and safety aspects, either by the microbial competition or by the production of antimicrobial substances, ensuring an innocuous traditional final product. The introduction of this innovative practice in Portugal should be considered in the short term in traditional products as it contributes to the increased use of endogenous resources combined with a more diversified offer.

The impact of a mixed inoculum use (four lactic acid bacteria isolated from either raw ewes milk or Serpa PDO cheese, previously characterized for their technological, probiotic, and antimicrobial properties[1,2]) for ovine cheese production was assessed. A pilot scale trial was developed using an autochthonous mixed inoculum (SF5C Test Inoculum) composed of the autochthonous strains *Lactococcus lactis* LLA17, *Lactobacillus paracasei* A2Lb1, *Lactobacillus plantarum* G1Lb5 e *Leuconostoc mesenteroides* LMA45. Four batches of cheese (performed in duplicate) were produced following the Serpa cheese specifications, except for the use of inoculum.

In the first batch (L1), used as the control group, only raw milk was used; in the second batch (L2), 2% inoculum was added to the raw milk, in the third batch (L3), 4% inoculum was added to the raw milk, and in the fourth batch (L4) 4% inoculum was added to pasteurized milk. The cheeses were evaluated (in triplicate), immediately after production (0 days) and after 15 and 35 days of ripening: physicochemical (pH, acidity, adhesiveness and hardness), microbiological (total mesophiles, enterobacteria, lactic bacteria - LAB and fungi) and sensory properties (panel with 9 tasters), were studied using standard techniques.

The physico-chemical and microbiological parameters were similar to those obtained in previous works for this type of cheese. The hedonic sensory testing point to a good acceptance of cheeses with "SF5C Test Inoculum": the batches with added inoculum (L2, L3 and L4) reached a higher score than batch without inoculum (L1), highlighted to the batch L3 (4% inoculum added to raw milk)(**Figure 1**). These results seem to indicate that the autochthonous inoculum "SF5C Test Inoculum" can be classified as "Valor Inoculum" within the scope of GO SERPAFLORA project.

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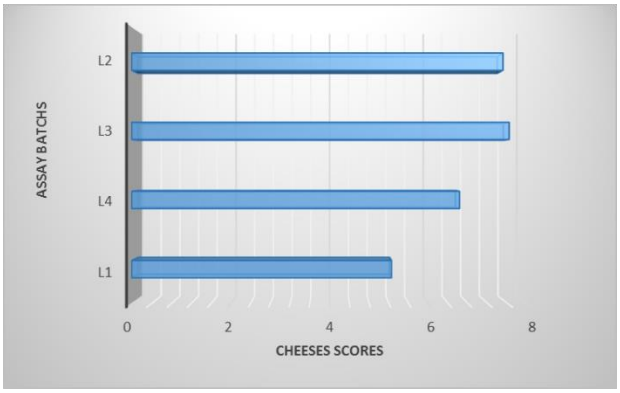

Figure 1 - Results obtained in the hedonic sensory test

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Microencapsulation of a phenolic-enriched fraction of *Gunnera tinctoria* with natural polymers: starch, pectin, and a starch/pectin complex

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Polyphenols are secondary metabolites responsible for numerous biological, physiological, and chemical activities. The properties of polyphenols make them a remarkable natural product for the prevention of several diseases, due to their antioxidant, anti-inflammatory and antitumoral effects, but the low bioavailability and high structural sensitivities limit their *in vivo* functionalities. Meanwhile, encapsulation plays a significant role in protecting sensitive bioactive compounds in industrial processes, storage conditions and biological media (controlled release) against environmental and biological conditions such as temperature, light, pH, humidity, high oxygen level and digestive system. Therefore, encapsulation techniques have been developed widely as efficient delivery carriers in the food, cosmetic and pharmaceutical industries.[1]

Gunnera tinctoria, also known as giant rhubarb, is a flowering plant species native to South America, where it is used for culinary purposes. *G. tinctoria* is rich in phenolic compounds such as gallic acid, ellagic acid, catechin, epicatechin, and quercetin, with reported antioxidant and antitumoral activities.[2]

In this work, a phenolic-enriched fraction of *G. tinctoria* was encapsulated using pectin, starch, and a mixture of both, using a spray-drying methodology.[1,2] The encapsulated particles were evaluated, regarding product yield, particle size (regarding internal and external layers), surface charges, and surface morphology (by scanning electron microscopy, SEM).

The product yield of encapsulated particles varied between 34.6 and 68.6%, with starch achieving the highest yield and pectin the lowest one. The starch/pectin complex slightly improved the product yield (38.0 %) compared to pectin. The particle size of the internal layer varied between 509.1 and 1196.5 nm, in volume, and between 355.0 and 916.5 nm in number. Starch promoted the smallest particles, while pectin originated the biggest ones. In addition, the starch/pectin complex acted as a size-reducing agent. Regarding the surface charges, they varied between -29.72 and -55.01, in the inner layer, and from -16.21 to -53.47, in the external layers, all in an acceptable and stable range. SEM images confirmed the particle sizes of the external layer (which varied between 1 and 20 µm) and the surface morphology of the coating polymers. Furthermore, the schematic formation of the polymeric layer was estimated by the particle size distribution of the inner layers.

The natural polymers herein studied, together with the spray-drying methodology, proved to be suitable to successfully microencapsulate a phenolic-enriched extract. Moreover, the differences found between polymers highlight the adaptability of the process for different applications. Thus, this green procedure can lead to the development of innovative and safe microencapsulated ingredients to be used, for example, in food or dietary supplements.

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Evaluation of coagulation kinetics using cardoon flower extracts and rennet in sheep milk from different origins in the Alentejo region

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Milk coagulation is the essential step in cheesemaking production process, for which preparations of aspartic proteases have been used for thousands of years. The Iberian Peninsula has the largest variety and production of cheeses using cardoon flower extracts as coagulants, which are normally produced on an artisanal scale. Those extracts show slight differences in milk coagulation when compared to coagulation with rennet or chymosin, the most widely used coagulating enzymes in cheese making. These differences can be important for cheese properties which are defined as early as milk coagulation. The main objective of this essay is to evaluate the kinetics of ewe's milk coagulation, as an additional and useful tool for monitoring the traditional cheese manufacture.
Twenty-eight samples of sheep milk from 4 different sheep milk farmers in the Alentejo region (Beja) were used to monitor milk coagulation process of the vegetable coagulant from *Cynara cardunculus* (cardoon flower aqueous extracts, obtained as described by previous work [1, 2] in comparison with a fermentation-produced chymosin preparation (a Maxiren DS coagulant, DSM Gist-Brocades, 2600 MA Delft, The Netherlands). The composition (fat, protein, lactose, total solids and non fat solids), pH and acidity of the milk samples from the different farmers (collected along 6 different sampling dates and frozen at -80°C) was assessed using a Milkoscan 133B (Foss Electric, HillerØd, Denmark), a potentiometer and milk titration (NP-470, 1983), respectively. The milk technological behavior throughout coagulation process by the action of the two coagulants was evaluated by determining the milk coagulant activity according to ISO 23058/IDF 199 (ISO/IDF, 2006), using a sheep milk as substrate and with an Optigraph (Alliance, Frépillon, France), which is based on the real time measurement of near infrared signal attenuation caused by micellar aggregation. All assays were accomplished in duplicate.
Preliminary results confirm the findings of previous work, showing that the nature of coagulant influences milk coagulation properties of sheep milk, which must be considered in cheesemaking. When cardoon extract was added to sheep milk the curd tended to be less firm than the curd obtained with chymosin solution, which can be related to the higher non-specific proteolytic activity of cardoon aspartic proteases. Although clotting time and curd properties can be related to the coagulant specificity, other factors, like milk properties and composition, can affect these properties and this can be observed when we consider the variability on monitoring of curd evolution. This highlights the need to control the different technological factors involved in the coagulation process and which will have a further effect on the cheese quality.

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Physical characterization and preservation studies of a clean label mayonnaise containing carrot powder

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Consumers are increasingly informed and aware of the decisions they make regarding food and the environment around them, so they seek to have a healthy and sustainable diet, in which there is opportunity for the growing trend of clean label products.

The main objective of this study was to evaluate the stability and preservation of a clean label mayonnaise, in which carrot powder was used to replace sugar, potassium sorbate and beta-carotene, present in standard mayonnaise. In addition, reducing the amount of oil maintaining the physical properties of the mayonnaise similar to those of the standard one was also a goal. A maximum amount of carrot powder used was 5% w/w, the threshold for no change in texture caused by the powder particle size.

The surface response methodology, coupled with a central composite rotatable design, was applied to study the effect of the content of carrot powder (0 – 5% w/w) and oil/water ratio (1.12 - 2.24) on the mayonnaise properties (color, pH, texture, viscosity and viscoelastic properties). It was observed that the mayonnaise samples with greater similarity to the standard one were those produced with 2-3.5 %w/w of carrot flour and with an oil/water ratio between 1.8 and 2. For the preservation studies was selected a mayonnaise sample containing 3 %w/w carrot powder and an oil/water ratio of 2. This sample presented an oil content of about 7.26 % lower than that of the standard product.

In the preservations studies, two types of products were studied: (i) the selected mayonnaise sample without preservation additives, and (ii) the selected mayonnaise sample with potassium sorbate. Both products were subjected to two preservation conditions: (i) under accelerated storage conditions at 40ºC with light, and (ii) under refrigerated conditions (at 4ºC) simulating the consumer handling. The mayonnaise samples were analyzed in regular time intervals in terms of microbial growth (molds and yeasts, mesophilic and psychrotrophic microorganisms, *L. monocytogenes* and *Salmonella sp.*), antioxidant activity (DPPH, FRAP and ABTS) and total phenolic content. In addition to that, samples were also characterized in terms of color, pH, texture, viscosity and viscoelastic properties over time.

The selected mayonnaises containing carrot powder presented similar physical properties to those of the standard product and good preservation capacity. It is envisaged a good sensory acceptance in a future sensory analysis study.

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Brewer's spent yeast as an emulsifier for vegan and clean label sauces

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Consumer's demand for clean labels, compelled industries to find natural options to replace food additives. [1] Brewer's Spent Yeast (BSY) is a byproduct of the brewing industry rich in protein and polysaccharides, representing an affordable source of β-glucans and mannoproteins, compounds with emulsifying properties due to their thickening and amphipathic properties, respectively. [2] Based on these capabilities, BSY was tested as a source of emulsifiers for sauce formulations. To achieve this, BSY extracts were obtained by a subcritical water extraction using microwave followed by an isolation of the polymeric material with 80% ethanol precipitation. For comparison, alkaline extractions with KOH 1 M and 4 M were performed for selective polysaccharide extraction. The obtained extracts were characterized regarding their sugar composition and tested as an emulsifier in a model emulsion system at neutral and acidic pH characteristic of mayonnaises.

Subcritical water extraction allowed the solubilization of a fraction with similar amounts of proteins and polysaccharides, with similar percentage of mannose and glucose, indicating the presence of mannoproteins and glucans. The alkaline extractions solubilized more protein material with the increase in KOH concentration and revealed a higher mannose content On the other hand, a protein-rich precipitate fraction obtained from 4 M KOH extraction was mainly composed of glucans. The extracts that showed a better emulsion stabilization capacity were the protein rich extract obtained by 4 M KOH alkali extraction, and the polymeric fraction obtained by subcritical water extraction showing better results than xanthan gum, egg yolk and modified starch. Mayonnaises were successfully prepared replacing egg yolk and modified starch with all yeast extracts. Only one third of the protein rich extract obtained by 4 M KOH alkali extraction was needed to develop a mayonnaise with the same texture parameters of the standard one. In this way, yeast extracts were able to produce mayonnaises with the same texture properties as the standard mayonnaise, replacing food additives and egg yolk and creating a vegan and clean label sauce.

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Effect of different edible coatings on the preservation of whole apples

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Apple is one of the most popular and consumed fruits worldwide.[1] Its appearance and texture are the most important criteria determining consumer preference.[2, 3] Thus, special attention should be paid by stakeholders of the supply chain once apples postharvest quality during storage and shelf life is of utmost importance to assure quality until the moment of consumption.[2] Several preservation techniques have been used to extend the shelf life of fresh fruits and the use of edible coatings is one that are quite popular. Edible coatings have been used successfully, such as some waxes used to preserve fruits, preventing water loss and enhance their aesthetic appeal and even preventing microbial contamination. Recently there has been growing interest in replacing these waxes which are products of the petroleum industry with more natural organic coatings to improve their healthiness and sustainability.[1]
In this study, edible coatings prepared with a solution of alginate and essential oils were tested to understand their preservative power in preventing colour and texture changes along their shelf life. To investigate the effectiveness of this treatments, green apple samples were analysed: control 1 (without biofilm); control 2 (without biofilm); control 3 (with 2% alginate); treatment 1 (with 2% alginate and 1% essential oil of oregano); and treatment 2 (with 2% alginate and 1% essential oil of thyme). Instrumental methods to measure apple quality based on appearance (such as fruit shape, size, colour) and texture attributes are important tools to monitor that quality.[1, 3] Samples were analysed in terms of weight loss, shape, and colour changes (CIE L*, a* and b* colour parameters).[1] Firmness was also evaluated by a compression test performed on unpeeled apples using the texture analyser equipped with a 4 mm-diameter probe.[1]
Results showed that weight values varied slightly over storage time as well as diameter that varied between 6.3-7.0 cm and 6.7-7.0 cm. There were no significant differences in the weight and size of the apples within the studied period. However, the visual analysis revealed that, in 3 months, all control samples started showing brown spots, and both treatments resulted in rotted apples. In the beginning of the assay, the treatment with 1% essential oil of oregano also showed brown spots. Concerning colour changes L*, a* and b* parameters were largely affected when compared to control and alginate coated samples, and as referred the browning spots are the major reason for the observed low L values. Texture was also affected as loss of firmness was observed, mainly due to dark spots also. Generally, the typicity of the inside colour and textural appearance is preserved despite the apparent loss in external quality. These results show that the use of edible coats with alginate and the essential oils are promising as it is possible to prevent water loss and maintain the texture by avoiding the use of waxes, although in this case the solutions used do not promote total control of browning.

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Compostos Bioativos

Flavonoids profile by UPLC-MS/MS of taperebá (*Spondias mombin* L.) fruit peel from Cerrado biome - Brazil.

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The Cerrado biome, in Brazil, is considered one of the 34 "hot spots" for conservation in the world, containing numerous fruits with medicinal benefits that have not yet been studied scientifically. The bioactive substances found in these fruits provide environmental protection and health benefits. Taperebá (*Spondias mombin* L.) is a member of the Anacardiaceae family, and it is found in this biome. Then, this study aimed to characterize the flavonoid compounds profile of taperebá peel extract by UPLC- MS/MS. According to results, a total of 14 flavonoid compounds were quantified, demonstrating a significant content of flavonoids in taperebá peel. Among these compounds, quercetin was the most abundant, followed by myricetin and kaempferol- 3-glc. Interesting values were also verified for syringetin and isorhamnetin-3-glc. These results reveal taperebá as natural source of flavonols, presenting a peculiar and interesting values of these bioactive compounds, that can be used in research to generate new bioactive molecules, and to show the benefits of consumption of this fruit.

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Anisophyllea boehmii food potential: Chemical composition and antioxidant activity

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Edible fruits are rich sources of both anthocyanins and flavonoid glycosides, which are responsible for the red, violet, purple, and blue color of the fruits [1], showing a wide range of antioxidant protection and preventive benefits including the integrity of genomic DNA, potent cardioprotective, neuroprotective, anti-inflammatory and anticarcinogenic properties [2],

The loengo (*Anisophyllea boehmii* Engl.) is a fruit growing mainly in Zambia, Tanzania, the Democratic Republic of the Congo, Uganda and Angola, where special edaphoclimatic characteristics can be found, with the highest rainfall in the African continent [3]. This is a stone fruit, plum-shaped, dark blue or carmine when ripe and its fleshy pulp has a pale reddish yellow color, with a sweet and acid taste, it is edible and contains only one seed. The chemical composition, nutritional properties and antioxidant activity of the loengo peel, pulp and seed was determined. The polyphenolic profile of loengo and quantification of the most representative polyphenols was analyzed through HPLC-DAD, table 1. Regarding anthocyanins, malvidin-3-glucoside and petunidin-3-glucoside were predominant with high values. Nevertheless, flavonols such as isorhamnetin-3-hexoside, quercetin-3-rhamnoside and quercetina-3-(6-acetyl)-hexoside presented the highest results. Besides, total polyphenol content by Folin-Ciocalteau assay and total anthocyanin content by differential pH assay and antioxidant capacity by DPPH and FRAP assays were also determined. In what nutricional composition is concerned, potassium and phosphorus were found in loengo as the major mineral nutrients and the seed was found to be rich in protein table 2. It is emphasized that *A. boehmii* can be consumed not only for its sweet taste, but also for its chemical and nutritional composition, for being rich in many minerals and having a considerable antioxidant potential, that are beneficial for health. Further work will be carried out for the well understanding of this fruit composition, representing a challenge for the rural development with impact on food security and health benefits.

Table 1. Concentration of the main antioxidant compounds (μmol.g^{-1} Fresh Fruit) found in loengo pulp (*Anisophyllea boehmii* Engl.).

Compound	RT~	mean (mg/kg)	SD
Anthocyanin			
Delphinidine-3-glucoside	4,62	3,13	0,10
Cyanidine-3-glucoside	6,74	18,69	0,47
petunidine-3-glucoside	8,44	0,87	0,01
malvidine-3-glucoside	13,02	0,86	0,07
Total Anthocyanin		∑=23,55	
Hydroxicinnamic acid			
Chlorogenic acid	13,07	0,98	0,12
acido cafeic	17,78	2,95	0,29
Flavonols			
myricetin-3-glucoside	18,51	1,26	0,05
myricetin-3-rutinoside	19,01	5,22	0,42
quercetin-3-(6-acetyl)-hexoside	21,49	2,33	0,04
quercetina-3-(6-acetyl)-hexoside 2	24,56	0,43	0,01
quercetin-3-rhamnoside	25,39	1,82	0,22
isorhamnetin-3 glucoside/rutinoside	36,6	1,30	0,12
Total Flavonol		∑=16,28	

Table 2. Mineral and nutritional composition from pulp and seeds of Loengo (*Anisophyllea boehmii* Engl.)[a].

Mineral and nutrients	Pulp (mg.100^{-1}g FW)	Seeds (mg.100g^{-1} FW)
K	266.10 ± 15.966	184.61 ± 11.078
P	139.59 ± 6.978	103.95 ± 4.158
Ca	43.48 ± 1.740	9.71 ± 0.485
Mg	36.18 ± 1.447	34.01 ± 1.700
Fe	2.20 ± 0.110	0.90 ± 0.04
Mn	4.50 ± 0.225	0.80 ± 0.03
Zn	0.20 ± 0.010	0.80 ± 0.03
Cu	0.03 ± 0.002	0.20 ± 0.02
Weight/fruit (g)	9.40 ± 0.376	3.00 ± 0.15
Moisture (%)	71.00 ± 2.840	51.30 ± 3.08
Protein (%)	7,96± 0,10	16,85 ± 0.21

[a]Values are the average of three replicates.

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Antioxidant, Anti-hypertensive and Anti- Alzheimer activities of *Porphyra* sp.: the effect of extraction time

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Research efforts are being devoted to the identification and characterization of compounds of natural origin with potential health benefits. Seaweeds are promising organisms as a source of these bioactive compounds and therefore can be used as ingredients for the formulation of functional foods and nutraceuticals. Antioxidants from seaweeds may be safer and more efficient than synthetic ones. Compounds such as carotenoids, polyphenols, sterols, carbohydrates vitamins E and C, proteins and peptides or aminoacids play an important role in the prevention of various diseases and ageing processes through protection of cells from oxidative damage (Farvin and Jacobsen, 2013). Hypertension is a significant chronic disease and the search for natural compounds to produce oral drugs with no adverse effects have been increasing. It was shown that peptides and phlorotannins released by seaweeds enzymatic hydrolysis exhibited Angiotensin-Converting-Enzyme (ACE) inhibitory activity (Seca and Pinto, 2018). Previous investigations also established that natural compounds (phlorotannins, phenolic acids and flavonoids) from seaweeds could be effective in the management of Alzheimer´s disease due to their strong neuroprotective potential (Olasehinde et al., 2019). In this work the effect of two extraction times (8 and 24h) on the antioxidant, anti-hypertensive and anti-Alzheimer properties of Porphyra sp. extracts prepared using Alcalase-assisted extraction was evaluated. Concerning total phenolic and flavonoids content the highest values (TPC=197.9 mg GAE/g dried extract and TFC=186.5 mg GAE/g dried extract) were observed in the extract obtained after 8h of Alcalase-assisted extraction (Table 1). Both extracts exhibited DPPH and ABTS radical scavenging activities but the extracts obtained after 8h of extraction had a higher DPPH and ABTS activities, as it can be seen by their lower EC_{50} value (Table 2). High Angiotensin-Converting-Enzyme (ACE) inhibitory activity (EC_{50}=0.9 mg/mL) was observed in both extracts and no significant differences were observed between them. In what concerns acetylcholinesterase (AChE) inhibitory activity the extract obtained after 8h did not attain 50% of inhibition for the concentration range tested, but the extraction for 24h had an EC_{50} value of 42.2 mg/mL. In conclusion, 8h of Alcalase-assisted extraction allowed to obtain extracts with high antioxidant, anti-hypertensive and anti-Alzheimer activities which make them useful as an ingredient in the preparation and development of functional foods and nutraceuticals.

Table 1 - Total phenolic and flavonoid content of Porphyra sp extracts prepared after 8h and 24h.

	8h extract	24h extract
TPC (mg GAE/g dried extract	197.9± 9.12	185.5± 1.35
TFC (mg QE/g dried extract)	10.55 ± 0.229	5.03 ± 0.168

Table 2- DPPH and ABTS radical scavenging activities, Acetylcholinesterase (AChE) and Angiotensin-Converting-Enzyme (ACE) inhibitory activities of Porphyra sp extracts prepared after 8h and 24h.

EC_{50} (mg/mL)	8h extract	24h extract
DPPH radical scavenging activity	14.9	19.7
ABTS radical scavenging activity	17.5	29.2
AChE inhibitory activity	42.2	---
ACE inhibitory activity	0.93	0.90

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Phenolic compounds from sea buckthorn leaves modulate ROS generation and inflammation markers in human cells

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Side streams of berries and woods offer diverse sources of various bioactive compounds. Especially berries are excellent and variable sources of phenolic compounds, such as phenolic acids, flavonoids, and proanthocyanidins. This research aimed to compare the maceration and pressurised hot water extractions to recover phenolic compounds from sea buckthorn leaves (SB) and analyse the cell-based antioxidant and anti-inflammatory effects in human cells. Maceration provided the extraction of a two-fold higher extraction yield (12.74 g/100 g), including total phenolics (TPC; 2.12 g/100 g) and condensed tannins (0.22 g/100 g), which also impacted higher antioxidant activity measured by CUPRAC (22992 mg ascorbic acid equivalent, AAE/100 g) and DPPH (3318 mg AAE/100 g). The cytotoxicity of the extracts was evaluated in lung adenocarcinoma epithelial cells (A549), human ileocecal adenocarcinoma cells (HCT8), and normal human lung fibroblast (IMR90). SB showed a slight decrease in cell viability in cancer cell lines (A549, IC_{50} = 1434 µg/mL; HCT8, IC_{50} = 1629 µg/mL), which suggests that this extract presented better antiproliferative activity. SB presented lethal concentration (LC_{50} = 1945 µg/mL) towards HCT8 cells. SB reduced the ROS generation towards H_2O_2-induced oxidation in both HCT8 and A549 cells, implying higher cellular antioxidant capacity. In lipopolysaccharide-stimulated THP-1 macrophages, SB increased TNF-α activation, thus indicating a pro-inflammatory effect. However, SB inhibited the ROS generation during the THP-1 cell respiratory burst. No cytotoxicity in THP-1 cells was observed between 7 and 1667 µg/mL. In conclusion, the freeze-dried extract obtained with maceration using water as the solvent was shown to exert antioxidant effects in human cells but had a pro-inflammatory activity in an acute cell-based model.

Evaluating phenolic compounds in ethanolic extracts of cherry pit

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The climatic conditions in Portugal favoured the adaptation of the cherry tree allowing its cultivation in several regions of the country. Studies made by the Portuguese Bureau of Statistics – INE [1], showed that the Portuguese sweet cherry production has an implemented area of 6,387 ha, producing 9,241 tons of this fruit. Its production extends mainly to two regions: the North (area of 3,099 ha and production of 6,586 tons) and the Centre (3,177 ha and 2,510 tons), while the rest of the country and islands accounts for only 1.6% of the cherry production. There are several varieties of cherry in Portugal, and the most important traditionally cultivated are: "Saco da Cova da Beira", "Saco do Douro", "Lisboeta", "São Julião", Big Burlat, Maring, Napoleon-big-foot and Big Windsor, being the first four varieties native from Portugal. Cova da Beira is the most important cherry production area in Portugal, either in terms of production volume, or also in area. Additionally, the evolution of technological indicators associated with culture reveals a high degree of specialization of the "new" farms, almost always associated with other fruit crops, which coexist in a very significant number of smaller farms, of a family type, that constitute the historical legacy of cherry production on the hillside north of the Serra da Gardunha. Sweet cherry seeds result from processing sweet cherry for sweets, juices and jams' production. Generally, seeds are considered a production waste, which gains a strong interest due to the environmental aspects related to waste disposal [2]. Additionally, it is well documented that production waste, such as peels, seeds, and pomace, contain high-value bioactive compounds [3]. Hence, the present work investigated the extraction of some bioactive compounds from cherry pits that originate from food manufacturing industries.

The waste management company Nutrofertil, located in Portugal, namely in the district of Viseu (Tondela), provided the Seeds of Sweet Cherry (SSC) for this study. The seeds were milled and dried for stability and then used for extraction with ethanolic solutions at different percentages (from 50 to 100% water v/v). Variable temperatures were also tested and the extracts were used for quantification of phenolic compounds through spectrophotometric techniques. The material was analysed to verify that it was exempt of hydrocyanic acid. Statistical techniques were used to treat the data: (a) Hierarchical cluster analysis using squared Euclidean distance and average linkage between groups method; (b) Principal component factor analysis with Varimax rotation.

The results indicated that extraction at 40 ^{o}C with magnetic stirring and using aqueous solutions of ethanol (water:ethanol ratio = 80:20, % v/v) constitute a separate cluster. Also, extracts obtained with similar conditions but for the temperature of 35 ^{o}C constitute another isolated cluster. Factor analysis revealed a grouping structure with four clearly distinct clusters (Figure 1). Group G1 accounts for the samples with water:ethanol 80:20 (% v/v) and a temperature of 35 ^{o}C, corresponding to the extraction of higher amounts of anthocyanins. Group G3 includes the extractions with 100% water at 70 ^{o}C (G3), with lowest contents of anthocyanins and flavonols. The remaining groups are divergent according to the values of total phenolic compounds. In group G4 are included samples in which were quantified high values for total phenolic compounds, flavonoids, proanthocyanidins, ortho-diphenols and phenolic acids, while G2 corresponds to samples with smaller amounts of those compounds. In conclusion, investigating the extraction potential of different conditions it was allowed to optimize the experimental conditions more favourable to maximize the recovery of certain bioactive compounds, which can have multiple applications as antioxidant substances after rigorous quality control regarding possible concentrations of hydrocyanic acid.

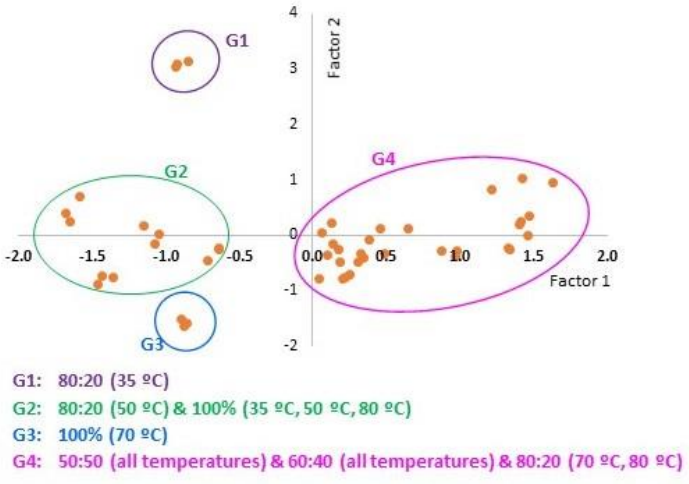

Figure 1: Results of factor analysis to the phenolic compounds extracted from cherry pits.

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Effect of harvesting time on the composition and biological activities of *Alaria esculenta*

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Alaria esculenta is a common edible brown seaweed species found on exposed rocky shores of the Atlantic Ocean in north Europe and America rich in phenolic compounds responsible for their high antioxidant potential (Farvin and Jacobsen, 2013). These seaweeds also contain bioactive compounds with anti-hypertensive capacity useful as food ingredients and nutraceuticals (Seca and Pinto, 2018). The time of harvesting is well documented as determining the biochemical composition of seaweeds and influencing the content of bioactive compounds that could be extracted (Rioux et al., 2009). Thus, in order to improve the management of brown seaweed *Alaria esculenta* production, the influence of the seeded long-lines deployment date and harvesting time on the total phenolic content (TPC) and biological activities (antioxidant and anti-hypertensive activities) of seaweed extracts prepared with Alcalase was evaluated. The extraction yields varied between 31.8 and 44.0% and in general, no significant differences were obtained between the extracts prepared with different samples. TPC of the extracts ranged between 57.4 and 120.0 mg GAE/g dried extract and decreased from March to June. With regard to the Total Flavonoids Content (TFC) Alaria extracts showed values between 15.5 and 29.1 mg QE/g dried extract. The highest TPC and TFC was obtained in the extract prepared with *A. esculenta* deployed on 28th November and collected on 20th March. All extracts exhibited high ABTS radical scavenging activity with EC_{50} values between 3 and 6 mg/ml. The lowest values were observed with extracts prepared with *A. esculenta* collected on 20th March and 3rd April. Concerning DPPH radical scavenging activity some extracts did not attain 50% of inhibition for the concentration range tested and the highest activity was obtained with *A. esculenta* collected on 3rd April. The highest reducing power were measured in extract prepared from *A. esculenta* collected on 20th March and deployed on 28th November. The lowest Cu^{2+} chelating capacity were recorded in extracts from *A. esculenta* collected on 20th March (EC_{50} = 2.36 and 2.40mg/mL for 15.10.2019 and 28.11.2019, respectively). Regarding Fe^{2+} chelating activity no noticeable trend was observed with the sampling dates, but in general, the extracts prepared with samples deployed on 15th October had lower EC_{50} values. All extracts exhibited ACE inhibitory activity and those prepared from A. *esculenta* collected on 20th March and 22nd April presented higher ACE inhibitory activity (ca 50%). Moreover, no significant differences were observed between the two deployed dates. The extracts prepared from seaweeds harvested on 20th March exhibited higher biological activities and it was also at this date that the extracts had the highest phenolic and flavonoid contents. Due to these biological activities these seaweeds could be used as ingredient in the preparation and development of functional foods and nutraceuticals.

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The impact of extraction temperature and solution concentration on the antioxidant activity of sweet cherry seeds' extracts

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Sweet cherry seeds, a valuable lignin-cellulose raw material for the production of polyurethane foams [1], are also a significant source of different phenolic compounds [2] and can be a good source of natural antioxidants, which can play an important role in preventing the formation of free radicals and protection against degenerative diseases.

Considering sustainability, the main objective of this project was the use of cherry by-products (seeds) to produce extracts rich in antioxidant compounds.

In this work, the seed extracts were obtained with the addition of different combinations of ethanolic solution (water:ethanol ratios - 50:50; 60:40; 80:20; 100:0 v:v) and at different temperatures (35, 50, 70 and 80 °C), all under magnetic stirring for 40 minutes. Then, the antioxidant activity of the extracts was evaluated through spectrophotometric methods, using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid)) radicals, and also the Ferric Reducing Antioxidant Power Assay (FRAP). All measurements were replicated at least in triplicates and were expressed as mg Trolox equivalents per gram (mg TE/g), after verifying that the material is exempt of cyanide acid. Statistical analysis was performed using the JAVA software.

ANOVA tests show that there is a statistically significant effect of temperature, water percentage and temperature on the antioxidant activity evaluated by the three methods used ($p > 0.001$ in all tests). The percentage of water is the variable that most contributes to this effect. Individual Post Hoc comparisons show, for all tests, that in general the temperatures induce differences in antioxidant activity, except 70 °C and 80 °C in DPPH and FRAP, and 80 °C and 50 °C in ABTS. Regarding the percentage of water, it was found that all samples are different from each other, except the FRAP, in which no significant differences between 50 and 60% of water were found.

In conclusion, no major differences between the ABTS, DPPH and FRAP methods were found. Temperature and percentage of water have a significant effect on the concentration of antioxidant activity in all methods. In that way, the cherry pit is a good by-product to produce extracts with high content of antioxidant activity, being that the 70° C with 50:50 and 60:40 water:ethanol solutions are the most favorable conditions to potentiate the antioxidant activity.

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Nutritional and chemical study of the fruits of *Rubus fruticosus* L. var. 'Triple Crown' as a food source with high antioxidant capacity

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Blackberries are fruits of great interest in the food, agricultural, and pharmaceutical industries due to their high content of bioactive compounds, which greatly vary according to the species. Therefore, to determine the benefits that each fruit species can bring to the consumer, its nutritional, chemical, and bioactive characterization becomes essential.[1] *Rubus fruticosus* L., specifically the 'Triple Crown' cultivar (**Figure 1**), is original from and grown primarily in Central and North America.[2] The fruit has a balanced taste between sweet and sour, which makes it well accepted by the consuming public, in addition to the high content of vitamins, minerals, and anthocyanins, as well as other phenolic compounds, carotenoids, glutathione, and endogenous metabolites, which contribute to its high antioxidant capacity.[3] In this study, *R. fruticosus* fruits were evaluated for their nutritional value (AOAC methods), free sugars (HPLC-RI), organic acids (UFLC-PDA), and fatty acids (GC-FID) composition, as well as for their anthocyanin content (HPLC-DAD-ESI/MS) and antioxidant properties (TBARS and OxHLIA).

The blackberry fruit revealed a balanced nutritional profile, presenting carbohydrates as the main macronutrients. Regarding the chemical composition, three free sugars were found, being glucose the one present in the highest concentration; six organic acids, with malic acid and quinic acid as the most abundant ones; and seventeen fatty acids, with a prevalence of polyunsaturated fatty acids. In addition, three anthocyanins were identified, all cyanidin derivatives (mostly cyanidin-3-*O*-glucoside).

The fruits also revealed a high antioxidant capacity, showing potential to be used in the development of new products in the food industry, such as natural dyes, juices, jams, and jellies, among others.

Figure 1: Fruits of *Rubus fruticosus* L., cultivar 'Triple Crown'.

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Bioaccessibility of phenolic compounds from white quinoa flour

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Quinoa (*Chenopodium quinoa* W.) is a pseudocereal rich in nutrients and in a wide variety of compounds with functional properties, such as phenolic compounds. The grains present different colors: white, black, yellow and red. Quinoa is consumed in whole grains or processed as flour and flakes (Lin et al., 2019).[1] Considering the widespread consumption of quinoa and its correlation with potential health benefits, as well as the use of its flour for the production of functional foods, this study aimed to evaluate the bioaccessibility of phenolic compounds in white quinoa flour.

To guarantee the authenticity of the analyzed flour, samples of white quinoa grains were acquired (in commercial establishments in Salvador-Bahia-Brazil), crushed and homogenized in a granulometric sieve (32 mesh). An *in vitro* simulation method of gastrointestinal digestion [2] with some modifications (**Figure 1**) was used to evaluate the bioaccessibility of phenolic compounds.

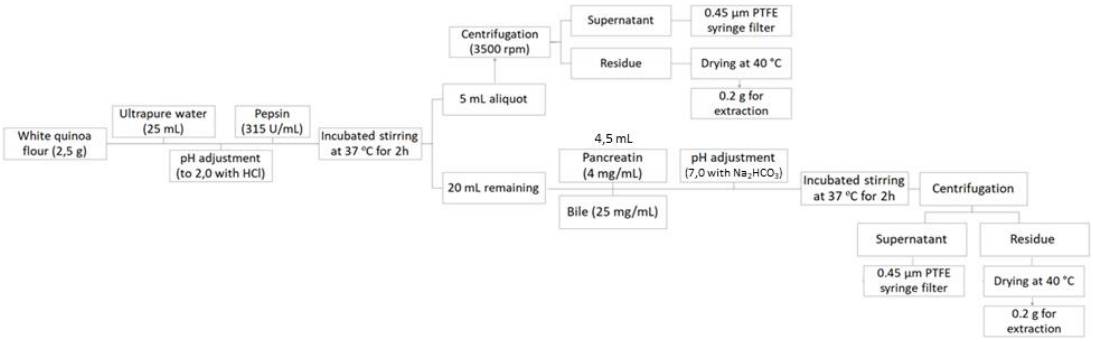

Figure 1: Schematic representation of gastrointestinal digestion simulation.

After the simulation of each digestion stage, the supernatants were separated from the residues, which were dried and subjected to extraction [3]. Blanks, supernatants and methanolic extracts of residues from the gastric and intestinal phases of the digestion simulation were analyzed by UV-Vis spectrophotometry to quantify the total contents of phenolic compounds [4].

To determine the bioaccessible fraction, the ratio between the content of digested phenolics (present in the supernatant aqueous phase) at the end of each digestion step and the total phenolic content, ie digested plus undigested (present in the residue) at the end of the last digestion stage (intestinal phase), was calculated. At the end of the gastric phase, 38.0% of the phenolic compounds became bioaccessible. For the intestinal phase, the bioaccessible fraction of phenolic compounds was 55.2%. According to the results obtained, it is indicated that most of the biaccessible phenolic compounds of white quinoa flour became bioaccessible during and after gastric digestion.

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Evaluation of the potential preservative capacity of pumpkin (*Cucurbita maxima Duchesne*) by-products

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Large amounts of fruits and vegetables are lost or wasted along the food supply chain. This occurs at the harvest level, for not meeting the sales standard, in the failure of transport and logistics, at the household, due to underutilization and discards, and many others.[1] A considerable portion of this waste generation is from the food processing industry, which has different leftovers such as peels, seeds, bagasse, leaves, fibers, and stalk, that are generally not recovered for reuse. Despite little explored and with low commercial value, these bioresidues and by-products have been shown to contain important high value-added compounds[2]. These compounds, such as polyphenols, alkaloids, flavonoids, carotenoids, etc., are originated in the secondary metabolism of the plant, therefore generally presenting bioactive and functional proprieties. The potential of recovery of these compounds and their application in foodstuff as natural additives has been increasingly demonstrated in the literature[3]. The present word proposed to evaluate the by-products obtained in the pumpkin industrial processing as a source of preservative compounds. Pumpkin seeds are consumed as snack in some regions; however, this part of the fruit, as well as the peel and fibers, are poorly utilized, being a cheap and promising matrix to be explored. In this sense, the by-products of pumpkins cultivated in Tunisia were evaluated for their bioactive properties, more specifically, in terms of preservative capacity. For that purpose, the hydroethanolic extracts of the peel and the mix of seeds and fibers from the "Batati", "Karkoubi", and "Bejaoui" varieties were assessed. For the antioxidant activity evaluation, the cell-based method of the inhibition of lipid peroxidation (TBARS) was applied. The antibacterial and antifungal activity was tested against 10 microorganisms of interest in food preservation. Moreover, non-tumor cells of a primary culture of porcine liver (PLP2) were used to assess the cytotoxicity, through the sulforhodamine B (SRB) colorimetric assay. Through this study, all the samples presented great preservative potential, since they protected at least 5 of the 10 tested strains of microorganisms, such as *Aspergillus brasiliensis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella 5 enterocolitica*, and *Yersinia enterocolitica*, in concentrations up to 10 mg/mL, and showed great antioxidant results, reaching values about just 2 times higher than the positive control Trolox. The highest antioxidant activity was presented by the seeds and fibers of "Karkoubi" and "Bejaoui", while for the "Batati" variety, the results were quite similar between the peel and the mix of seeds and fibers. Regarding the antimicrobial activity, the peel presented better results than the seeds and fibers in the antibacterial assay, and the opposite was noticed in the antifungal evaluation. All the mix samples protected against the 2 tested strains of fungi, the peel of "Batati"and of "Karkoubi" inhibited 6 of 8 bacterial strains, and none of the samples presented bactericidal nor fungicidal effect. Furthermore, the safety of food application of the samples was verified by the absence of toxicity in the primary culture of non-tumor porcine liver cells (PLP2), at the maximum concentration tested (400 µg/mL).
These results corroborate the purpose of valuing pumpkin by-products as a source of natural preservative compounds with interest for application in food products, thus promoting the replacement of synthetic additives by a natural alternative obtained from underexplored matrices.

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Dietary polyglycosylated anthocyanins, the smart option? Towards their stability and bioavailability

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Over the years, anthocyanins have emerged as one of the most enthralling groups of natural phenolic compounds. The interest raised around anthocyanins goes way beyond their visually appealing colors and their acknowledged structural and biological properties have fueled intensive research towards their application in different contexts. However, the high susceptibility of monoglycosylated anthocyanins (MGA) to degradation under certain external conditions might compromise their applications and health properties. In that regard, polyglycosylated anthocyanins (PGA) might offer an alternative to overcome this issue, owing to their peculiar structure and consequent less predisposition to degradation [1].

PGA from different food sources (purple sweet potato, red wine, *Dianthus Caryophillus* and *Viola Tricolor* edible flowers) were isolated and structurally characterized. The stability at different pH was evaluated by means of pH jumps. Thermal stability was also evaluated. Furthermore, their stability to the digestion processes was performed in vitro at the oral, gastric and intestinal level. Transepithelial transport assays were performed using gastric and intestinal celular models to evaluate the absorption and role of food matrices. Also, a nano-gene-silencing technology with gold nanoparticles allowed the evaluation of the molecular mechanism of absorption of these anthocyanins. Laser Scanning Confocal Microscopy was used to track PGA in gastric and intestinal cells.

The results suggested a higher stability at a broader range of pH values (with lower hydration and higher acidity constants) when compared to the already published kinetic and equilibrium parameters of MGA, and a higher thermal stability up to 100 celsium degrees and pH 7 (with more than 50% of total PGA detected when compared to MGA).
The digestions studies revealed a higher resistance of acylated PGA at the different levels (with significant differences up to 30% when compared with MGA) and the transport studies revealed a structure-absorption efficiency relation at both gastric and intestinal level in the presence and absence of food matrix, with the more complex structures presenting a lower transport efficiency. Also, specific food matrix components such as proteins and glucose had a tremendous negative effect on the transport efficiency of the anthocyanins.
Furthermore, the involvement of Glucose Transporters 1 and 3 (GLUT1 and GLUT3) on the transport mechanism of PGA was observed, through a significant reduction of the transport efficiency of the anthocyanins up to 50% upon the silencing of the transporters. And upon incubation, PGA seems to be concentrated in specific cell areas, suggesting localized bioactive actions.

These results elucidate new insights on PGA stability and bioavailability and suggest that this subclass on anthocyanins may be more appealing for both nutritional, health and technological applications.

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Microwave Assisted Extraction of *Pinus pinaster* bark under different process conditions

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Pinus pinaster Aiton subsp. *atlantica*, commonly named as maritime pine, is the conifer with the most extended dissemination in the West of the Iberian Peninsula where it covers more than 28% of the whole forest surface[1] and is the third most important forest specie in Portugal.

Pine bark is an abundant waste from the timber industry. Being a source of polyphenolic compounds have received considerable attention in the fields of nutrition, health, and medicine owing to their physiological and biological activities, namely antibacterial, antiviral, anticarcinogenic, anti-inflammatory and cardiovascular system diseases prevention[2,3]. Great interest has been recently focused on the addition of pine bark extracts to food matrices, as a preservative, based on the polyphenols well-known abilities to scavenge free radicals, i. e. antioxidant power.

The aim of this study was the extraction of phenolic compounds from pine bark (*Pinus pinaster* Aiton subsp. *atlantica*) by Microwave Assisted Extraction (MAE) using a SK-12 medium pressure rotor (ETHOS X, Milestone, Italy). Pine bark samples were collected from a certified plantation, in Minho region, and they were first dried at 40°C for 72 hours and ground to a particle size of 200-850 µm. The irradiation occurred at a microwave power of 1600 W and was performed with three different solvents: water, water: ethanol [50:50 (v/v)] and ethanol, in a ratio plant/solvent of 1:20; at three temperatures: 90, 110 and 130°C and two extraction times: 15 and 30 minutes. The extraction yield ($g_{\text{dry extract}}/g_{\text{dry bark}}$), total phenolics, condensed tannins[2], antioxidant activity (DPPH and ABTS assays), pH and colour evaluation were performed on the extracted materials (Table 1).

Higher extraction yields were obtained with hydroethanolic solvent at 130°C followed by ethanolic and aqueous extracts. and no significant differences in extraction percentage were found (time extraction of 15 and 30 minutes, led to 11.1 ± 0.0 and 10.5 ± 0.1 % (w/w), respectively). The pH of the extracts obtained varies depending on the used solvent, i. e. aqueous extracts showed the lower pH values (3.60 to 3.90) and an increase were observed in hydroethanolic and ethanolic extracts (4.08 to 4.41 and 4.21 to 4.30, respectively), however, the extracts continued to have an acid character. The colour of the extracts also varied depending on the used solvent. Values of L, a* and b* were higher in the aqueous extracts. Higher lightness values (L) may be due related to the efficiency of this solvent in the extraction- less extracted pigments and so a light appearance. The hydroethanolic mixture proved to be the most efficient solvent in the total phenolics extraction. Higher values were obtained with T= 110°C; t= 30 min followed by T= 130°C; t= 15 min and no differences were found between both extraction conditions ($p>0.05$). The antioxidant activity (DPPH and ABTS assays) was also higher with hydroethanolic solvent in the condition T= 130°C; t= 15 min. Results from DPPH and ABTS were 276.9 ± 1.1 µmol $_{\text{eq trolox}}/g$ and 546.5 ± 8.6 µmol $_{\text{eq trolox}}/g$, respectively. In relation to condensed tannins, higher concentrations were found in the hydroethanolic extracts at T= 130°C; t= 15 min, and T= 110°C; t= 30 min (39.9 ± 0.5 and 37.1 ± 0.3 mg CE/g extract, respectively).

A Principal Component Analysis (Figure 1) was also performed, graphically showing that ethanolic and hydroethanolic extracts presented the most satisfactory results in all tested parameters.

Temperature/time binomial are a key variable in this process. In this study high temperature increased the extraction efficiency and consequently decreasing the required exposure time, i. e. with the condition T= 130°C and t= 15 min it is possible to obtain pine bark extracts with a high extraction yield, higher concentration of polyphenols and condensed tannins and, also, presenting the higher antioxidant activity. Overall, it can be concluded that MAE extraction with hydroethanolic solvent mixture [50:50 (v/v)] can be chosen as it led to bark extracts with higher polyphenolic content and great antioxidant power.

Table 1: Extraction yield, Total phenolics, Antioxidant activity (DPPH and ABTS) and Condensed tannins of pine bark MAE extracts (average of at least three replicates).

Sample	Extraction yield in dry pine bark (% w/w)	Total phenolic content (mg GAE/g extract)	DPPH (µmol eq trolox/g extract)	DPPH EC_{50} (mg/mL)	ABTS (µmol eq trolox/g extract)	ABTS EC_{50} (mg/mL)	Tannins (mg CE/g extract)
110_15_W	4.8 ± 0.0	26.7 ± 1.0	172.0 ± 1.8	0.07 ± 0.00	194.2 ± 0.1	0.24 ± 0.02	21.3 ± 0.2
110_15_WE	**8.3 ± 0.0^{d}**	**53.2 ± 4.6^{b}**	**257.7 ± 3.0^{c}**	**0.02 ± 0.01^{b}**	**351.9 ± 1.6^{d}**	**0.13 ± 0.00^{d}**	**34.0 ± 0.2^{c}**
110_15_E	7.6 ± 0.1	47.8 ± 3.8	253.3 ± 2.0^{a}	0.02 ± 0.01	369.0 ± 3.3	0.13 ± 0.00	30.9 ± 0.5
110_30_W	5.0 ± 0.1	32.0 ± 5.5	245.4 ± 5.1	0.06 ± 0.00	301.8 ± 1.6	0.23 ± 0.01	21.0 ± 0.8
110_30_WE	**8.9 ± 0.0^{c}**	**59.0 ± 3.2^{a}**	**267.8 ± 2.1^{b}**	**0.01 ± 0.00^{a,c}**	**371.3 ± 3.3^{c}**	**0.11 ± 0.00^{c}**	**37.1 ± 2.3^{b}**
110_30_E	8.0 ± 0.1	52.1 ± 3.1	256.3 ± 4.1	0.03 ± 0.00	357.7 ± 6.7	0.12 ± 0.01	34.0 ± 0.7
90_15_W	4.2 ± 0.1	28.4 ± 5.9	180.3 ± 4.0	0.07 ± 0.00	154.7 ± 16.2	0.24 ± 0.02	6.6 ± 1.2
90_15_WE	7.2 ± 0.0	51.0 ± 4.6	251.1 ± 4.8	0.03 ± 0.00	317.1 ± 1.3	0.15 ± 0.01	14.0 ± 0.3
90_15_E	7.1 ± 0.2	46.4 ± 5.1	237.6 ± 5.1	0.03 ± 0.01	296.9 ± 2.6	0.14 ± 0.00	13.2 ± 0.2
90_30_W	3.5 ± 0.0	22.3 ± 4.2	196.2 ± 4.3	0.08 ± 0.00	199.9 ± 1.9	0.27 ± 0.01	1.4 ± 0.5
90_30_WE	7.3 ± 0.0	49.8 ± 3.6	251.4 ± 3.5	0.03 ± 0.00	330.5 ± 4.9	0.14 ± 0.01	15.8 ± 1.0
90_30_E	6.8 ± 0.1	46.9 ± 3.3	253.4 ± 4.4	0.03 ± 0.01	330.0 ± 3.3	0.13 ± 0.01	16.2 ± 2.7
130_15_W	6.4 ± 0.1	36.0 ± 3.5	192.8 ± 2.6	0.06 ± 0.00	247.6 ± 8.6	0.12 ± 0.01	19.3 ± 2.7
130_15_WE	**11.1 ± 0.0^{a}**	**58.0 ± 3.7^{a,c}**	**276.9 ± 1.1^{a}**	**0.00 ± 0.00^{a}**	**546.5 ± 8.6^{a}**	**0.08 ± 0.01^{a}**	**39.9 ± 0.5^{a}**
130_15_E	8.2 ± 0.1	46.7 ± 3.3	265.0 ± 6.5	0.02 ± 0.01	441.9 ± 14.8	0.11 ± 0.01	27.5 ± 1.6
130_30_W	5.6 ± 0.1	17.3 ± 1.5	169.3 ± 12.9	0.10 ± 0.01	224.5 ± 5.4	0.19 ± 0.02	9.0 ± 0.4
130_30_WE	**10.5 ± 0.0^{b}**	**53.1 ± 2.6^{b,c}**	**269.9 ± 1.1^{b}**	**0.01 ± 0.01^{b,c}**	**490.5 ± 3.0^{b}**	**0.10 ± 0.01^{b}**	**32.1 ± 0.8^{c}**
130_30_E	7.8 ± 0.1	50.1 ± 4.8	257.9 ± 0.1	0.04 ± 0.00	434.9 ± 5.9	0.11 ± 0.00	24.8 ± 0.4

a,b,c Items in the same solvent with different temperature (110 and 130 °C) and different time (15 and 30 min.) with different superscripts are significantly different ($p < 0.05$).

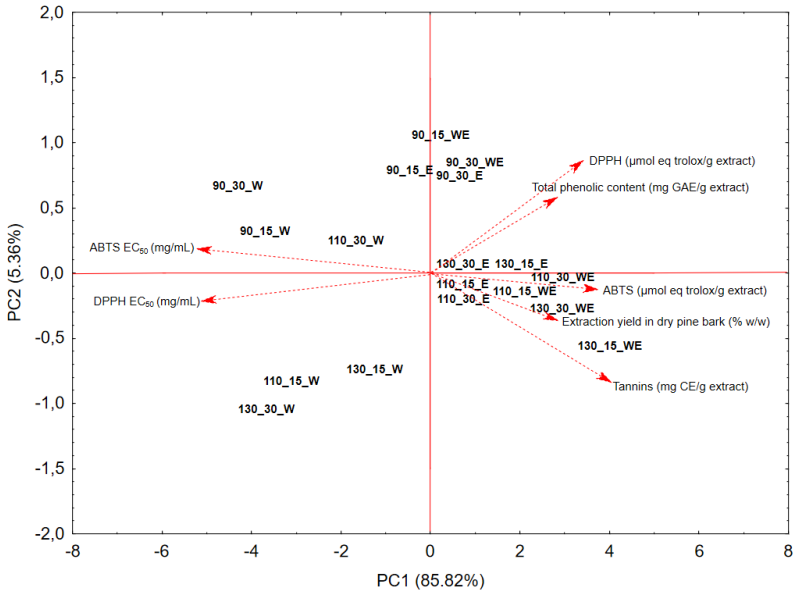

Figure 9: Principal component analysis of physicochemical parameters (PC1 vs PC2 – 85.82% vs 5.36%) for the different extraction conditions: Temperature (ºC)_Time (minutes)_Solvent (W - Water; WE – Water:Ethanol [50:50]; E – Ethanol).

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Screening Methodologies to Extract Polyphenols from Olive Oil Pomace

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Olive oil is a product from the fruits of the plant *Olea europaea* L. About two million ton of olive oil are produced annually, whereas Spain, Italy and Greece are the major producers. Its production worldwide causes 30 million m^3 of olive mill waste each year [1], which consist of olive oil pomace and olive wastewater. Following the goals of the ONU agenda for 2030, under the topic of circular economy that includes "zero waste production", an economy model uses the residues as resources to be valorized [2]. The olive oil pomace contains bioactive compounds, such as the hydroxytyrosol, tyrosol, oleuropein [3], which have shown to present antimicrobial and antioxidant proprieties, with applications in the food industry. This works aims to test and optimize different extraction techniques to obtain bioactive compounds from the olive oil pomace byproduct. Techniques such as maceration in water or hydroalcoholic solutions, with or without enzymes, assisted or not by ultra-turrax, ultrasound or microwave. Preliminary results showed that the use of enzymes in extraction allowed a higher yield of extraction. Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were used to identify the bioactive compounds present in the extract. Relevant compounds with antimicrobial and antioxidant proprieties will be used in packaging as films or coatings for food applications.

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Comparison of theaflavin-3,3'di-O-gallate content, as a 3CLPro SARS-CoV-2 inhibitor in different *Camellia sinensis* tea plantation zones

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The rapid spread of SARS-CoV-2 worldwide has caused countries to impose lockdowns and undertake preventive measures. It is well known that SARS-CoV-2 viral spike protein (S1) binds to the host cell's receptor ACE-2 for initial entry, followed by S2-mediated membrane fusion.[1] The SARS-CoV-2 spike (S1) protein is a heavily glycosylated transmembrane glycoprotein containing 1273 amino acids. Recently, studies have demonstrated that SARS-CoV-2 spike protein has 22 N-linked and 2 O-linked glycan sites. Mostly these N-glycans are composed of oligomannose, hybrid and complex type, and the O-glycans are core 1 derived structures, which may play a significant role in protein folding, receptor binding, and immune escape.[2] On the other hand, the molecular constituents of *Camellia sinensis*, in particular epigallocatechin-3-gallate (EGCG) and, more remarkably, the galloylated theaflavins, mainly theaflavin-3,3'-di-*O*-gallate (TF-3,3'-DG), have been reported to inhibit SARS-CoV-2 3CL-protease, an enzyme required for the cleavage of its polyproteins to produce vital individual functional proteins for viral cell replication. The aim of the present study was to investigate the variability of TF-3,3'-DG content in *C. sinensis* of different Tea Plantation zones for further optimization the horticultural and processing conditions in order to maximize the content of TF-3,3'-DG. The determination of TF-3,3'-DG was achieved by HPLC following the Paiva *et al*.[3] methodology. The tea samples were collected in the following altitudes of tea plantation (Zone 1: 122 m; Zone 2: 136 m; Zone 3: 272 m; Zone 4: 286; Zone 5: 306 m, above the sea level).
The results of TF-3,3'-DG were presented in **Figure 1** and revealed a huge difference, more than 150%, between zone 1 (3.85 mg/g of tea DW) and zone 5 (9.79 mg/g of tea DW), that can be explained by different plantation zones altitudes interfering with soil pH that have impact on the amount of the polyphenol oxidase enzyme affecting the catechin conversion to theaflavins. The zones 1 and 2 have lower altitude presenting lower values of 3.85 and 4.67 mg/g of tea DW, respectively, followed by zone 4 and 3 with values of 6.86 and 8.03 mg/g of tea DW, respectively. The zones 3, 4 and 5 have also different solar exposition than zones 1 and 2. The next step will be the investigation of the processing conditions to maximize the TF 3,3'-DG in zones that presented higher theaflavin content.
Conclusion: This study revealed the possibility to create a novel Azorean antiviral tea, investigating the best tea plantation zones that show higher TF-3,3'-DG content as an inhibitor of $3CL^{pro}$ enzyme and, consequently, reducing the SARS-CoV-2 infectivity as already been reported by several research teams.

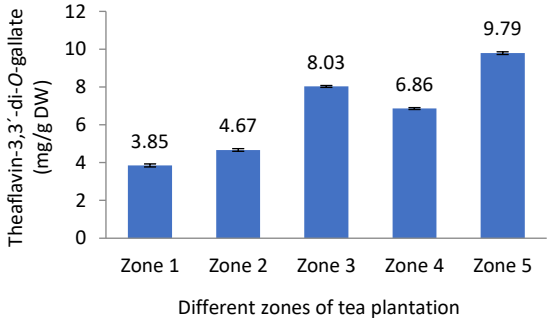

Figure 1: Theaflavin-3,3'-di-*O*-gallate content in different zones of tea plantation.

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The surplus value of bromelain as a potential therapeutical agent for COVID-19 and SARS-CoV-2 infectivity: Bromelain content in different parts of pineapple plant

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Bromelain is a generic name for the mixture of different thiol-endopeptidases, found in the tissues of the *Bromeliaceae* plant family of which pineapple (*Ananas comosus* L.) is the best-known source.[1] Recent research has shown that bromelain presents anti-inflammatory activity, inhibition of platelet aggregation, interference with malignant cell growth, fibrinolytic activity, as well as may reduce the risk of cardiovascular diseases.[2] Recently-based on the research by Sagar *et al*.[3] (**Figure 1**), bromelain can resist SARS-CoV-2 infection by targeting ACE-2 and TMPRSS2, as well as SARS-CoV-2 S-proteins. On the other hand, the global COVID-19 pandemic is an emerging respiratory illness due to severe coronavirus 2 acute respiratory syndrome (SARS-CoV-2) affecting more than 549. 286 million people and causing more than 6.339 million deaths globally (Data from Johns Hopkins Coronavirus Resource Center on April 22, 2022).Taking this into consideration, the objective of this study was to compare the bromelain content in different parts of pineapple plant (fruit, stem, and leaves) using an easy-to-scale up process with ethanol precipitation and size exclusion, followed by cation exchange chromatography.[1]
Results: The **Table 1** shows that stem extracts from fruitless pineapple give better bromelain yield (2.60%) followed by fruitful pineapple that showed a value of 1.75%, and the slip/sucker and leaves the lower values of 1.2% and 0.29%, respectively. Cold ethanol extraction presented better yield as compared to bromelain extraction/purification using size exclusion chromatography followed by cation exchange chromatography (0.68%). The extraction of bromelain from pineapple wastes will be a valuable addition, allowing its use for various applications, and may have impact in the economy of the Azorean pineapple producers.
Conclusion: The results showed that bromelain rich stem extracts, particularly from fruitless and fruitful stem pinneaplle, may be used as an antiviral agent against COVID-19 reducing the SARS-CoV-2 infectivity as already have been reported by several research teams.

Table 1. Bromelain extraction from fruit, stem and leaves of pineapple plant using

cold ethanol precipitation, and size exclusion chromatography combined with cation exchange chromatography[a].

Sample	Bromelain Extration yield (%)	Weight (g/100 Start. Mat.)
Fruitless stem of pineapple (a)	2.60 ± 0.15	3.51 ± 0.17
Fruitful stem of pineapple (a)	1.75 ± 0.18	2.56 ± 0.14
Slip/sucker pineapple fruit (a)	0.03 ± 0.01	0.02 ± 0.01
Leaves (a)	0.29 ± 0.04	0.33 ± 0.05
Fruit (a)	0.81 ± 0.12	1.28 ± 0.10
Fruitfull stem extraction (b)	0.68 ± 0.11	0.99 ± 0.04

[a]Values are mean ± SD (n=3); (a) Precipitation with ethanol; (b) Extraction using size exclusion and cation exchange chromatography.

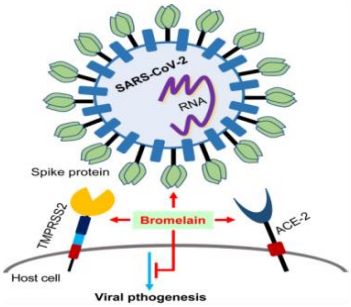

Figure 1: Adapted from Sagar *et al.*[3]

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Optimization extraction of natural antioxidants from Galega kale by-products using response surface methodology

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The observed rising trend in brassicas crop production and consumption is supported by Brassica vegetables' excellent nutritional traits and by its classification as functional foods, as Brassicaceae plants are a rich source of nutrients and antioxidants compounds. Circular economy principles are based on using by-products from one industrial processing as the raw materials in another. This study aimed to obtain extracts with high antioxidant activity for Galega kale (*Brassica oleracea* L. var. *acephala* cv. Galega) by-products from fresh-cut which could be wisely used for other industries. This study aimed to obtain extracts with high antioxidant activity for kale (Brassica oleracea L. var. acephala cv. Galega) by-products of the fresh-cut industry, which can be wisely used in other industries.To achieve a high-quality extract, an optimal extraction process is needed. Central composite design (CCD) was used for the experiment design, which contained two independent variables: the solvent ratio (water: ethanol) and the solvent volume. The evaluation of extracts was done by measuring the total phenolic compounds by the Folin-Ciocalteu method (TPC, mg GAE/100 g FW) and determination of antioxidant activity (AOx, mM ET/100 g FW) by ferric reducing antioxidant power (FRAP) and free radical scavenging (ABTS radical) methods. Process optimization was done using response surface methodology (RSM). Statistic software version 12.0 was used to design experiments and data analysis. Regression analysis showed that the model predicted a value of 95,4 mg GAE/100 g FW for TPC and 1062, 5 mM ET/100 g FW, and 1568,7 mM ET/100 g FW for AOx by ABTS and FRAP methods, respectively. Both independent variables had a similar influence on the extraction process. The optimal conditions that maximizes the antioxidant content were determined using the desirability function (**Figure 1**). The following conditions were obtained: water: ethanol solvent ratio of 75:25 and a solvent volume of 40 mL, with a desirability value of 0.84. The predicted and experimental values for the response variables TPC and AOx were compared. When the optimal extraction parameters were applied, maximum values of 204,4 ± 1,7 mg GAE/100 g FW, 1016,6 ± 19,6 mM ET/100 g FW, and 1085,1 ± 39,7 mM ET/100 g FW were obtained for TPC, ABTS and FRAP methods, respectively. The extraction time (in ultrasound bath) was also evaluated. This parameter did not influence the extract's total phenolic content or the antioxidant activity, as no differences in the respective values were observed over the time tested (30 min, with regular measurements every 5 min). In sum, the optimized extracts have a very high total phenolic content and antioxidant activity, proving the high value of Galega kale by-products, which are considered waste as raw material in obtaining the extracts, but also the efficiency of the optimized extraction process. Furthermore, the Galega kale by-product extract was analysed with HPLC to determine the phenolic profile under the optimised extraction conditions, confirming the prevalence of phenolic acids (caffeic and coumaric).

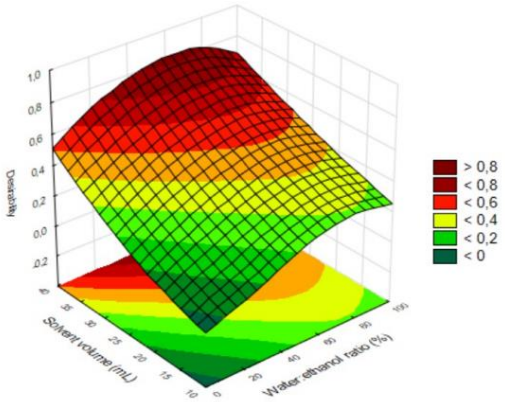

Figure 1: Desirability Function.

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Influence of olive anthracnose and olive fruit fly on bioactive compounds of Cobrançosa olive oils

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Phenolic compounds are the most important bioactive compounds in olives and since 2012 a health claim can be declared for the olive oil if it contains more than 5 mg of hydroxytyrosol (Hyt) and its derivatives per 20 g of oil[1]. In turn, β-carotene is a precursor of vitamin A that essentially functions in many biological processes including vision. However, the presence of bioactive compounds in olive oils depends on cultivar, agro-ecological conditions, harvest time, post-harvest, extraction technology and storage. Pests and fungal diseases of olive fruits, mainly olive fly (*Bactrocera oleae*) and anthracnose (*Colletotrichum* spp.), are among the main constraints that affect both olive production and oil quality. The most relevant fungal disease of the olive tree in Portugal is anthracnose, also known as "Gafa", resulting in a depreciating effect on the quality of olive oil, reflected mainly in increasing free acidity and in the presence of negative sensory attributes[2]. 'Cobrançosa' cultivar is considered moderately susceptible[3] to the disease and Cobrançosa oils are known for their high contents in phenolic compounds, that gives high intensity of bitter and pungent notes, that´s why this oil is usually used in the blends of "premium extra virgin olive oils".

The present study is focused on the effect of olive anthracnose and olive fruit fly on the concentration of bioactive compounds, namely in phenol composition, Hyt and its derivatives (health claim) and β-carotene in olive oils extracted from olives of the cultivar 'Cobrançosa', harvested in October and November in two consecutive years, in Castelo Branco. The olives were extracted in an Abencor laboratory system after harvest, without any storage time. The phenolic profile was evaluated by HPLC-UV and the total Hyt derivatives by HPLC-DAD; total carotenoids were evaluated by VIS spectroscopy.

The results show that the total concentration of Hyt and tyrosol (Tyr) is higher than 5 mg/20g, the minimum value that allows the use of this health claim, for all Cobrançosa olive oils, throughout ripening, in both campaigns and regardless of the incidence of pests and diseases. Although, also dependent on ripening stage, the lower content of β-carotene (1.58 mg/kg) was achieved for fruits with higher severity of fly attack (40%) and anthracnose (12%). From the sensory point of view, a decrease in all positive attributes was observed in Cobrançosa oils, corresponding to a decrease in oleacein and oleocanthal, when biotic stress increased. However, no sensory defects were observed. Both oleacein and oleocanthal are phenolic compounds highly related with the positive attributes of bitterness and pungency in virgin olive oils. Thus, the presence of pests and fungal diseases may compromise the use of Cobrançosa oils in award-winning olive oil blends, mainly due to the decrease in the intensity of these attributes.

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Extraction of bioactive compounds from *Mastocarpus stellatus* using ultrasound and microwave-assisted extraction: a comparative study

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The ocean environment is a rich and largely unexplored reservoir of biodiversity, comprising several species of seaweeds. Seaweeds are commonly classified in three main groups, depending on their main pigments, in green, brown, or red seaweeds. Red seaweeds (Rodophyta) include a wide variety of species, such as *Mastocarpus stellatus* (Stackhouse) Guiry, and are known as beneficial for consumers health due to their content in micronutrients, vitamins, omega-3 fatty acids and other bioactive compounds [1].

With this knowledge, the aim of this work was to optimize the bioactive compounds extraction from *M. stellatus* seaweed. For that purpose, microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE) and a combination of both methods (MAE + UAE) were tested. For each treatment, 1.2 g of homogenized *M. stellatus* seaweed were accurately weighted and 30 mL of 60% (v/v) distilled water-ethanol solution was added. Samples were submitted to MAE (MARS-X, 1500 from CEM), power 600 W, for 20 min at 90 °C, to UAE (Sonics & Materials VCX750), power 750 W, for 20 min at 70 °C, and to sequential MAE-UAE treatment using the previous conditions. The obtained extracts were analyzed (in triplicate) for carotenoids, chlorophylls, proteins and total phenolics (TPC) contents, as well as for antioxidant capacity through the $ABTS^{\bullet+}$ scavenging assay.

The results for the three extraction methods (MAE, UAE, and MAE + UAE) presented some differences. Concerning pigments extraction, the contents of carotenoids ranged between 0.4 µg/mL (UAE) and 0.8 µg/mL (MAE + UAE), while total chlorophylls concentrations ranged between 2.6 µg/mL (UAE) and 4.0 µg/mL (MAE +UAE). Regarding protein extraction, concentration values between 89 µg/mL (UAE) and 157 µg/mL (MAE + UAE) were achieved. About TPC results, values ranged between 154 mg/mL (UAE) and 177 mg/mL (MAE + UAE). Concerning antioxidant capacity ($ABTS^{\bullet+}$ results), values were between 61 µg/mL (UAE) and 86 µg/mL (MAE + UAE).

These results suggest that the combined MAE-UAE treatment is the best extraction technique to recover carotenoids, chlorophylls, proteins, phenolics and antioxidant compounds from the *M. stellatus* seaweed species. Further work should be done to optimize the best MAE-UAE conditions to maximize the extraction of valuable bioactive compounds from *M. stellatus.* The optimum extract will be also characterized regarding the carotenoids and phenolics profiles and *in vitro* bioactivities.

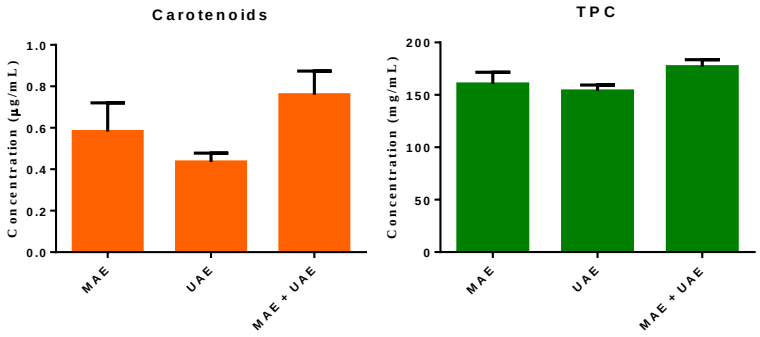

Figure 1: Concentration of carotenoids and TPC in the extracts from the three extraction methods (MAE, UAE and MAE+UAE).

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Chitosan/Alginate coating functionalized with essential oils: A bio-based proposal for meat preservation

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Coupled with the Ethical Meat project, whose objective is the development of an Integrated System for Sustainable Meat Production, based on innovative processes, technologies and information systems to ensure the production of meat products, the importance of formulating a sustainable and safe packaging system arises as a pivotal need.

The main objective of food-oriented packaging is to form a barrier between food products and the environment, minimizing or even preventing exposure to degenerative factors, such as changes resulting from the action of microorganisms, oxygen, temperature and humidity, in order to avoid or delay the loss of quality and nutrition, while preserving the sensorial properties of the products, often resulting in shelf life extension. In this context, preservation systems using biopolymers have emerged for improving packaging functionality and greater safety of packaged meat.[1] In order to develop films and coatings for meat preservation, polysaccharides such as chitosan and alginate have been highlighted. Essential oils and natural extracts have also been tested to enhance films and coatings' water vapor barrier properties or to serve as antimicrobial additives and natural antioxidants.[2,3] In order to select optimized formulations to preserve meat, a Central Composite Rotational Design, added of three replicates at the central point and six axial point essays, was performed including two polysaccharides – chitosan (CH) and alginate (AL) (independent variables) (0.5 - 2% and 0.25 - 0.75%, respectively) – and essential oils (oregano (OO), thyme (TO) and linseed (LO) oil) mixed primarily (0.01- 0.12%) in water and then in a green tea extract solution (2% w/v) (GTE) for the following responses (dependent variables): contact angle (coating vs meat surface) and pH. The final formulations were selected after performing contact angle analysis, in order to ensure that there is a balance between adhesion and absorption by the meat surface (**Figure 1**). In general, the formulations with chitosan presented contact angles with low magnitude, resulting in a high absorption of the coating formulation in the meat. However, when 0.1% LO was added to a 2% CH aqueous solution, the cohesive and adhesion forces were balanced, resulting in a contact angle (22.3^{o}) capable of attaching to the meat surface. Regarding to the AL formulations, it was selected one formulation for each oil, since no microbiological tests have yet been conducted to measure the impact on the meat shelf life (future work). Through contact angle analysis, droplets evaluation and the costs associated, AL-optimized formulations were selected. According to these selection parameters, the AL concentration was fixed at 0.75%, varying the essential oils in solution: 0.1% of TO, 0.03% of OO, and 0.03% of LO. The OO did not have significant effect on droplet spreading, since different oil concentrations with the same biopolymer amount resulted in similar contact angles. This is demonstrated by the contact angles of 28° and 28.7°, which correspond to the formulations with 0.03% and 0.1% OO in 0.5% AL solution, respectively. On the other hand, an increase in the TO concentration resulted in a contact angles increase between the formulation and the meat surface, for equal biopolymer concentrations. To finish, the optimized formulations will be analysed in terms of viscosity, flow and consistency index, storage (*G'*) and loss (*G''*) moduli, antioxidant and antimicrobial capacity, water vapor and oxygen permeability, as well as the analysis of the meat shelf life under different coating/packaging conditions.

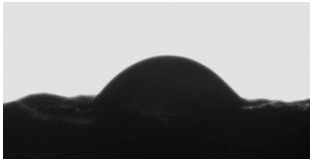

Figure 1: Contact angle of a chitosan formulation drop on meat surface.

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Application of soaking and cooking waters as prebiotics

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Pulses contain oligosaccharides with prebiotic effects, including galacto-oligosaccharides (GOS). GOS are prebiotic compounds composed of a variable number of galactose units, between 2 and 10 (e.g., degree of polymerization, DP = 10), linked via glycosidic bonds, and a terminal glucose (or sucrose) unit.[1] The processing of legumes before consumption by soaking and/or cooking enhances the bioavailability of certain nutrients, improves organoleptic characteristics such as texture or flavour, and reduces undesirable effects after consumption.[2] These treatments usually involve the use of large amounts of water, generating wastes and high levels of environmental pollution. Reusing industrial by-products or discards is an important strategy to improve the sustainability of food production. Lactic acid bacteria (LAB) are a group of probiotic microorganisms significant for the food industry. GOS can help in the growth of LAB.[3] The objective of the present work was to evaluate a possible application for different samples of waste waters obtained through the processing of lentils and chickpeas, on the growth of LAB.

GOS-containing extracts were obtained by either soaking, cooking after soaking, or cooking dry seeds of commercially obtained lentils (*Lens culinaris* Medikus var. variabilis) or chickpeas (*Cicer arietinum L.*) in water. The same seed to water was used in all experiments. Every extract was analysed using High Performance Liquid Chromatography with Refractive Index (HPLC-RI) for the determination of carbohydrates. Their content was estimated by comparison with Vivinal® GOS Syrup (Vivinal) as a standard.

The prebiotic effects of the obtained GOS were evaluated over two strains of LAB *Lactiplantibacillus plantarum* CIDCA 83114 (LP114) and *Lactobacillus delbrueckii subsp bulgaricus* CIDCA 331 (LB331). For this, the extracts were added at 0.3 % to de Man, Rogosa and Sharpe (MRS) culture medium (without glucose as carbon source) and then, bacterium were inoculated in a concentration of 2E+7 CFU/mL (1% v/v). These results were compared to the same experiment using conventional sugars or none.

Chickpeas appear to be a richer source of saccharides compared to lentils, both in terms of the overall number of species present in the extracts, (shown by the larger number of peaks detected in the chromatograms); as well as the larger amount in which each compound seems to be present. Although GOS can be found in higher quantities in the chickpea extracts, lentils provided far less monosaccharides and DP = 2 sugars, which usually require removal. The cooking methods produced extracts with higher GOS' contents than the soaking processes. The results obtained for the microbiological assays for LP114 were similar for all saccharides tested, i.e., the bacteria grew in all conditions. However, this was not the case for the assays carried out with the LB331 strain, in which only glucose and Vivinal were able to promote its growth.

In conclusion, GOS were detected in all waste waters derived from lentil and chickpeas, as well as other saccharides. These samples showed prebiotic potential towards LP114 strain, or at least did not inhibit its growth. This behaviour was not shown towards LB331.This work proved the wastes produced during domestic and industrial treatments of legumes present bioactive compounds with possible applications in the food or pharmaceutical industries, such as prebiotic supplements.

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Assessing green tea catechins' effect on gluten-driven activation of intestinal CD4+ T cells from celiac disease patients

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Latest researches estimate that approximately 1.4% of the world's population has CD. CD is a T-cell mediated enteropathy triggered by ingestion of gluten proteins from wheat, barley, and rye by genetically predisposed individuals carrying the HLA-DQ2/DQ8 haplotype. As with many other autoimmune conditions, CD has emerged as a major public health problem, whose incidence is considerably increasing over time (around 7.5% each year) so that nowadays it has an extensive epidemiological distribution, affecting almost all countries and ethnicities. Currently, the mainstay of treatment for CD depends on a strict life-long adherence to a gluten-free diet (GFD). Nonetheless, doubts still exist as to whether gluten exclusion completely restores the intestinal mucosa of CD patients or whether consequences of the previous strong immune response persist despite adherence to GFD [1]. At this point, a question arises. Could the consumption of specific foods, rather than a GFD, contribute to the so much-needed relief for CD? Compelling observational and interventional evidences are now available on the benefits of consuming plant-based foods and beverages, with current dietary advices for optimal health and longevity, suggesting a high consumption of fruits, vegetables, and minimally processed grains. Among the bioactive constituents of natural matrices that have been under the spotlight, polyphenols are undoubtedly the most promising ones, being actively researched for their health care potential in the prevention of several chronic diseases, such as diabetes, cardiometabolic and neurodegenerative disorders, cancer, low-grade inflammation, and symptoms associated with ageing and menopause [2]. In most cases, however, the biological significance and mechanisms of action of polyphenols on human disease conditions remain largely unknown, especially in CD, for which the immunomodulatory function behind the therapeutical potential of polyphenols has never been explored before [3]. These unknowns, namely of how and by which mechanisms the diet (with its bioactives) interact with the body to affect human heath, are holding back the field of nutrition and considerably delaying the improvement of the life of CD patients.

Herein, the immunomodulatory effect of green tea catechins (GTE) has been assessed, *in vitro*, on gluten specific intestinal T-cell lines generated from biopsy specimens of HLA-DQ2 CD patients through detection of IFN-γ production by ELISA. The specificity of GTE effect on peripheral blood mononuclear cell activation, proliferation and death was additionally evaluated by flow cytometry, in response to the mitogen phytohemagglutinin (PHA). Overall, the results obtained to this point demonstrate the ability of green tea phenolics to reduce, in a dose-dependent manner, gluten capacity to stimulate a T-cell mediated immune responses in CD.

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Phenolic composition and antioxidant properties of Cowpea Portuguese Landraces

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Cowpea (*Vigna unguiculata*) is a robust crop that endures heat, drought and marginal land conditions with the added benefit of improving soil quality through nitrogen fixation. Cowpea grain is an alternative source of protein, but other parts of the plant are also nutritious for humans and livestock. Hence, cowpea fits in the of goal sustainable food security under a scenario of climate change. In addition, cowpea is also relevant in terms of bioactive compounds that may be a source of healthy dietary and functional nutrition. Polyphenols are an important group of bioactive compounds in cowpea and are mostly concentrated in the seed coat affecting its coloration. Extensive use of commercial varieties and disregard for cowpea landraces is causing genetic variability loss, compromising breeding efforts. To contribute to the evaluation of Portuguese cowpea germplasm, 21 landraces (from different national origins and morphology) and a commercial variety (CV) were analyzed in terms of polyphenolic content (Folin–Ciocalteu method followed by high performance liquid chromatography (HPLC, Thermo Scientific Vanquish with diode-array, fluorescence and electrochemical detection), antioxidant activity (oxygen radical absorbance capacity, ORAC) and response towards processing.

Total phenolic content (TPC) of the 22 samples analyzed varied between 50 and 263mg gallic acid equivalents (GAE)/ 100g of grain flour, values about half than those described for white and brown common bean varieties, respectively[1]. The CV corresponding to the common white phenotype, has an average phenolic content of 70 mg / 100g dry grain, which is quite approximate to that of all white samples analyzed. The analyzes of the TPC of several legumes, resulted in a classification according to content[2], low: <100, medium: 100-200 and high: > 200 mg EAG / 100 g. Following this classification, for the 22 samples analyzed, there are 16 samples with low TPC (all white except a grey landrace), two samples with medium content (one brown and other of mixed color grains of white and brown) and 4 samples with high phenolic content (salmon; black; and two red/brown). These results were corroborated by the HPLC analysis.

In terms of antioxidant capacity, values ranged between 23.5 and 168.3 µmol trolox equivalents antioxidant capacity (TEAC)/g of flour. Samples with the highest TPC were also those with highest antioxidant activity. A significant correlation was found ($R=0.961$; $p<0.001$) for both parameters (Figure 1).

Despite the absence of a direct relationship between color intensity and phenolic content, we find a color sequence that reflects phenolic content and hence, antioxidant activity. That sequence follows quite closely the sequence reported by Qui et al.[3] who described decreasing antioxidant activity from red cowpea to black to brown to blue and finally white/cream seeds. The authors suggest that in breeding programs aiming for high antioxidant activity, breeders should select darker colour coated seed. However, further considerations must be accounted for because when considering the soaking process that precedes pulses cooking habits and the cooking process itself we found a significant loss of these compounds to the soaking and cooking water. When analyzed after an overnight soaking period, the white/cream cowpea seeds seem to conserve better their phenols and antioxidant activity, loosing only 15% of its phenolic content to the soaking water and 20% to the cooking water. On the other hand, the landrace with highest amount of phenols, had a much higher loss, losing 48% of its phenolic content for the soaking water and 40% for the cooking water, resulting in a total of about 88% after soaking and cooking. Such high and variable values of phenolic loss into soaking water had already been described for common bean[1] and have to do with cellular

permeability to phenols. We offer an indication that cowpea soaking water from industrial processing is an underexplored and promising byproduct.
Ongoing HPLC and Mass Spectrometry analysis is reveling which specific flavones, phenolic acids and flavonols most contribute to the observed antioxidant activity and will differentiate among national landraces.

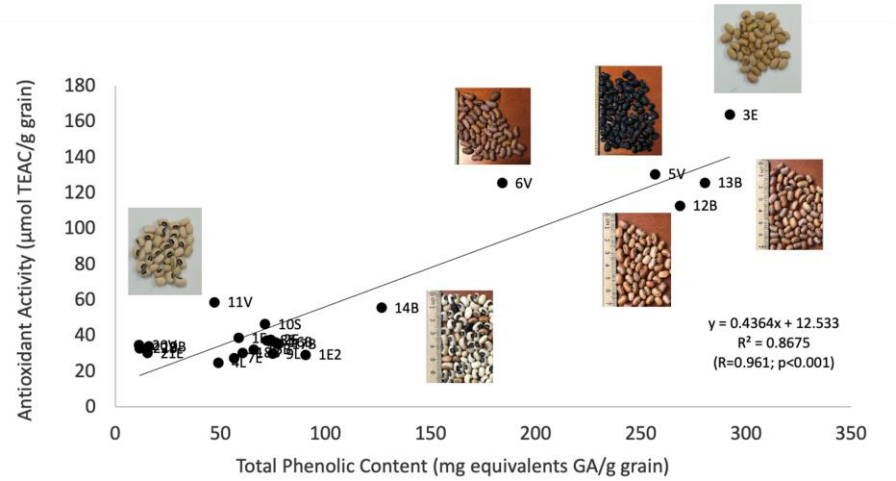

Figure 1: Correlation between total phenolic content (expressed as gallic acid equivalents (GEA)) and antioxidant activity (expressed as trolox equivalents with antioxidant capacity (TEAC)) with representative grain morphology. (Regression coefficient significant with $p < 0.001$)

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Solid lipid nanoparticles produced with beeswax as oral carriers of quercetin

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Quercetin is a flavonoid and an excellent free radical scavenging antioxidant. It can be used as a nutritional compound to maintain the general health or to prevent various diseases, like obesity, diabetes and cardiovascular diseases[1]. However, hydrophobic molecules such as quercetin, cannot be easily incorporated into foods, due to their low solubility, low stability (to heat, light, etc.) and low bioavailability.[1] Therefore, quercetin is easily degraded during food processing and shelf life, which could reduce the commercial value of the final product. In order to overcome these drawbacks, quercetin can be encapsulated within lipid-based nanoparticles such as solid lipid nanoparticles (SLN) and nanoemulsions. SLN are composed of a solid lipid mixture dispersed in an aqueous solution of surfactant. They have been proposed as potential carriers for oral delivery of bioactive compounds possessing low solubility.[2]
The objective of this work was to formulate and characterize quercetin-loaded SLN to enhance quercetin's solubility, stability, bioavailability and preservation of its functional properties.
In this study, beeswax was selected as a solid lipid to produce SLN because it is biodegradable, non-toxic and a low-cost material **(Figure 1)**. SLN was prepared by high-speed homogenization mixing the lipid solution - beeswax, PHOSPHOLIPON® 90G (lecithin) and quercetin - and the aqueous solution containing Tween® 80. The effects of process variables such as lipid, emulsifier or quercetin content and, homogenization time or speed on quercetin-loaded SLN size, polydispersity (PDI), ζ-potential and storage stability were assessed. Additionally, the *in vitro* digestibility of SLN and quercetin bioaccessibility were evaluated according to an *in vitro* harmonized static digestion method[3], consisting on oral, gastric and intestinal phases.
SLN containing quercetin were successfully formulated. SLN formulation produced with 3% beeswax, 2.5% PHOSPHOLIPON® 90G, 2% Tween® 80 and 0.1% quercetin has been chosen based on the dynamic light scattering (DLS) results. SLN mean particle size was 143.43 ± 4.48 nm, polydispersity index (PDI) was 0.251 ± 0.008 and ζ-potential was -13.13 ± 0.84 mV. SLN exhibited good physical stability after 65 days of storage at 4 °C (mean particle size was 147.83 ± 1.98 nm and PDI was 0.271 ± 0.001). *In vitro* digestion results showed a decrease of SLN stability after gastric digestion phase (e.g., particle size increased).
In summary, this work provides an insight on developing effective lipid-based matrices, namely SLN, for encapsulating quercetin for potential food applications.

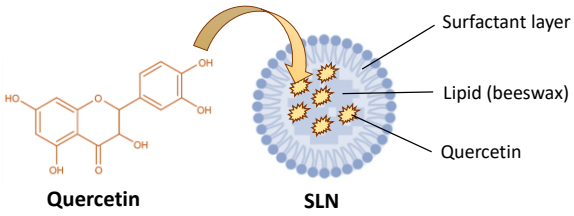

Figure 1: A schematic illustration of solid lipid nanoparticle (SLN) produced with beeswax incorporating quercetin.

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LC-MS profiling and biological evaluation of methanolic extracts from local varieties of *Brassica* species

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The *Brassica rapa* spp. rapa species develops a broad, white root with thin, light green, slightly pubescent leaves. The species is consumed in Asia, Europe and North America for its nutritional benefits with low caloric value due to its low protein and lipid content, and has been used in the preparation of soups and stews. The flavor of root and leaves is slightly bitter and has been related to the degradation of sulfur-containing compounds called glucosinolates (GLS). The isothiocyanates produce a pungent taste and sulfurous aroma when the plant tissue is bruised, playing a significant role in the organoleptic characteristics[1]. In addition to flavor formation, GLS has been reported to be implicated in anti-nutritional and health-promoting effects. GLS content varies depending on species, cultivar, plant part, climatic conditions, agronomic practices, insect attack and microorganism intrusion[2].
Brassica napus subsp. napobrassica (synonymous:B. napus subsp. rapifera) grown as an annual vegetable, most are upright with alternate, often glaucous leaves and long taproots, which is well known by the names Swede and Turnip, amongst others. It is a root vegetable commonly consumed.
In 2019, a collection of 17 accessions of turnip roots (15 accessions of *B. rapa* subsp. *rapa* and 2 of *B. napus* subsp. *napobrassica*), locals varieties, conserved at the Portuguese Germplasm Bank (BPGV) that were collected in north and center regions of Portugal, were grown at same environmental conditions in Braga and assessed for GLS content determination. The objectives were to determine the profile of GLS and to evaluate the biological activity for breeding purposes.
Methanolic extracts of 7 turnip accessions from different geographic origin were analysed by HPLC-HRMS. A typical glucosinolate profile is shown in Figure1 for B. *rapa* accession BPGV03990. Gluconapin, gluconasturtiin and neoglucobrassin are the more abundant GLSs identified in extracts of the 7 turnip tubers accessions. Minority forms of GLS included gluconapoleiferin, glucobrassicanapin, glucoerucin, glucobrassin and 4-hidroxyglucobrassin. The 3 D graph shown in insert (a) illustrated the variation of the peak areas of GLSs as a function of 7 turnip accessions.
The content of total phenolic compounds (TPC), for the methanolic extracts of turnips, ranged between 12.10 and 5.38 mg GAE. g^{-1}, with accession BPGV05875 showing the highest value. The antioxidant power, obtained by the FRAP method, varied between 106.41 and 51.03 µmol. g^{-1} and showed a strong correlation with the TPC method (R^2 = 0.86), with sample BPGV06987 presented the highest value.
The antioxidant activity obtained by the DPPH method, expressed as EC_{50}, showed values ranging from 1.08 to 1.66 $mg.mL^{-1}$, higher than ascorbic acid (EC_{50}=0.04 $mg.mL^{-1}$), with accession BPGV05875 showing the highest antioxidant activity.
The antibacterial activity was determined in the methanolic extracts of turnips against five Gram-negative bacteria, namely *Enterobacter cloacae* (ATCC 49741), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica* subsp (ATCC 13076), and *Yersinia enterocolitica* (ATCC 8610) and three Gram-positive bacteria, namely *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19111), and *Staphylococcus aureus* (ATCC 25923). The extracts revealed a strong activity against *S. enterica* and *S.aureus* (MIC values: 5 – 2.5 $mg.mL^{-1}$) and most of them also revealed the capacity to inhibit *E. coli* and *Y.enterocolitica* (MIC values: 10 – 5).
The results indicate that the methanolic extracts of the 17 accessions of turnip roots with a specific GLS content, are good sources of natural antioxidants and antimicrobial compounds that can provide producers with plant sources with nutritional quality and strong potential for breeding programs.

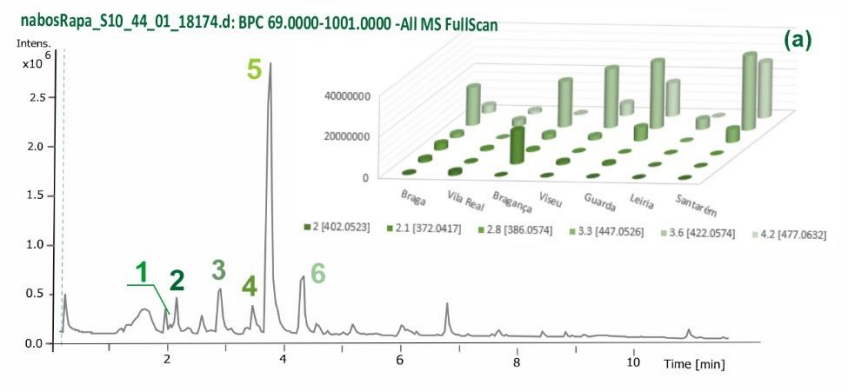

Figure 1: Total ion chromatogram obtained by HRMS in the ESI negative mode of a methanolic extract from turnip material BPGV03990: 1) gluconapoleiferin (t_R 2.0 min; *m/z* 402.0523) ; 2) gluconapin (t_R 2.1 min; *m/z* 307.0475); 3) glucobrassicanapin (t_R 2.8 min; *m/z* 386.0574); 4) glucobrassicin (t_R 3.3 min; *m/z* 447.0526); 5) gluconasturtiin (t_R 3.6 min; *m/z* 422.0574), 6) neoglucobrassicin (t_R 4.2 min; 477.0632). Insert a) 3D graph showing the variation of the peak areas of GLS as a function of 7 turnip accessions.

Key-Words: *Brassica rapa* var. rapa; phenolic compounds; antibacterial activity, antioxidant activity.

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Valorization of citrus by-products through the evaluation of their antioxidant capacity

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Introduction: The genus Citrus *L.* is one of the most popular fruits crops in the world. However, more than half of the fresh fruit mass are peels, seeds, pulps, rejected by food industry and considered waste. Those citrus by-products could represent a huge economic value, since they contain considerable levels of bioactive compounds[1]. In fact, citrus by-products contain more bioactives than the corresponding edible parts and these are associated with health-promoting activities [2].

Aim: This work aims to assess the antioxidant capacity of citrus pomace, a by-product from juicy industry, of three species: orange (*Citrus sinensis* L.), lemon (*Citrus limon* L.) and lime (*Citrus aurantiifolia* L.) and study their potential as antioxidant additives.

Materials and methods: For the characterization of the antioxidant properties of citrus by-products, orange, lemon and lime pomace from Portugal were collected from industry. The extraction process was followed as previously described with some modifications. For each sample, 4 g of the homogenized by-products was extracted with 40 ml ethanol. The process was followed by agitation for 30 min by using a shaker. After centrifugation, the supernatant was collected and filtered using a filter paper. The filtered extract was then concentrated until dryness by using a rotavapor. Lastly, the dry residue was reconstituted by dissolving it in ethanol (3 mg/ml) [3]. The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were determined by Folin–Ciocâlteu reagent assay and aluminum chloride method, respectively. The extracts were also tested for their antioxidant capacity using the β-carotene bleaching assay and the DPPH radical scavenging assay, as previously describe [3].

Results: The lemon by-products extract presented the highest TPC (48.45 ± 0.03 mg gallic acid equivalents (GAE)/g) from the studied extracts. The TPC of lime and orange were 47.29 ± 0.13 mg GAE/g and 41.53 ± 0.06 mg GAE/g, respectively. Regarding the TFC of lemon, lime and orange were 5.55 ± 0.29 mg epicatechin equivalents (ECE)/g, 4.98 ± 0.75 mg ECE/g and 3.68 ± 0.16 mg ECE/g, respectively. Lemon extract showed the highest TFC and orange extract showed the lowest TFC. Concerning the DPPH radical assay, the lemon extract presented the highest antioxidant activity (7.78 ± 0.46 mg trolox equivalents (TE)/ g), followed by the lime extract (4.38 ± 0.29 mg TE/ g) and orange extract (3.04 ± 0.36 mg TE/ g). In β-carotene bleaching assay, the orange extract presented the highest AAC (1878.81 ± 0.02) and the lime extract showed the lowest AAC (1727.31 ± 42.90).

Conclusions: In general, all three citrus by-products presented a good antioxidant capacity. However, the lemon extract was revealed to have the highest potential as an antioxidant extract, with higher TCP, TFC and scavenging activity against DPPH radical. These results support the valorization of this fruits by-products as potential source of natural antioxidants. These dry extracts are rich in antioxidants and could be used as food additives or ingredients to increase the shelf life of foods, developing functional foods and/or active food packaging, with potential health benefits, within the concept of circular economy and sustainability.

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Valorization of by-products: comparison of the antioxidant capacity of pistachio (*Pistacia vera* L.) and peanuts shells (*Arachis hypogaea* L.)

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Introduction: Pistachios (*Pistacia vera* L.) are a popular nut crop due to their flavor and nutritional quality. Peanuts (*Arachis hypogaea* L.) are a leguminous crop, rich in protein, and popular consumed along with nuts. Pistachio and peanuts are high-value foods, consumed worldwide roasted or in processed foods. Consequently, a huge amount of shells are produced every year, most of which are discarded, and thus cause environment problems. However, pistachio and peanuts shells have a high content of bioactive compounds (such as polyphenols, tocopherols, dietary fibers, essential oils, and unsaturated fatty acids) with antioxidant properties and health-promoting effects [1,2]. Within the framework of circular economy, there is a need to focus on valorization of those by-products.

Materials and methods: For the characterization of the antioxidant properties of by-products, one sample of peanuts and 3 samples (A,B,C) of pistachio were collected from supermarket and then the shell was manually separated from the edible part. The extraction process was followed as previously described with some modifications. For each sample, 4 g of the homogenized by-products was extracted with 40 ml ethanol. The process was followed by agitation for 30 min by using a shaker. After centrifugation, the supernatant was collected and filtered using a filter paper. The filtered extract was then concentrated until dryness by using a rotavapor. Lastly, the dry residue was reconstituted by dissolving it in ethanol (2 mg/ml). The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were determined by Folin–Ciocâlteu reagent assay and aluminum chloride method, respectively [3]. The extracts were also tested for their antioxidant capacity using the β-carotene bleaching assay and the DPPH radical scavenging assay, as previously described [3].

Results: The peanut shell extract presented the highest TPC (415.66 ± 0.43 mg gallic acid equivalents (GAE)/g). The TPC of the three pistachio shells was lower and range between 49.84 ± 0.13 to 127.67 ± 0.10 mg GAE/g. Regarding the TFC, peanut shell also shown the highest content (558.66 ± 2.94 mg epicatechin equivalents (ECE)/g). Among the pistachio's shells, the C sample has the higher TPC and TFC. Concerning the DPPH radical assay, the peanut shell extract presented the highest antioxidant activity while the pistachio shell samples presented lowest activity through this assay (B sample: 134.45 ± 1.39 mg trolox equivalents (TE)/ g) and the D sample with the highest activity: 179.28 ± 0.67 mg TE/ g). In β-carotene bleaching assay, the pistachio C shell extract presented the highest antioxidant activity coefficient, AAC, (4060.61 ± 85.71) and the other extracts presented similar AAC.

Conclusions: The peanut shell extract revealed to have the highest antioxidant capacity, although the pistachio shells samples also presented a good antioxidant capacity. The results support the valorization of these shells, with low economic value to date, as promising source of natural antioxidants. These extracts could be used as additives in active food packaging, that could allow a controlled release of the bioactive compounds to the packaged foods to delay the deterioration and consequently to increase food shelf life, within the concept of a circular economy and sustainability.

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Implementation of an innovative methodology, FPSE, for extraction of free polyphenols from food matrices

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Polyphenols present in foods encompass a wide range of secondary metabolites that exhibit various effects on human health, namely in the prevention of metabolic disorders associated with oxidative stress, including cancer, cardiovascular, and neurodegenerative diseases. In recent years, scientific and industrial interest in this group of compounds, supported by the growing and increasing consumer demand for foods that promote health and prevention of certain diseases, namely those etiologically related to oxidative stress, increased exponentially.

The objective of the present work was to validate an emerging analytical methodology, the FPSE, for the extraction of free polyphenols from food samples, followed by ultra-performance liquid chromatography equipped with a PDA detection system (UPLC-PDA) analysis. Several analytical parameters influencing the efficiency of the extractive process were optimized, including extraction time, ultrasound-assisted agitation (US), extraction temperature, nature of the extraction solvent, and back-extraction time and solvent. The optimal extraction conditions were used to validate the methodology, FPSE/UPLC-PDA, obtaining the merit figures of the method. The most suitable FPSE parameters for extraction of selected polyphenols are 10 min US assisted extraction at room temperature and back-extraction with the US for 15 min using methanol as extraction solvent. In general, MeOH was the best solvent, however, it was observed that the effectiveness of the procedure depends on the nature of the polyphenols to be extracted. Satisfactory results were obtained for the different analytical parameters of the method with R2 values greater than 0.9, values for inter- and intra-day precision less than 15%, low detection and quantification limits, and recovery percentages that varied between 55 and 90%. The applicability of the method to food matrices was evaluated using saffron, orange juice and lemon juice as real samples.

As an emerging analytical technique in the food field, FPSE revealed great potential in the extraction of free phenolic compounds. In addition, it proved to be a fast and sensitive analytical approach, using small volumes of sample and extraction solvent, in the order of μL.

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Production of biodegradable edible coatings from algae polysaccharides

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Seaweeds are a source of valuable compounds (polysaccharides, phenolic compounds) with bioactive properties that can be introduced in the Portuguese diet through add-value tasteful products. As such, marine underexploited resources, like seaweeds, might be used under bioeconomy and biorefinery concepts to obtain healthy food while exploiting the oceans potential for economic development. Edible coatings (EC) are thin film structures that cover the surface of food products and act as barriers to physical, chemical, and biological modifications. When applied on a food product surface before frying, these EC may prevent oil absorption and decrease degradation of polyunsaturated fatty acids, while maintaining sensory features of fried products, leading to healthier and tasteful fried products. Additionally, they can be used as vehicles of incorporation of additives, like antimicrobial and antioxidant compounds. EC may be produced from different materials, such as polysaccharides and proteins. In this work, *Codium tomentosum*, a green seaweed widely present in Mediterranean Sea, was used as a source of polysaccharides and bioactive compounds to produce functional EC. In this context, a simultaneous hydrothermal extraction of polysaccharides and bioactive compounds was performed, followed by the characterization of the extract. The extraction process showed a yield of 13% $w/wl_{yophilized\ matter}$. The extract contained 32% $w/w_{dry\ extract}$ of polysaccharides and a total phenolic content of 26.56 ± 3.16 mg gallic acid Equivalents/g. Envisaging its application to produce active EC, the extract capacity to form stand-alone films was firstly studied and the films were characterized. Films were produced by casting and drying a filmogenic solution composed of 2% w/v of dry extract and 35% $w/w_{dry\ extract}$ of glycerol. The obtained stand-alone films presented a dense and non-porous surface. In terms of mechanical properties, they have shown a stress at break, an elongation at a break and a Young modulus of $5.875 \times 10^8\ Pa$, 28.13 ± 4.85 % and $2.050 \times 10^9\ Pa$, respectively. Regarding barrier properties, though a high water vapour transfer was observed (water vapour permeability of $2.595 \times 10^{-10} mol.m/m^2 sPa$), films have shown a good resistance to oil transfer and a quite high barrier to UV radiation. In addition, films presented active properties by showing antioxidant capacity (4.78 ± 0.15 mg Trolox Equivalents/g film by ABTS method; 14.08 ± 0.66 mg Trolox Equivalents/g film by FRAP method). In conclusion, the simultaneous extraction of polysaccharides and phenolic compounds from *Codium tomentosum* produced an extract that was successfully used to develop stand-alone films with good properties. It is envisaged the application of such extract to produce an EC to be applied in food products (e.g. fish fillets and potato) before frying to avoid oil absorption.

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The effect of transition metals on coniferaldehyde oxidation in wine spirits model solutions

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Wood is known to be a complex biological system composed mainly of cellulose, hemicelluloses and lignin. Cellulose is commonly degraded by the thermal treatment of wood during coopering giving rise to HMF and 5-methylfurfural, while hemicellulose can generate pentose by hydrolysis, and posteriorly, furfural and its derivatives. Lignin transformations during ageing process of distillates are among the most important factors that may influence the quality of aged wine spirit (WS). Lignin generates two distinct groups of the phenolic aldehydes, one consisting of syringyl-type compounds (sinapaldehyde (sipde), and syringaldehyde), and the other composed of guaiacyl-type compounds (coniferaldehyde (cofde), and vanillin, vanil).[1] The oxidation of sipde gives rise to syringaldehyde, which is oxidized to syringic acid, similarly, cofde converted to vanillin and, in turn, oxidized to vanillic acid.[1,2] It is widely assumed that the aldehydes phenolics contribute to the aroma and colour of aged wine spirits, which are determinates of consumer choice. However, few is known about the oxidation reactions that occur during the WS ageing and the involvement of phenolic aldehydes in these reactions. This work aimed to evaluate the behaviour of target phenolic aldehydes (cofde and sipde), that are among the major components of WSs, and to understand their oxidation pathway, using an oxidative catalysis approach in synthetic medium (MOS) and considering some variables, such as the presence of transition metals (copper (Cu) and iron (Fe)) and the presence of an oxidizing agent. The transition metals, Cu and Fe, are extracted from wood and are present in the wine distillate as well, may play an important role on oxidation reactions of phenolics.[3]

The combinatory reactions were performed with a model solution (EtOH/H_2O, 75:25 v/v); target phenolic aldehyde (cofde), which was selected according to its relevant behaviour during technological assay[3]; metals (Cu^{2+}, Fe^{3+}); oxidant (H_2O_2) and temperature (40 ± 1 °C). MOS was conducted as follows: five glass tubes containing 2 mg/mL of cofde in each were coded as Control$^-$ (without metals or oxidant); Control* (only oxidant); CM* (Cu^{2+}/Fe^{3+} and oxidant); CF* ($Fe^{3+}$and oxidant) and CC* (Cu^{2+} and oxidant). The reaction vessels were placed in a heating magnetic stirrer for 45 days. Substrate consumption was determined by HPLC-DAD and the chromatic properties during kinetic analysis were examined (7, 14, 30 and 45 days). The kinetic analyses showed that the system with the presence of Cu^{2+}/Fe^{3+} consumes the cofde quickly, i.e. approximately 82% of the initial concentration of cofde was consumed after 7 days (Figure 1A). In contrast, a system containing only Fe^{3+} or the oxidizing agent (H_2O_2) consumed only nearly 18% of the initial cofde. After 45 days, all systems containing the oxidizing agent had completed the conversion of cofde. The oxidation products were identified by ESI-MS, and the most relevant were: ferulic acid, vanillin, ethyl ferulate, and the ferulic acid phenoxyl radical dimer product which is stabilized by a keto-enol tautomerism. In addition, the chromatic characteristics of the samples were determined (in duplicate) using the CIELab/CIELCh method. The results for chromatic characteristics of the samples (Control$^-$, Control*, CM*, CF*, CC*) during kinetic are shown in Figure 1B-D. In general terms, the samples containing oxidant and metals (Control*, CM*, CF*, CC*) showed a continuous evolution of lightness (L*), chroma (C), green-red hue (a*), yellow hue (b*) in relation to the sample Control$^-$ during the MOS. After 30

days, CM* increased significantly the +a* values (red hue) and +b* values (yellow hue). Chroma was significantly lower in the Control^{-}, while the samples (Control*, CM*, CF*, CC*) displayed a gradual increase during kinetics, being significantly greater in CM* than CF* and CC*. In summary, there is a greater evolution of the chromatic characteristics in the sample with metals (Cu^{2+}, Fe^{3+}) and oxidant than samples containing the metals added separately with oxidant. Cofde, their metabolites, and interaction with Cu or Fe, in catalytic percentages (5-10 mol/%), seem to be correlated with increased of the red and yellow hues, which are characteristics of aging process of WS. Therefore, this study provides information on role of these metals in phenolic aldehyde oxidation processes and their contributions to the colour development of aged WS.

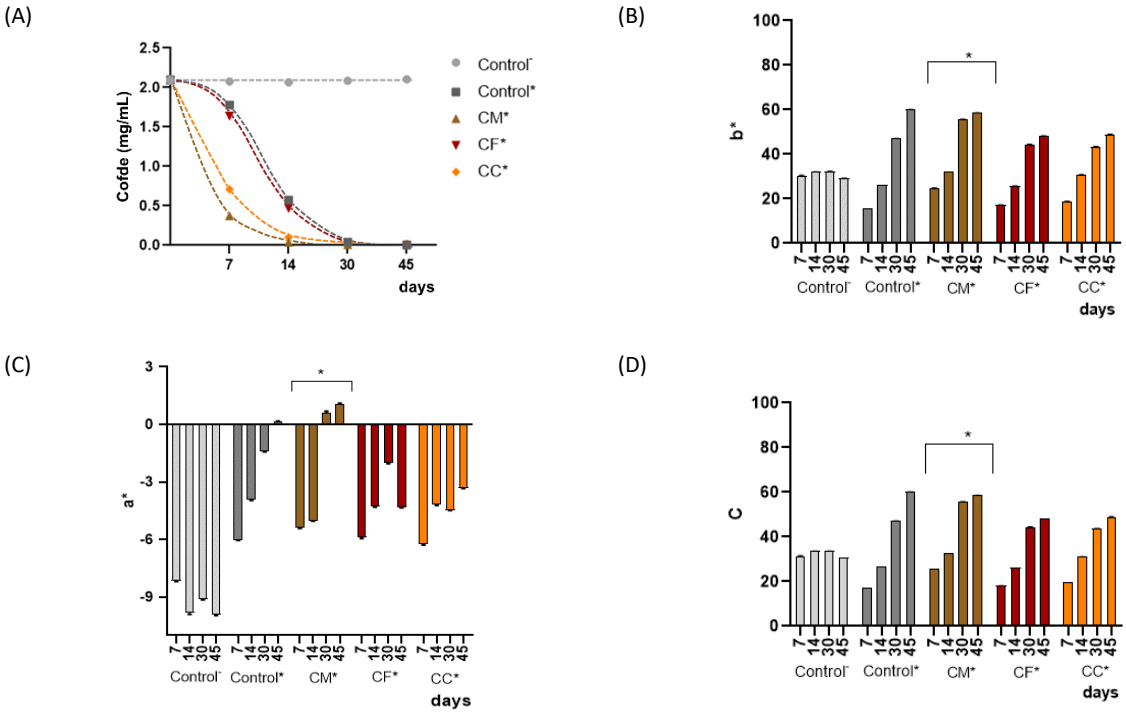

Figure 1: Kinetics curve and chromatic characteristics of samples (Control-, Control*, CM*, CF*, CC*) at 7, 14, 30 and 45 days using a modelling oxidation reaction in synthetic medium (MOS). (A) kinetics curve of consumption of cofde; (B) chromaticity coordinate b*; (C) chromaticity coordinate a*; (D) chroma. *Significance of means comparison (ANOVA, Tukey's test; $p < 0.05$) of samples is shown in each above graphics.

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Valorization of a food industry orange waste as biostimulant plant growth: use of vibrational spectroscopy to early access their chemical composition

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Orange peel is one of the highest wastes obtained from the orange processing industry. These wastes contain a balanced amount of sugar, cellulose, pectin's and hemicellulose, as well as an interesting amount of bioactive compounds. Such composition increases the interest of this product with biological activities in different food and non-food application. Some of the application of the citrus waste include food additive, prebiotic, pectin source, polyphenol, dietary fiber, or essential oil. Nitrogen fertilizers are applied in most of the agricultural crops since soils didn't have enough available N to crop needs. In addition, the manufacture of N fertilizers is high energy consuming, since the Haber-Bosch process used to capture de atmospheric N_2 into NH_3, has an energy foot print of 12.1 kWh kg^{-1} of NH_3-N. So, the recovery of nitrogen from food industry wastes or livestock effluents is now a relevant approach to contribute for the circularity of the nutrients in agriculture, and also to save natural resources. The process used to recover N was by the gas-permeable membrane (GPM) technology. This GPM technology recovered the N of the effluent by its volatilization in the form of NH_3 and recovered it at an acidic H_2SO_4 solution in the form of NH_4. During this process, other volatile organic compounds can also cross the membrane and be recovered in the acidic solution. The presence of low amounts of organic matter has been detected in the acidic solution, and it can have a beneficial effect on crop development. So, the early chemical characterization of this liquid N fertilizer can help to a better understanding about its potential agronomic effect. This work aimed at evaluate the accuracy of the FT-RAMAN spectroscopy to the early characterization of the chemical composition of a liquid N recovered fertilizer. The effluent used in this work to recover N was obtained by the co-digestion of an orange peel effluent from an orange juice food-industry mixed with pig slurry. This N recovered solution (N-rec) was used as the N source to fertilize triticale (×*Triticosecale* Wittmack, var. Misionero), and its effect on the biomass production was compared with the use of a mineral nutritive solution (Hoagland – Hoag) and a control treatment (water – W) with any fertilization.

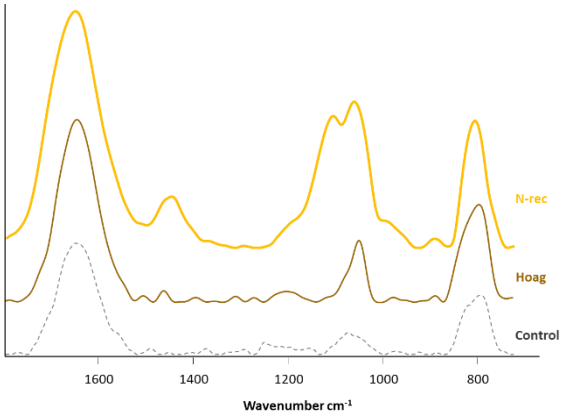

Figure 1: Raman spectra of aqueous fertiliser solution with and without orange waste. (adapted from 2)

The spectral results acquired with FT-RAMAN (Figure 1) showed that the liquid N recovered fertilizer is rich in bioactive compounds namely phenolic compounds, sugars and organic acids, compared with the Hoag or the W treatments. At the end of the experiment the biomass of the triticale increased 29% compared with the Hoag treatment. These results highlighted the agronomic value of this liquid N bio-based fertilizer. In addition, the results highlighted the role of the organic compounds from the N-rec solution as potential biostimulants, which can be early assessed by the FT-RAMAN spectroscopy.

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Comparison of two HPLC methods with derivatization to assess γ-aminobutyric acid (GABA) contents in brown rice flours and rice bran

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Rice is a staple food and an important source of energy for the world's population. Although the highest compound in white rice is starch (~70-90%), brown rice and rice bran contain greater nutritional value and also bioactive compounds that may have positive effects on chronic diseases control. The γ-aminobutyric acid (GABA) is such a compound and it is known to be a neurotransmitter found in the central nervous system with important tranquilizer and a hypotensive effects. Protective actions of GABA on diabetes control is known to control insulin secretion by pancreatic β-cells, through glucagon inhibition. Several animal studies have shown that regular consumption of GABA-rich foods can contribute to the increase of insulin secretion, and a decrease of blood glucose load[1]. GABA thus demonstrates to be a potent bioactive compound, and its quantification is important as it will be useful for the production of healthy rice products and for differentiation of rice varieties with greater GABA concentration. In rice grain, GABA accumulates essentially in the germ and bran layer (10.7 to 350.0 mg/100 g). GABA is also present in brown rice, and its concentration may increase with the germination process of the grain. The amount of GABA may differ, depending on the method used to quantification[3]. Three principal methods have been commonly used by authors: colorimetric, enzymatic and chromatographic methods. The high-performance liquid chromatography (HPLC) with derivatizing methods or by amino acid analyzer have been reported[3]. Within HPLC methods, several derivatization reagents can be adopted, but o-phthaldialdehyde (OPA) is the most widely used, due to the simple sample preparation and short derivatization time. However, the OPA amino acid derivatives are very instable when compared with other reagents.

The aim of this work is to test and compare the efficiency of two HPLC methods with different pre-column derivatization procedures for GABA quantification in brown rice flour and rice bran. In one method, the 2-hydroxynaphthaldehyde (HN) derivatization reagent, a photodiode array detector with a C18 column and a gradient mobile phase of methanol : water were used. In the other method, the OPA derivatization reagent, a fluorescence detector with a C18 column and a gradient mobile phase of 0.1M sodium acetate buffer (pH 7.2) : methanol : tetrahydrofuran were used. For both methods GABA was extracted from bran and brown flour fractions from the Ariete variety (*Japonica* variety) using 70% ethanol, centrifuged 3-times and analyzed after derivatization. The calibration curves were found linear over a concentration range of 0.01-1.0 mg/mL for GABA with a correlation coefficient (R^2) of 0.995 for HN method and 0.01-0.25 mg/mL with R^2 = 0.989 for OPA method. In these conditions, it is verified that the OPA-derivatives are less stable than HN derivatives, but the OPA method showed better sensitivity due to the more sensitive fluorescence detection. The retention time obtained for GABA in HN method was lower than in OPA method. Results showed differences in GABA content between the two methods due to different GABA derivatives procedures. Highest GABA concentration was found in rice bran.

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Phenolic compounds as mycorrhization signaling molecules in micropropagated chestnut (*Castanea sativa* Mill.) seedlings

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MiChestnut3 is a project of the company DEIFIL whose main objective is to produce more resistant and productive hybrid chestnut (*Castanea sativa* Mill.) seedlings through micropropagation and mycorrhization techniques. This extensive work aims at the development of protocols for the micropropagation of different chestnut rootstocks and producing varieties; the isolation and identification of ectomycorrhizal fungi capable of establishing effective symbiotic associations with chestnut rootstocks, which make it possible to overcome or minimize agroecological problems inherent to their planting/propagation; and the establishment of the best conditions for acclimatization in substrate for the mycorrhizal seedlings obtained by micropropagation. In this work, in addition to the agronomic traits of the micropropagated mycorrhizal chestnut seedlings, it was also important to evaluate the changes induced by mycorrhization in the phenolic profile of these plants. Phenolic compounds are plant secondary metabolites involved in plant-microbe interactions/symbiosis and act as signaling molecules in the establishment of arbuscular mycorrhizal symbioses, as well as in plant defense mechanisms.[1] Mycorrhizae are formed in symbiotic interactions between plants and fungi and, therefore, mycorrhizal plants grow better than non-mycorrhizal plants. According to the literature, considerable increases in phenolic compounds in host plants as a result of arbuscular mycorrhizal fungus inoculation have been reported during the progression of the infection.[1] Therefore, this work was caried out to study the impact of the type of fungal inoculum and the period of mycorrhization (before or after potting) on the qualitative and quantitative profile of phenolic compounds in the roots and leaves of the chestnut seedlings produced by DEIFIL. After collection and lyophilization of the plant material, hydroethanolic extracts were prepared and the phenolic compounds were characterized by HPLC-DAD-ESI/MS.[2] Ellagic acid derivatives and *O*-glycosylated flavonoids were the major phenolic compounds in both plant roots and leaves, which agreed with previous reports.[2,3] A statistical analysis showed that the type of inoculum and period of mycorrhization significantly ($p < 0.05$) affected the phenolic profile of the chestnut hybrids. In general, the mycorrhizal seedlings with the fungi *Amanita caesarea* and *Boletus edulis* were those that presented the highest levels of phenolic compounds. Relationships between the levels of these signaling compounds and the agronomic performance of the chestnut seedlings were also found. These results bring new perspectives into the future production of a hybrid chestnut tree resistant to ink disease, mycorrhized and highly productive.

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Evaluation of bioactive coatings in post-harvest physical and mechanical properties of cherries

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In the Beira Interior region, cherry production is one of the most representative crops, revealing a high economic value for the region. The cherry, being a seasonal fruit, has great temporal demand, not only due to its nutritional properties but also highly appreciated for its flavor and texture. However, it is a very perishable fruit, and immediately after harvesting, it must be kept at low temperatures and high relative humidity. In an attempt to avoid this degradation, in recent years, there has been the development of edible coatings/films with bioactive properties to extend the shelf life of foods, namely perishable fruits.[1] On other hand, the increasing concern about pollution and food waste leads to the development of alternatives for this problem. Edible coatings based on biopolymers, such as chitosan, correspond to a perfect profile to solve part of this problem, as they are abundant in nature, are biodegradable, non-toxic, and have good physical and mechanical protection characteristics.[2] Additionally, to contribute to the bioactive properties of coatings, as an antioxidant and antimicrobial, plant extracts such as essential oils have been used as a functional additive.[3]

The objective of this work was to develop and characterize formulations of edible coatings with the incorporation of *Lavandula stoechas subsp. luisieri* essential oil, and its application in cherries, evaluating some physical and mechanical properties during the storage time at two different temperatures. Four formulations were made based on chitosan and other textural ingredients (Figure 1). Formulation FA was composed of chitosan 1.5% (w/w), 0.2% (w/w) glycerol, and 0.5% (w/w) tween 20; FB was composed of 1.5% (w/w) chitosan, 0.2% (w/w) glycerol, 0.5% (w/w) tween 20, and 0.5% (w/w) *L. stoechas* subsp. *luisieri* essential oil; FC was composed of 1.5% (w/w) chitosan, 0.2% (w/w) glycerol, 0.5% (w/w) tween 20, and 4% (w/w) beeswax; FD was composed of 1.5% (w/w) chitosan, 0.2% (w/w) glycerol, 0.5% (w/w) tween 20, 4% (w/w) beeswax, and 0.5% (w/w) *L. stoechas* subsp. *luisieri* essential oil. The water vapor barrier, solubility, thickness, color, surface tension, and elasticity were evaluated in these four formulations. Based on previous properties, we selected two formulations to apply to cherries. After immersion in the formulation solutions, the cherries were placed at room (22°C) and refrigeration (6°C) temperatures, with controlled relative humidity (90%). Weight loss (g), soluble solids content (°Brix), and firmness (N) of each treatment (Control, FA, and FB) were measured at day 0 (fresh fruits, T0), after 4 days (T1), and after 12 days (T2).

The results obtained for the films of formulations indicated that the coatings based on chitosan, FA and FB, showed better color (L*25.09 to 26, a*-1.52 to -0.94, b* 4.47 to 4.63) characteristics, thickness (0.2 to 0.1 mm), surface tension (15 to 48 MPa), elasticity (46 to 57), and water vapor barrier (5.67 E^{-7} to 9.46 E^{-7} g m^{-1} s^{-1} Pa^{-1}). As expected, in coated cherries it was found that the temperature significantly influences the textural properties of the fruit, being the refrigeration temperature the one that preserved the conservation conditions. The coated cherries with FA and FB, at both temperatures, showed better results in weight loss and firmness results, compared to the control (without coating).

Key Words: Cherry, Chitosan, Fruits conservation, Essential oil, *Lavandula stoechas* subsp. *luisieri.*

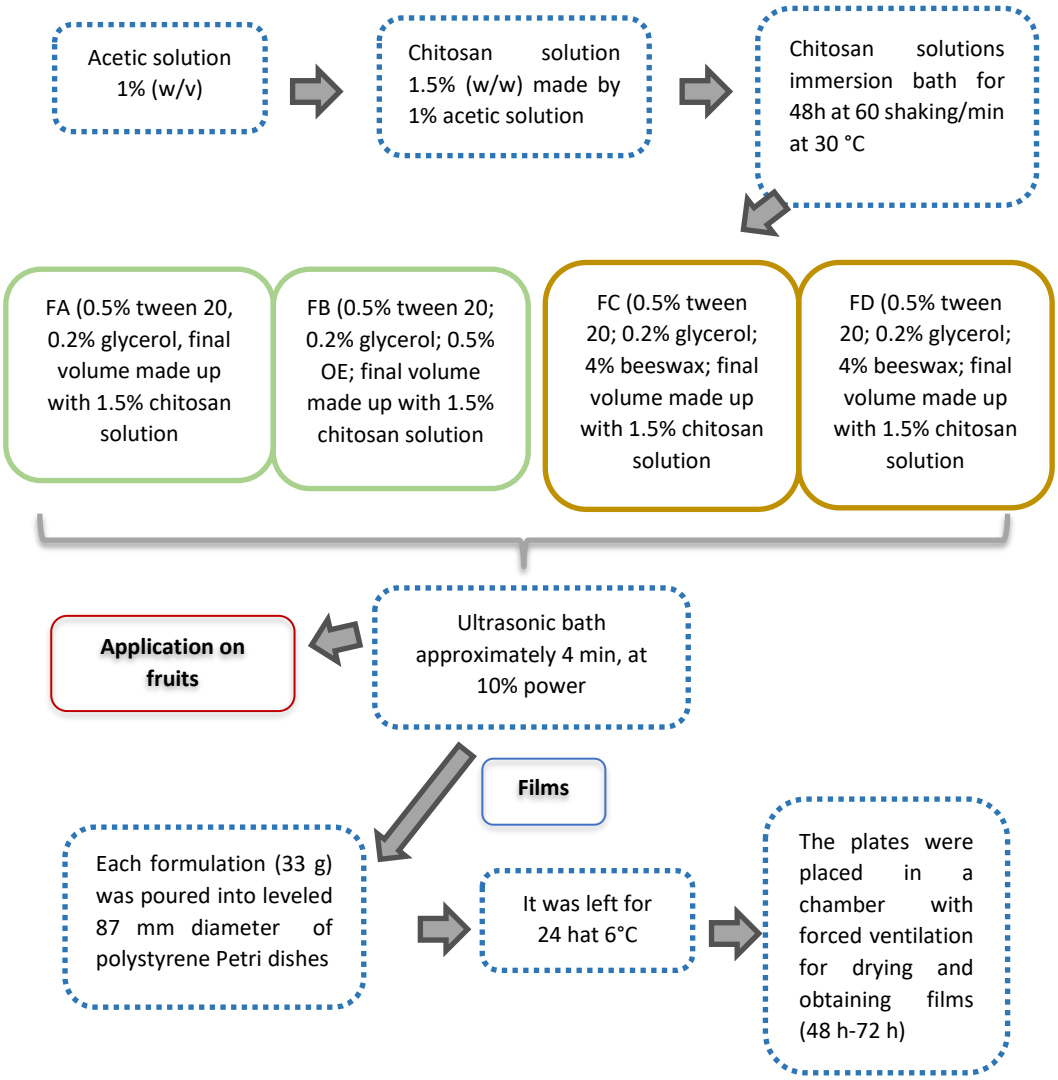

Figure 1: Experimental scheme for the preparation of formulations FA, FB, FC, and FD, and the respective films.

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Influence of the extracting solvent on the antioxidant activity and bioactive compounds of grape stems

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Portugal is one of the most renowned wine producers in the world. Furthermore, there are over 400,000 hectares of vines in the country, with more than 250 grape varieties recognized. It is of common knowledge that winemaking produces a large quantity of by-products and wastes in a short period of time, corresponding to approximately 30% w/w of the beginning grapes, represented by grape pomace, seeds, stems, and wine lees as well as wastewater.[1] Despite being the less valorised residue from grapes derived from the winery industry, grape stems are a source of phenolic compounds, celluloses, hemicelluloses, and lignins.[2]

In this study, the influence of the solvent on the extraction of antioxidant compounds from grape stems was studied. Having in focus the green chemistry principles, only Generally Recognized as Safe (GRAS) solvents were employed. Thus, 100% water, 100% ethanol and a 1:1 hydroethanolic mixture were used to prepare extracts of stems from four different grape varieties (Touriga Nacional, Touriga Franca, Viosinho and Roriz). These were then analysed regarding total phenolics (TPC) and total flavonoids contents (TFC).[3] The antioxidant activity was also determined by *in vitro* assays, namely, ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging activity.

In most of the studied parameters, the dependence of the bioactive compounds recovery on the extraction solvent was clear. The hydroalcoholic solution was the one that presented the best results. Indeed, it allowed a 6 to 9-fold increase in total phenolics extraction compared to 100% ethanol, and a 2-fold raise compared to 100% water. A similar behaviour was also observed for the total flavonoids content. In contrast, ethanol proved to be the least efficient extracting solvent (regarding the type of phenolic compounds present in the grape stems), presenting significant lower values (p<0.05) compared to the other solvents. Moreover, TPC was positively and significantly correlated with FRAP and $DPPH^{\bullet}$ scavenging activity for all the types of extracts.

Concerning grape stems, the information obtained based on these GRAS extractions encourages their use as potential ingredients for new functional foods. Additionally, valuable technological applications need to be developed based on the current knowledge.

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Coffee by-products as sources of bioactive compounds: a comparative study

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Coffee cherry comprises various anatomical parts, namely skin, pulp, mucilage, parchment, silverskin, and beans. [1,2] Wet or dry methods can be used to obtain green coffee beans from coffee cherries, resulting in the generation of chemically and anatomically different by-products.[1] In the dry method, commonly used in *Coffea canephora* producing countries, the cherry is sun-dried and then mechanically dehusked. From this process, coffee husks are obtained, which are composed of dried skin, pulp, mucilage, parchment, and parts of silverskin. In the wet method, the cherry is depulped, being coffee pulp (skin and pulp) separated, the parchment coffee beans are fermented to remove the mucilage, and then dried to remove the parchment. This method is frequently employed to process *Coffea arabica*.[2] Coffee husks and pulp account for approximately 45 and 39 % of the coffee cherry, respectively, and are generally discarded, constituting a source of environmental contamination due to the presence of anti-nutritional substances such as tannins, caffeine, and polyphenols (chlorogenic acids). [1,2] These by-products must be exploited and used in the creation of new products in order to increase the sustainability of the coffee chain.

Thus, the aim of this study was to compare the chlorogenic acid profile and caffeine content of two coffee by-products: coffee husks (*Coffea canephora*, from Panama) and coffee pulp (*Coffea arabica*, from Colombia). Dried samples were kindly provided by Colombian and Panamanian producers through a national coffee importer and roaster company (JMV-José Maria Vieira, SA).

For this, 0.500 g of sample were extracted with 25 ml of ethanol: water (1:1) by shaking in a multi-rotator for 60 min. After centrifugation, the residue was re-extracted with 10 ml of ethanol: water (1:1) for 30 min. After new centrifugation, the supernatants were mixed. Caffeine and chlorogenic acids (caffeoylquinic acids [CQA] and feruloylquinic acids [FQA]) were analyzed by RP-HPLC-DAD and monitored at 274 and 320 nm, respectively. [3]

The results showed that 5-CQA was the most abundant chlorogenic acid in both samples. The coffee pulp (2.54 mg/g dry weight [dw]) contained significantly higher amounts of 5-CQA compared to the coffee husk (0.80 mg/g dw). FQA were also found in these coffee by-products although at lower levels, ranging between 0.15 and 0.02 mg/g for 5-FQA and 4-FQA, respectively. In terms of caffeine content, coffee pulp (12.28 mg/g dw) had a higher content than coffee husk (6.48 mg/g dw). These differences can be due not only to the coffee species and variety, but specially to the place of planting, fruit maturity, and type of post-harvest processing, among other factors.[1] It should be noted that chlorogenic acids and caffeine have been associated to antioxidant and anti-obesity activities, as well as a beneficial effect on plasma total cholesterol and triglyceride levels.[2]

In conclusion, coffee by-products, particularly coffee pulp, can be considered potential sources of chlorogenic acids and caffeine. The extraction of these bioactive compounds, as well as their use in the dermocosmetic industry or in the development of functional foods, should be the focus of future studies. In this way, a treated residue with a lower environmental impact can be generated and reused in other fields, producing new sources of income, and adding value to the coffee industry.

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By-products from seaweed production: protein content and amino acids profile

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Porphyra spp. is one of the most valuable seaweed species in the world. Its life cycle involves two distinct phases: the microscopic, filamentous sporophyte called the conchocelis stage, and the gametophyte that corresponds to the macroscopic leafy blades phase[1]. In Portugal, the company Algaplus produces *Porphyra spp*. at a commercial scale during the whole year, overcoming seasonality matters. Prior being transferred to outdoor tanks, the conchocelis are grown under controlled conditions in the nursery. The process of transferring the conchocelis from the nursery flasks to the external tanks generates a by-product that is discarded. Thus, this work aimed at characterizing this by-product regarding its protein fraction, following a freeze-drying step.

Protein-, non-protein and total nitrogen were determined by a modified macro-Kjeldahl method[2]. The amino acids (AA) profile was determined by RP-HPLC-FLD analysis[3], following an hydrolysis step performed with 6 M HCl at 110 ˚C, for 24 h, or 4 M KOH at 110 ˚C, for 4 h (for tryptophan). The hydrolysates were submitted to an automatic pre-column online derivatization with OPA/3-MPA and FMOC prior HPLC injection. The amino acids were identified by comparing their retention time with those of standards. Quantification was performed using norvaline as internal standard.

The by-product presented about 5% dry weight (dw) of protein nitrogen (~25 g protein/100 g dw, by using the conversion Nx5 factor for seaweed protein determination). Total AA content was 29.7±0.3 g/100 g dw, from which 11.29±0.1 g/100 g dw correspond to essential AA and 18.5±0.2 g/100 g dw to non-essential AA. All essential AA were identified in the hydrolysate. The most representative essential and non-essential AA were Leu>Lys>Val and Glu>Asp>Ala, respectively. Branched chain AA represented about 46% of essential AA (20% Leu, 16% Val and 10% Ile).

This by-product was herein characterized by the first time regarding its protein and amino acids profile. Based on the results, it is possible to recognise it as a potential novel source of protein and/or protein-derived bioactive compounds, especially branched chain AA, to be used in the formulation of new food products. It is important to remind that this biomass is currently discarded and has no commercial value. Therefore, more studies are being conducted to thoroughly characterise this potential co-product, bearing in mind the perspective and principles of sustainability and circular economy.

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Development of highly effective growth strategies aiming at improving *Dunaliella salina* biomass production for food applications

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Dunaliella salina is one of the most promising saline microalgae with huge industrial potential, namely in the food, cosmetics and pharmaceutical industries. This great interest is mainly due to its ability to generate high levels of β-carotene. The β-carotene in *D. salina* can reach 14% of dry weight, making these microalgae one of the main sources for the global market, being this pigment widely used, for example, in the cosmetic industry as protection against UV radiation and oxidative stress and in the food industry as a food colorant or source of pro-vitamin A. The traditional source of carotenoids for industrial applications is chemical synthesis. In the case of β-carotene, chemically synthesized compounds represent approximately 90% of the products in the market. However, the microalgal pigments present several advantages when compared with those from the traditional sources, revealing, for instance, higher bioaccessibility. The aim of this study was to evaluate the impact of 4 cultivation variables (salinity, airflow, and nitrogen and phosphorus medium's concentration) on the microalgae growth rate through the application of a central composite rotatable design (CCRD) – through which 28 different cultivation conditions were tested. Based on the results obtained, the salinity and phosphorus concentration of the culture medium were found to have a positive effect on the *D. salina* biomass productivity, which varied between 0.019 and 0.109 $g.L^{-1}.d^{-1}$. Regarding the maximum biomass production achieved, 1.75 $g.L^{-1}$, it was observed using a salinity and a phosphorus content of 35g/L and 0.15 mM respectively. The conditions tested promoted significant variations in the biochemical composition of the microalgae cells, with differences in the content of proteins, carbohydrates, and lipids between 8.5-23.5%, 24.7-72.3%, and 5.7-16.7% respectively.

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Comparison of roasted coffee and coffee silverskin extracts: effects on a malignant pancreatic cell line (AsPC-1)

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In 2020, the average number of newly diagnosed cancer cases exceeded 19 million worldwide, with about 10 million cancer deaths.[1] Meanwhile, an increased interest in natural resources emerged from epidemiological research showing a link between a high polyphenol consumption and a lower risk of cancer.[2] In this regard, roasted coffee, and coffee by-products such as coffee silverskin arise as natural source of several phenolics and bioactive compounds. Additionally, recycling food wastes into health-promoting products is of great importance, and it has a great socioeconomic and environmental impact. The aim of this work was to investigate and compare the antitumoral properties of roasted coffee and coffee silverskin (the main by-product of coffee roasting) on a malignant pancreatic cell line.

Green coffee beans (*Coffea canephora*) from Cameroon were subjected to a controlled roast procedure (210ºC; ~10 min). Coffee silverskin, which detaches from the beans at this stage, was carefully collected after the thermal procedure. The samples were stored at room temperature in a dry place and protected from light until extracts preparation. Both roasted coffee beans and silverskin were ground and an aqueous extraction was performed using a SONOPULS ultrasonic homogenizer HD4050 (BANDELIN electronic GmbH & Co. KG, Heinrichstrasse, Germany) using the following conditions: extraction time of 10 min; resonant frequency of 20 ± 0.5 kHz, and constant amplitude of 50% of the system's capacity. The extracts were filtered, freeze-dried, and the powder used for the cellular assays.

A human pancreatic cell line with high metastatic rate (AsPC-1) was used to evaluate the antitumoral effects of the samples. The AsPC-1 cells were exposed to both extracts (prepared at 1 mg/mL) or to their vehicle for 24 h. After that, cell viability was determined by measuring cellular leakage of lactate dehydrogenase (LDH) to the extracellular culture medium[3]. Cell proliferation rates were determined by quantification of ^{3}H-thymidine incorporation[3]. Culture growth was determined with the sulforhodamine B (SRB) assay, which reports on intracellular protein content[3]. Cell cycle was analyzed by flow cytometry. VEGF-A (angiogenesis) and MDA (malondialdehyde - oxidative stress) levels were quantified using commercial kits.

Both extracts showed no effect on cell viability (no cytotoxicity). Moreover, culture growth (SRB) was not disturbed by any of the extracts, but the coffee extract showed a significant reduction in VEGF-A (angiogenesis) levels in AsPC-1 cells, showing a dissonant effect to that of silverskin extracts. Interestingly, this cell line had its proliferation rate significantly reduced by both extracts (silverskin: to 52% of the control; roasted coffee: to 38% of the control). Also, the roasted coffee extract reduced the MDA level in AsPC-1 cells, showing again a different effect compared to silverskin. In addition, silverskin provided a significant increase in the number of cells occupying the G2/M phase, while roasted coffee was able to generate a significant increase in the number of cells occupying the G1 phase and a decrease in the S and G2/M phases, showing significant differences compared to silverskin in all phases.

Overall, these findings suggest that the effects of silverskin and roasted coffee extracts (1 mg/mL) on neoplastic cells exist but differ. Indeed, both inhibited proliferation of AsPC-1 cells, but roasted coffee also demonstrated antioxidant capacity in AsPC-1 cells. Significantly different from silverskin, the coffee extract also induced an anti-angiogenic action in AsPC-1. Furthermore, both extracts altered the cell cycle distribution, with different impacts at all stages.

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Bacterial biofertilization improves production and modifies organic chemical composition of blueberry

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Blueberry production has strategic importance in Portuguese agriculture due to its possibility of spreading to inland territories and its economic importance, devoting a large part to export. Thus, the production requires new solutions to obtain a more sustainable and nutritious final product where biofertilization using plant growth promoting bacteria (PGP) can be an important solution, as has been seen in other red fruits [1,2]. In the present work, the qualitative and quantitative effect of four bacteria-based biofertilization treatments was analyzed, one using native bacteria (*Paenibacillus* sp. VMFR46), non-native bacteria (*Rhizobium* sp. PEPV16), lactic acid bacteria (*Lactiplantibacillus plantarum* QSE79) and a combination of these last two. The trial carried out in Covilhã showed that all treatments were capable of improving production by up to 60% (VMFR46), and this treatment also showed an increase in sweetness (total soluble solids-TSS) from 11.75 to 14.55 (Brix degree). The study of sourness (titratable acidity-TA) and their maturity index (TSS/TA ratio) showed an improvement in all treatments of between 17% and 44%, without detecting differences in the firmness of the fruits. In this way, we decided to evaluate the physicochemical characteristics, the phenolic profile by HPLC-DAD-ESI/MSn and the volatile compounds by SPME/GC-MS of the yielded fruits. A total of 31 phenolic compounds were identified showing that Delphinidin 3-*O*-galactoside (between 5000 and 1500 µg/g of dw) was the most abundant compound, while treatments with *Rhizobium* sp. PEPV16 alone or in combination increased the concentration of the non-coloured fraction of phenolic compounds, while all treatments showed an increase in the concentration of the coloured fraction of phenolic compounds. In turn, in the control treatment, the presence of 5-Feruloylquinic acid, Myricetin 3-O-glucoside, Myricetin 3-O-pentose only present in the fruits from the control, while Quercetin 3-O-rutinoside, Myricetin aglycone was detected. and Quercetin derivative appear in the biofertilized treatments. In turn, an improvement in the antioxidant capacity of the fruits against DPPH, NO and SO radicals was detected. Regarding the volatile composition, a total of 39 volatiles we detected, qualitative and quantitative differences were observed between control and biofertilized treatments. Among compounds, 3-methyl-butanal, hexanal, 4-methyl-octane, decane, limonene, trans-*β*-ocimene, linalool, *α*-terpineol were the main ones. These data reveal that biofertilization employing PGP bacteria is an effective tool to improve blueberry production at a quantitative level by improving the yielded fruits together with a qualitative improvement through the increase of sweetness, maturity index, and volatile and phenolic compounds concentration with influence on its antioxidant capacity, obtaining more production, healthier and more attractive to the consumer.

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Development of bold naturally colored ice creams using organic Spirulina

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Abstract

Ice cream flavors compatible with organic ingredients as well as unusual colors are a market differential. At the same time, the full use of renewable sources is a global concern linked to sustainable pathways. This has also been occurring under increasing demand to substitute synthetic food dyes, sometimes related to carcinogenicity, hyperactivity, and hypersensitivity. This work aimed to produce natural blue and green colored ice creams applying C-phycocyanin (C-PC) and *Spirulina* residual biomass (RB) after C-PC extraction, respectively. The ice creams were sweetened using maltodextrin (M) or sucrose (S) to compare the color stability alongside 182 days. C-PC and the RB from *Spirulina* could be applied as a stable blue and green dye in ice creams. RB with sucrose formulation is the more suitable for scaling up due to its price and sustainability.

Introduction: *Spirulina* (*Arthrospira platensis*) biomass contains 57.5% proteins, 23.9% carbohydrates, and 7,72% fatty acids[1] and C-PC, the major compound with antioxidant activity and a brilliant blue color, followed by allophycocyanin and phycoerythrin. After the C-PC's extraction, the naturally green colored RB remains nutritionally attractive and can enrich food products instead of being discarded. "*Spirulina* extract" is recognized as a safe ingredient, regulated for use as a colorant permanently listed for food use[2]. Also, the color is closely linked to sensorial acceptance of food products which motivated the study of applying these natural dyes in an ice cream.

Objective: Producing four bold formulations of natural blue and green colored ice creams applying C-PC or RB. The basis was fermented milk varying the sweetener between maltodextrin (M-CPC; MRB) or sucrose (SC-PC; SRB) to compare the color stability alongside 182 days. The controls were MC and SC.

Material and Methods were compiled in **figure 1**. Analytical methods for C-PC concentration, purity and yield were evaluated according to previous works[3] and colorimetric analysis were performed by using a Colorimeter (Minolta, model CM25D, Japan). The values of L*, a*, and b* were determined The Hue angle (h, Equation 1) indicates the color angle (0° - red, 90° - yellow, 180° - green, 270° - blue, and 360° - black). L* indicates brightness (0 – 100b), a* indicates red (positive values) or green (negative values), and b* indicates yellow (positive values) or blue (negative values). The color difference (ΔE, Equations 2 - 5) was calculated from the initial (0) and final (t) values for SC, SC-PC, SRB, MC, MC-PC, and MRB.

$$h\,(°) = 180 + \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (1) \qquad \text{when } (-a^*, +b^*) \text{ or } (-a^*, -b^*)$$

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (2)$$

$$\Delta L^* = L_0^* - L_t^* \quad (3)$$

$$\Delta a^* = a_0^* - a_t^* \quad (4)$$

$$\Delta b^* = b_0^* - b_t^* \quad (5)$$

Results and Discussion: For all the samples (**Figure 2 a to f**), the L* a* b* and Hue angle values varied little over the 182 d, even with different sweeteners. The luminosity values were the most drastically changed until 21 d for SC, MC, until 42 d for SC-PC, and MC-PC and until 49 d for SRB and MRB. After the maturation period, the color was stable for both sweeteners. As sucrose is more cost effective, it is the best option for producing naturally colored ice creams. The challenge of C-PC's color stability was addressed, probably due to the low temperatures of storage and lack of high temperature in the production process. C-PC maintained color stability, and did not influence the starter culture,

improved the texture, and decreased syneresis. The sensory evaluation demonstrated that 4% C-PC added yogurt had the best overall acceptability.

Conclusion: After the maturation period, the ice creams' colors were stable for both C-PC and RB. The present work overcame the challenge of keeping C-PC and RB as stable food dye for six months in a complex matrix. RB with sucrose formulation is the more suitable for scaling up due to its price, sustainability and human health. The results are significant starting points for new products containing natural pigments with functional appeal. Further research should evaluate sensorial acceptance and antioxidant activity.

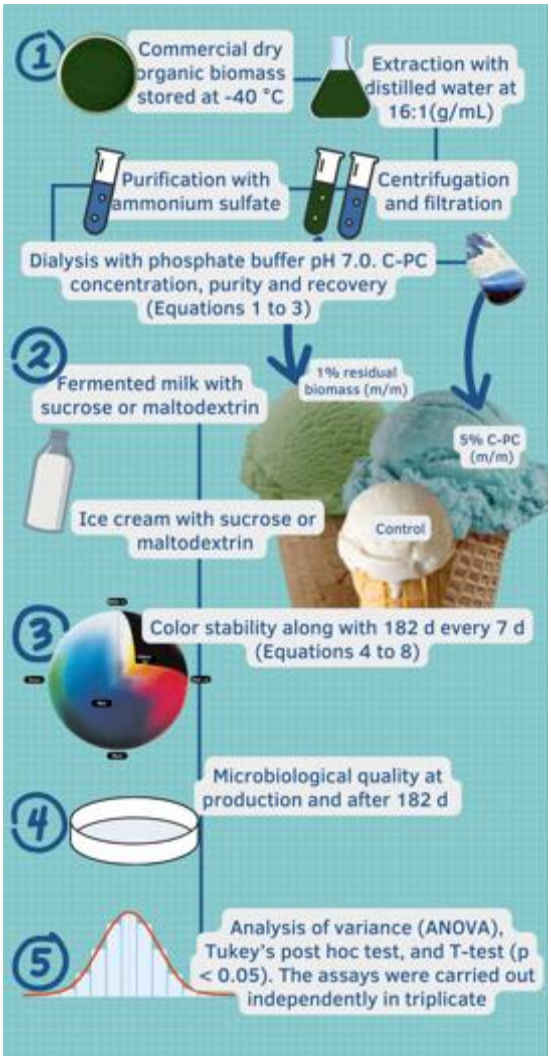

Figure 1: Material and methods

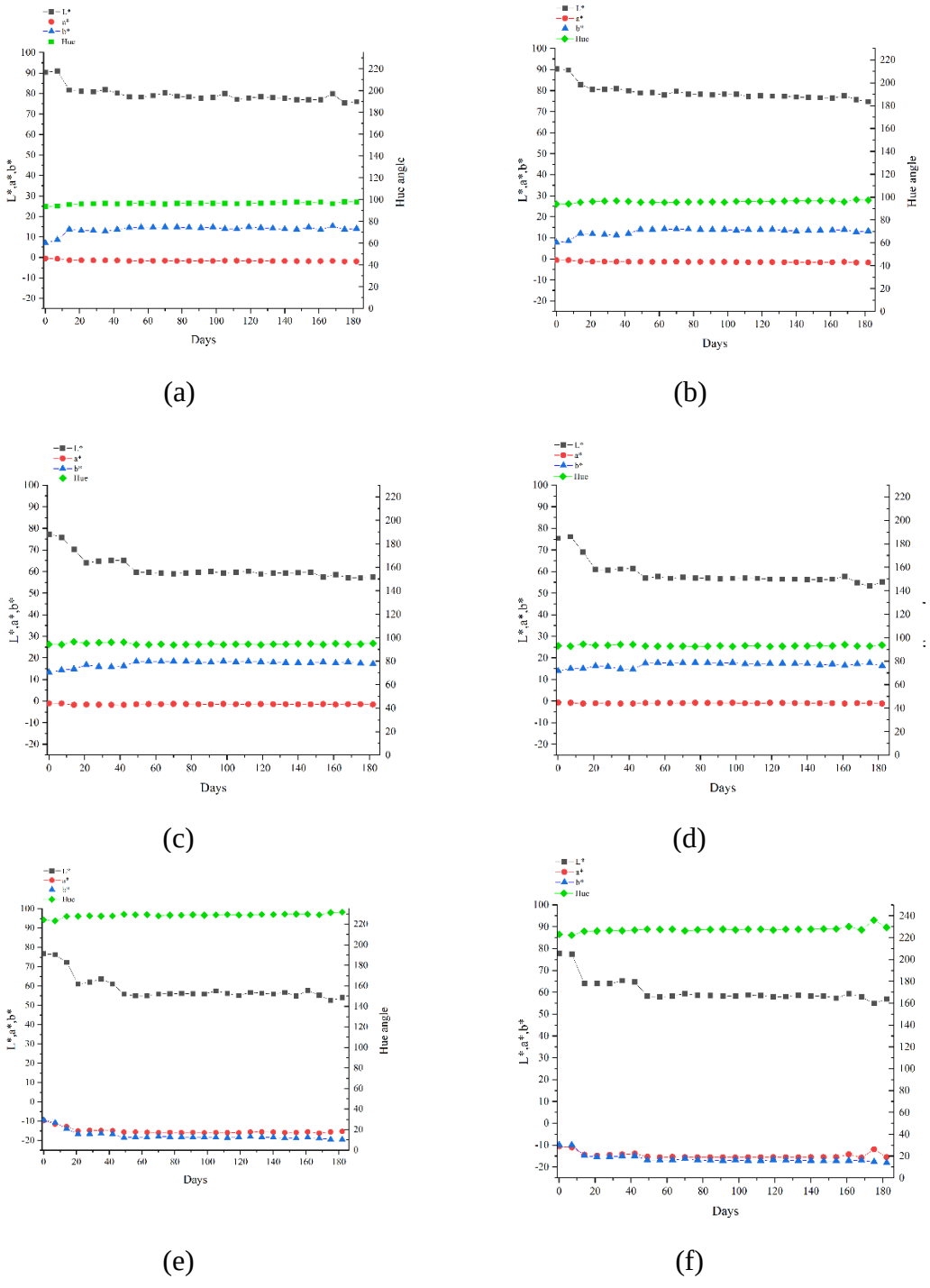

Figure 2. Color variation of the formulated ice creams over the time: a - sucrose control (SC); b - maltodextrin control (MC); c - sucrose with residual biomass (SRB); d - maltodextrin with residual biomass (MRB); e - sucrose with C-PC (SC-PC); f - maltodextrin with C-PC (MC-PC).

Keywords: Circular economy, bioactive compounds, natural food dye, colorimetric analysis.

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Conflicts of interest

The authors declare that they have no financial interests or personal relationships that could influence the work reported in this paper.

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Autenticidade e Rastreabilidade dos Alimentos

Ethical Food Entrepreneurship (EFE) - Erasmus+ Project: Food for People – Planet – Profit

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The main objective of the present work is to present the Ethical Food Entrepreneurship (EFE) Project, an Erasmus+ Project. It is Europe's first Higher Education Institution (HEI)-led ethical food entrepreneurship programme. The main objective of the EFE project is to contribute to the professional development of food HEI & Vocational Education and Training (VET) educators. Its main aim is to increase HEI & VET educators' pedagogic skills to develop and teach new food entrepreneurship supports, based on the triple-bottom-line: planet, people, and profit. The logotype of the project is represented in **Figure 1**.

ethical food entrepreneurship

Co-funded by the Erasmus+ Programme of the European Union

Figure 1: Logotype of the Ethical Food Entrepreneurship (EFE) - Erasmus+ Project.

We hope to empower a new generation of food entrepreneurs to start, grow and adopt new ethical food enterprises and consequently accelerate progress across key elements of the Sustainable Development Goals (SDGs). Among these are SDG 8 - Food producers to be drivers of equitable and sustainable growth and SDG 10 - Reducing inequalities & creating employment. If we empower small-medium enterprises (SMEs) and start-ups, we are directly helping to breed the innovative food concepts and thriving new businesses that the European Union (EU) needs.
Savonia University of Applied Sciences (Finland) is the Coordinator of the EFE project. As partners, Instituto Politécnico de Bragança (Portugal), Antalya Bilim Universitesi (Turkey), Momentum Marketing Services Limited (Ireland), European E-learning Institute (Denmark) and Bia Innovator Campus CLG (Ireland) must be mentioned. The project started on 01st January 2022 and will end on 31st December 2023.
Regarding the tasks, the EFE project consists of four, namely: Task 1 - Development of an Educators Guide on Drivers and Enablers for Innovation of Ethical Foods; Task 2 - Development of the Innovating Ethical Foods, Entrepreneurship Manual; Task 3 - Development of the EFE Open Education Resources (OERs); and Task 4 - Development of an Ethical Food Entrepreneurship Sharing and Mentoring Platform. With these tasks, we intend to: (*i*) present both policy and market/consumer trends, drawing conclusions about the opportunities, benefits and challenges that food SMEs will face when developing innovative, ethical foods; (*ii*) the Entrepreneurship Start-up Manual specifically will help students and potential food entrepreneurs to understand the steps involved in starting/ diversifying into an ethical food business; (*iii*) suggest an Ethical Food Entrepreneurship programme to enable HEI & VET providers to deliver an Ethical Food Entrepreneurship course to their students/potential entrepreneurs of SMEs including curricula, learning objectives, evaluation techniques and recommended content for classroom-based courses; and (*iv*) develop an Ethical Food Entrepreneurship Sharing and Mentoring Platform, which will function as a circular classroom and a peer-learning and knowledge-sharing platform. To obtain more information about the EFE project, please consult https://www.facebook.com/efe.erasmus.project/.

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Botanical identification of honey from Natural Park of Montesinho: improving honey DNA extraction methodology

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Honey is a sweet natural food produced by bees from floral nectar that bees collect from plants and store it inside the beehive. Before storing, collected nectar is broken into simple sugars by enzymes that can be found in bee's saliva. Constant fanning of bee's wings cause water evaporation from nectar and the sweet tacky liquid is created. The use of honey has a long history as it has many nutritional values and health related properties. Honey is composed mainly by water and sugars, however, contains other minor components such as amino and organic acids, vitamins, and minerals that enhance biological and organoleptic properties. The presence of these compounds in honey is related with the floral origin and its distribution confer to honey different and distinct organoleptic and antioxidant, antibacterial, and anti-inflammatory properties. For example, antioxidant capacity depends on the amount of phenolic acids in honey which depends on the honey's floral source.[2] This strict relation of botanical origin and its biological and organoleptic properties lead to a quality distinction of honey types. Usually, monofloral honey (honey with at least 45% of pollen grains of one single plant species[3]), attains high market values due to its particular flavor and taste and specific biological properties, as well as some honey produced in specific regions attains the denomination of Protected Designation of Origin (PDO), as honey produced in Natural Park of Montesinho (NPM). The NPM honey is mostly collected from heather, chestnut and rosemary. The natural vegetation of plants is affected by particular conditions of NPM therefore, all of that reflects in the flavor and properties of honey.

The botanical origin of honey is assessed trough melissopalynology studies.[3] This technique consists in the analysis of pollen content and fungal spores in honey. However, some of the pollen content in honey can be found from contamination. Besides, these methods require appropriate skills and high-end equipment and is time-consuming. As alternative, DNA-based methods are more promising, as these methods are simple, fast and precise. Moreover, DNA molecule proved to be stable at adverse conditions, including food processing. Briefly, DNA based methods consist in DNA isolation and extraction, PCR amplification of particular genetic markers containing DNA fragments, and identifying DNA profiles defined by those markers.

Thus, it is important to find the best method for DNA extraction from plants and honey with the best yields and purity possible. Honey is a complex matrix with high amounts of sugars and other compounds that could inhibit the PCR reaction. Thus, it is necessary to prepare honey samples for the DNA extraction. In this work, a pre-treatment was performed according literature[1], and other similar 2 pre-treatments using less quantity of sample and different times of ultrasounds (3 and 1 min) were tested in honey samples from Natural Park of Montesinho (NPM). For the DNA extraction, the commercial kit NucleoSpin Plant II (Machery-Nagel) was used in honey and in plant samples collected in the NPM. The quality of DNA extracts was assessed by spectrophotometry and electrophoresis. Results revealed that honey pre-treatment using ultrasound for 3 minutes produces the best yields and purity. Additionally, a PCR amplification using universal primers targeting the gene 18S rRNA was performed to evaluate amplifiability of the extracted DNA. As expected, all extract samples revealed to have amplifiable DNA. Combining a simple and easy to perform honey pre-treatment with an effective DNA extraction method, allows us to get desired good extracts for honey. Thus, this methodology is the first step for further studies involving botanical identification of honey and quality studies regarding honey quality.

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Optimization of molecular methods for the identification of commercial strains of *Saccharomyces cerevisiae*

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The yeast Saccharomyces cerevisiae is widely used in the production of fermented foods, due to its ability to perform alcoholic fermentation at aerobic and anaerobic conditions. It metabolizes important secondary metabolites which provides the final product with flavour and aroma. The diversity and proportion of these depend largely on the strain used. Thus, it is of great importance to the producer to use the right strain according to their aim, which makes important the identification of strains and therefore the optimization of efficient and fast methods to this purpose. The aim of this work is to optimize the methods for strain identification analysing 10 commercial strains through interdelta polymorphism. Two polymerase chain reaction (PCR) amplification conditions and two primers pairs were tested. Six out of ten commercial strain were identified. Phenotypic differences were verified by evaluating the growth of the strains. The DNA extraction method proved to be efficient for the amplification of DNA from the interdelta regions. The band patterns of the samples obtained by the primer δ2 - δ12 present an average of 6 bands, while by the primer δ12 - δ21 an average of 8 bands are obtained, however, none of the two pairs of primers used is significantly more efficient than the other for the identification of strains. The results were confirmed using GelAnalyser program. Conditions A, exhibited higher resolution of the band patterns obtained through agarose gel and the primers pair did not reveal significative differences. This technique might not be sensitive enough to identify the set of samples (1, 2 and 3). These results may be of interest for future characterizations approaches.

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Differentiation of Arabica coffee from distinct geographical origin based on integration of volatilomic profiles and chemometrics

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Coffee is a popular beverage worldwide, that is produced from the seeds of plants belonging to the Coffea genus. It is estimated that, during 2020, coffee production exceeded 169 million 60 kg bags, proving coffee's economic significance. Aroma is an important characteristic that influences coffee quality in addition to consumer preference. It depends on several factor, such as variety and geographic origin. The main coffee varieties with commercial relevance are *Coffea arabica* and *Coffea canephora* var. Robusta. Since Arabica coffee beans produce a sweeter, softer beverage with a better taste when compared to the stronger, harsher tasting coffee obtained from Robusta coffee beans, it is considered more valuable and, consequently, more expensive. The authentication of foods and food-derived products, namely those with high commercial value, has become of rising concern, motivating the development of high-resolution analytical tools to mitigate the situation.
In this context, the aim of the present study was to differentiate Arabica coffee (*Coffea arabica* L.) from five distinct geographical origins - Brazil, Colombia, Ethiopia, Guatemala, and Timor, using headspace solid phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME/GC-MS) and chemometric analysis (principal component analysis (PCA), and partial least square discriminant analysis (PLS-DA)) was used to obtain the most effective parameters on chemical changes. A total of 110 volatile organic compounds (VOCs) were identified in the roasted ground coffee samples - 93 in Brazilian and Ethiopian, 89 in Timorese, 87 in Colombian and 81 in Guatemalan coffees. From the different chemical families identified, furanic and nitrogen compounds were the dominant contributors to the volatile composition of all samples. Overall, higher volatile concentrations were observed in Ethiopian coffee and lower on Guatemalan coffee, while there were no significant variations among coffee from other origins. The VOCs that allowed the geographical discrimination of the investigated coffee samples were 2,3,6-trimethyl-1,5-heptadiene, methyl-2-butanoate, β-terpinene, o-cymene, 2,7-dimethyloxepine, 2-methyl-5-propylpyrazine, 2-ethyl-1-hexanol, 2,3-diethyl-5-pyrazine, 2-methoxy-3-(2-methylpropylpyrazine and m-tert-butylphenol.

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Assessment of volatilomic fingerprint of apple ciders as a powerful tool to find putative geographical biomarkers

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Apple cider is a traditional alcoholic beverage fermented from apple juice, with increasing consumption and production worldwide. Apples (*Malus domestica*) and their derivates, particularly cider, pose a significant impact in terms of global fruit cultivation, being the most ubiquitous and well-adapted fruit species in temperate regions. In 2017, across Madeira Island, around 130 ha were dedicated to the production of 2000 tons of apples, corresponding to the production of 3328 hl of apple cider. Moreover, opposite to evidence in the remaining country, the cider-making by traditional process has never been discontinued in Madeira Island. The traditional cider-making started with the harvest of selected apple varieties (e.g., Azedo, Branco, Calhau, Domingos, Festa, Rijo, Ribeiro, Vime and Verde apple varieties). Then, the apples are cleaned, crushed and pressing to obtain the fruit juice without solid parts. The fruit juice is submitted to a controlled fermentation (15 to 18 days) at 18 °C to obtain an ethanol content of 7–8% (v/v). Due to the edaphoclimatic and geographical characteristics associated with the organoleptic quality of the different endogenous varieties of cultivated apples, Madeira Island has all the natural conditions to produce excellent quality apple ciders.

Currently, the food-quality programme of the European Union encourages food-origin protection through Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI)) with the purpose of ensuring the quality of the final product. Therefore, it is necessary to develop analytical tools to establish the authenticity and genuineness of food-derived products.

In the current work, headspace solid-phase microextraction followed by gas chromatography-mass spectrometry (HS-SPME/GC–MS) combined with chemometric tools was used to establish the volatile fingerprint of apple ciders produced in different geographical regions of Madeira Island, in order to define their typicity and to identify putative geographical markers. A total of 143 volatile organic compounds (VOCs) belonging to different chemical families have been identified, of which 28 were found in all apple ciders independently of geographical region. Esters, terpenic and furanic compounds presented, on average, the highest contribution for the total volatile fingerprint in cider produced in northern region of the Island, whereas alcohols, acids, volatile phenols, carbonyl compounds and lactones in cider from southern region. Forty-three VOCs revealed statistically significant differences ($p < 0.001$) between the target geographical regions, and 11 between northern and southern regions. A clear differentiation among cider-producing regions was observed on the developed partial least squares-discriminant analysis (PLS-DA) model. Two alcohols (1-hexanol, 1-octanol), 6 esters (methyl acetate, (Z)-3-hexen-1-ol acetate, ethyl hexanoate, ethyl nonanote, ethyl octanoate, isoamyl octanoate) and 1 terpenic compound (limonene), can be considered putative geographic markers due to their discriminatory ability. The results obtained recognize the specific and typical geographical characteristics of the cider, which will allow the forthcoming guarantee for the construction of a sustainable platform for the establishment of the authenticity and typicality of the regional cider.

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Discrimination of Italian saffron samples using µQUEChERS-dSPE/UHPLC-PDA and chemometric analysis of their bioactive composition

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Saffron is eventually one of the most expensive spices in the world, easily reaching values over 5000€/kg. It is obtained from the dried red stigma of *Crocus sativus* L. flowers produced in just a small number of places in the world, mainly Iran, Spain, Greece, Morocco, Italy, and India. For this reason, control of saffron quality and origin is of utmost importance. In this context, we proposed a fast and simple untargeted approach to discriminate saffron samples from different origins. Accordingly, saffron samples cultivated in seven different places in Campania, Italy, were extracted by µQUEChERS-dSPE and processed by UHPLC-PDA analysis at 254 and 450 nm. This allowed us to obtain chromatographic profiles exhibiting 16 unidentified phenolics and carotenoids in saffron samples that show high variability among the samples analysed. This data was then subjected to chemometric analysis that demonstrated the ability to discriminate samples according to the relative abundance of certain compounds from the 16 detected. Despite the identify of the 16 compounds detected in the saffron samples is not yet known, this introductory work points that their relative levels can be used to discriminate the sample according to their origin. Such variations have a great economic potential because they affect the samples composition and quality. Finally, it is worthwhile to refer the successful miniaturization of QUEChERS procedure here described, only requiring 0.4g of sample by analysis, is of utmost importance given the cost of the saffron samples.

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Segurança Alimentar

Food and nutritional security in Brazil and the covid-19 pandemic: a literature review

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The objective of this work was to analyze food and nutrition security in Brazil during the COVID-19 pandemic from a narrative review in the main databases, including: the electronic libraries VHL (MEDLINE, LILACS, IBECS and BDENF) and SciELO. The criteria for inclusion of the studies were: articles published in full in the last two years (2020 to 2022), in Portuguese, English and Spanish in Brazil and abroad. The following descriptors were used: food and nutrition security; food insecurity; hungry; poverty. After the selection of bibliographic materials, a full reading of the works was carried out, where the central ideas, objectives, methodology and results were identified. The sample of this review consisted of 14 articles that met the inclusion criteria. The results revealed that the articles converge to the understanding that the pandemic not only worsened the food and nutritional insecurity of socially vulnerable groups in Brazil, but also included new groups, increasing the population contingent in situations of scarcity, hunger and poverty in the country. In this way, we believe it is necessary to intensify food and nutrition policies and programs in the country in order to guarantee the human right to adequate food at this critical health moment, with emphasis on the National Food and Nutrition Policy (PNAN), the National Program School Feeding Program (PNAE) and the Food Acquisition Program (PAA).

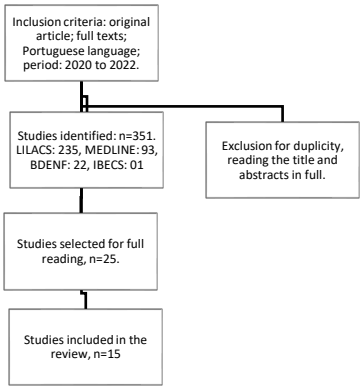

Figure 1: Stages of the bibliographic review, 2021.

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The application of the one health concept in meat and meat products supply chain

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Worldwide, 320,000 workers die each year from work-related infectious diseases, 5,000 of them in the EU. The risks generated by zoonoses and antimicrobial resistance, in the primary sector, are not usually included in the risk assessment. In 2020, the European Agency for Safety and Health at Work1 reported five groups of high-risk occupations concerning the biological agents and prevention of work-related diseases namely: animal-related occupations, waste and wastewater management, healthcare, arable farming, and occupations that involve travelling for work and contact with travellers, such as for example in customs work. During the 21st Century many global health threats have already emerged linked to zoonotic and (re) emerging diseases (e.g. SARS, COVID-19), climate change and environmental sustainability, but also several news stories associated with food safety issues derived from meat and meat products. These health threats have the focus on food safety, but in a one health perspective the issues associated with animal-related occupations exposure to biological agents and the resulting occupational health problems cannot be neglected. Both are complex and cannot be adequately addressed by any individual discipline acting in isolation. European Food Safety Authority and European Centre for Disease Prevention and Control presents every year the results of zoonoses monitoring activities in European countries. The analysis of this reports allows an analysis of the occupational exposure, in comprise abattoir and slaughterhouse workers, animal breeders/carers/handler, responsible for zoonose and antimicrobial resistance.

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Risk assessment of toxic metal contamination in the reuse of treated wastewater for urban vegetable irrigation

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Water is a natural resource essential to the maintenance of all kinds of life. Without water, the extinction of various forms of life will be inevitable. The reuse of treated wastewater (RTWW) for irrigation is as a possibility with challenges to be clarified at scientific level.[1] In particular, the use of RTWW for vegetables irrigation and the eventual contamination of these foods by toxic metals and other compounds harmful to humans present in these waters.[2] On the other hand, the RTWW may allow significant water saving and provide the cultivated species with the nutrients, which may also dispense the use of fertilizers.

The main goal of the present investigation relies on the assessment of whether the water from the wastewater treated plant (WWTP) can be used for irrigating agricultural fields without harming human health, thus promoting a sustainable use of this resource as an alternative to its discharge into the environment. In this work, vegetables such as, *Brassica oleracea* (cabbage) and *Lactuca Sativa* (lettuce), were planted in a greenhouse with controlled environment (temperature and humidity) located at ISEL (Instituto Superior de Engenharia de Lisboa) campus. Part of the vegetables is irrigated with potable water, and another part with RTWW from two WWTP, Chelas and Frielas, both located in Lisbon´s District. To ensure the viability of using RTWW in this process, it is important to determine the content of toxic metals such as, Pb^{2+}, Cd^{2+}, Ni^{2+} and Cr^{2+}, in the cultivated species as well as in the soil.

This analysis is carried out by Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS).

Additionally, the antioxidant activity in the vegetable species will be determined, assisted by microwave microextraction with methanol and applying the DPPH method. The total content of polyphenols and flavonoids present in the extracts will also be determined, using the Folin Ciocalteu method[3] and the aluminum chloride method [6], to infer if the presence of toxic metals affect these important parameters of vegetable's food quality.

The obtained results shown an increase in metals content in the soil and vegetables due to the irrigation with TW. On the other hand, in vegetables with higher metal content it was observed a decrease in antioxidant activity as well as an increase in the production of total polyphenols.

From the conclusions of this work, it will also be possible to design synergies with local authorities and WWTP to define strategies for RTWW for irrigation of urban gardens.

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Host-guest system based on sulfonatocalixarene and pyranoflavylium dye for biogenic amine sensing during food spoilage

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Food quality is a central issue in today's food economics mainly due to the growing demands of consumers. Information availability and environmental concerns are the reasons for the growing scientific research on this field, ultimately leading to an increase in food quality. The rise of health problems due to food spoilage or toxicity has motivated researchers to focus on the development of innovating new methods for controlling food quality, serving the purpose of public health.[1] Biogenic amines are biologically active nitrogen-containing compounds, formed in the normal metabolism of animals, plants and micro-organisms. The presence of these biomolecules in food products is undesirable and their ingestion in significant amounts can cause headaches, respiratory distress, heart palpitations and several allergenic disorders.[2]

The main goal of this work was the development of a colorimetric host-guest molecular switch based on interactions between a bioinspired 10-methylpyrano-4'-hydroxyflavylium[3] guest dye and the *p*-sulfonatocalix[8]arene macrocyclic host with sensing ability for biogenic amines. The interaction between macrocycle and pigment as well as biogenic amine detection was evaluated through UV-Vis spectroscopy. This non-covalent interaction in a host/guest system has shown promising results for applications regarding functional materials or switch systems as covered in multiple scientific papers.

The macrocycle-dye system was optimized in terms of molecular ratio and the working pH was taken from the maximum differences between the free pigment (p*K*a 6.72) to the one complexed with the macrocycle (pK_a 8.45). Overall, in a phosphate buffer solution (pH 7.6), the complex was able to encapsulate putrescine in solution with concomitant release of the quinoidal base species of dye to the bulk, with spectral variation from yellow to pink-red (**Figure 1**). This host-guest system demonstrated great potential for the detection of biogenic amides, one of the main indicators of food spoilage.

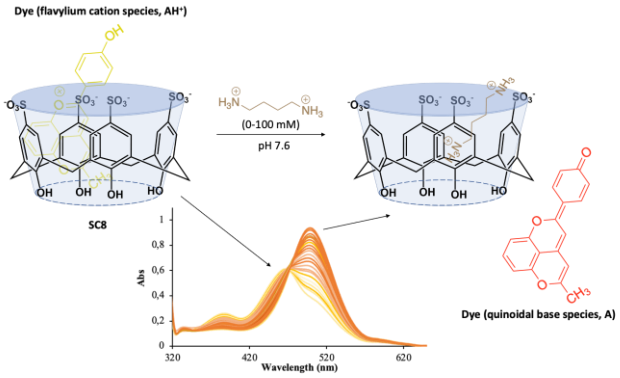

Figure 2: Example of the Dye-SC8 (0.05:0.5 mM) system in the presence of increasing concentrations of putrescine (0-100 mM) at pH 7.6.

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Microbiological quality assessment of two portuguese PDO cheeses - Serra da Estrela and Azeitão

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Cheese manufacture is a complex process, home to a dynamic and highly selective three−dimensional microbial ecosystem composed by bacteria, yeasts and/or moulds, which are ultimately responsible for the development of its organoleptic characteristics.[1] Artisanal cheeses are generally perceived to yield higher quality cheeses then those obtained in industrialized large-scale productions.[1] This results mainly from the use of raw untreated milk that harbours a rich microbiota, whose metabolic interaction during cheese manufacture leads to the production of large amounts of volatile and non-volatile compounds, which translates into organoleptic complex cheeses packed with flavours and aromas.[1]

Portugal harbours a rich cheese heritage, accounting up to almost 100 traditional cheeses made from cow, goat and ovine milks or milk mixtures. So far, 11 cheese types were awarded with the Protected Designation of Origin (PDO) status. Of those, Azeitão and Serra da Estrela are two of the most popular and renowned PDO ovine raw milk cheeses.[2] Despite composed mostly by beneficial microbes, raw milk cheeses can also harbour a series of pathogens, that gain access from raw material transference and/or during manufacture due to faulty sanitary and/or hygiene practices.[3] In fact, in these last 40 years, over 60 food-borne outbreaks were reported worldwide linked to the consumption of contaminated cheese products, although none of the reports arose from the consumption of Portuguese cheeses.

The aim of this work was to perform an updated microbiological quality assessment of Serra da Estrela and Azeitão PDO cheeses at different manufacturing periods within 2020/2021 production campaign. To that end, cheeses from both regions were collected from local producers in December/January, March and April. Microbiological screenings included the enumeration of microorganisms at 30°C according to ISO 4833-1:2013; β-glucuronidase positive *Escherichia coli* according to ISO 16649-2:2001; *Enterobacteriaceae* according to ISO 21528-2:2017; *Bacillus cereus* according to ISO 7932:2004; coagulase-positive staphylococci according to ISO 6888-1:1999; *Listeria monocytogenes* and *Listeria* spp. according to ISO 11290-2:2017; yeasts and moulds according to ISO 21527-1:2008 and the detection of *Salmonella* spp. according to ISO 6579-1:2017. For each sample, the mean values of viable counts and respective standard deviation error were estimated and expressed as the logarithm of colony forming units per gram of product (log CFUs g^{-1}).

To assess the safety and hygiene of Serra da Estrela and Azeitão PDO cheeses, both the European (EC Nº 2073/2005) and Portuguese[3] guidelines establishing the microbiological criteria for foodstuffs and ready-to-eat food products were used. Concerning food safety, viable counts of *B. cereus*, coagulase-positive staphylococci and *L. monocytogenes* were not found, while *Salmonella* spp. was not detected in any of the cheese samples. Overall, these results attest the microbial safety of Serra da Estrela and Azeitão PDO cheeses.

Regarding hygienic standards, it was found that irrespectively of the period of production, Azeitão presented slightly higher total mesophilic microorganisms (TMM) than Serra da Estrela cheeses, just over 9.0 log CFU g^{-1} and 8.7 log CFU g^{-1}, respectively. These TMM values are above the national guideline threshold of 6.0 log CFU g^{-1}. However, they result from the high load of Lactic Acid Bacteria (LAB), over 8.4 log CFU g^{-1} and 8.8 log CFU g^{-1} in Serra da Estrela and Azeitão cheeses respectively. Corresponding to acceptable TMM/LAB ratios (≤100), as stated in the same document. *Listeria* spp. was absent from Serra da Estrela cheeses but found in one Azeitão cheese sample from December, at a concentration of 2.36 ± 0,03 log CFU g^{-1}, above the limit of 1.0 log CFU g^{-1} stated in the national guideline. Also, *Enterobacteriaceae* were found in all analysed cheeses samples, between 3.8 to 6.09 log CFU g^{-1}, with *E. coli* comprising most of the counts ranging from 2.83 up to 5.14 log CFU g^{-1}. Furthermore, in both cheese types is possible to observe an increment of the *Enterobacteriaceae* load across the production season, that is also replicated by *E. coli*. According to the European guidelines, from all analysed cheese samples, only the Serra da Estrela cheese sample obtained in January presented a satisfactory *E. coli* content, not exceeding the limit of 3.0 log CFU g^{-1}. Finally, both European and Portuguese guidelines are omissive on the threshold values of yeasts and moulds in foodstuffs and ready-to-eat food products. The here analysed cheese samples presented a yeast and mould load that ranged from absent up to 5.0 log CFU g^{-1}. Concerning Serra da Estrela cheeses moulds were absent from January and March cheeses and only recovered from April samples. On the contrary, yeasts show a tendency of decrease across the production season, being absent from April cheeses. Regarding Azeitão cheeses yeasts and mould were both recovered from all analysed samples, with moulds presenting a higher abundance volatility between manufacturing periods.

This study showed that Serra da Estrela and Azeitão are free from potentially pathogenic microbiota. However, considering the quantification obtained for environmental and fecal indicators, their manufacture might benefit from an action at multiple levels. First, enforce the implementation of effective and modern sanitary and hygiene practices

in head management and supply of raw ovine milk by dairy farmers, strengthening sanitary and hygiene practices at the production line, preventing punctual contaminations, due to microbial transfer from the factory environment and cross contamination events. Lastly, consider a manufacture process update, extending the ripening stage until the obtention of admissible levels of specific groups of microorganisms (*e.g. E. coli*).

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Parasites and its Zoonotic Potential in Fishery Products from the Portuguese Markets

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The presence of parasites in fishery products threaten their safety and quality and may represent a consumers' health hazard. A total of 697 samples from different seafood products, whole fish (n= 199), fish steaks (n= 188), fish fillets (n=3), fish fingers (n=278), ray wings (n= 3), shredded salt cod (n= 1 290.70 g) and common octopus (n=18) from the Portuguese markets were analysed.

External surface, internal organs, visceral cavity and muscle of fresh or thawed whole fish were visually inspected for detection of parasites. The muscle tissue was directly observed under stereomicroscope, submitted to candling method (fillets and belly flaps) and artificial digestion. Thawed fish steaks and fish fillets were also visually inspected and the muscle was weighed, sliced, observed by candling and artificially digested. Wet mount preparations of muscle tissues from fresh and thawed fish, thawed fish steaks and fish fillets were prepared and microscopically examined. Parasitological analysis of each fish finger was performed on the fish muscle, after removing the batter coating, through macroscopic observation, stereomicroscope examination, slicing and candling. Fresh smears of the muscle were examined microscopically. Third stage larvae of anisakid nematodes in cephalopods were searched in the mantle muscle. The collected parasites were morphologically identified[1] and their viability evaluated.

The occurrence, distribution and viability of anisakids (viable and dead larvae) which represent a risk to the consumers' health and those of other parasites that are associated with economic losses in the fish market were reported. Viable *Anisakis* and *Pseudoterranova* L3 larvae were collected from different fish samples, although live larvae were only found in fresh fish. Other nematodes, *Huffmanela lusitana* and *Philometra* sp.; cestodes, pleurocercoids of *Gymnorhynchus gigas* and *Hepatoxylon trichiuri*; crustacean copepods, *Pennella instructa* and *Lernaeocera branchialis*; mixosporean spores of *Kudoa* sp., *Kudoa thyrsites* and *Henneguya salminicola*; microsporidean *Spraguea americana*, *Spraguea lophii*, and *Dasyatispora levantinae* gen. et sp. nov. were also observed. No *Anisakis* larval specimens were detected encysted inside the mantle tissue of common octopus.

Fish are often placed on the market as fresh fishery products and they may contain parasites. If these parasites could be observed macroscopically in commercially valuable fishery product, it makes the fish disgusting, leading to the withdrawal of the fish from the supply chain, with consequent economic losses.

On the other hand, *Anisakis simplex* s.s. and *A. pegreffii* larvae of the genus *Anisakis* are the most frequently reported and the ones that are most important from a health point of view because they have zoonotic potential, i.e., they can cause disease to humans. Human infection (anisakiasis) occurs when raw or poorly prepared fish is consumed, containing live L3 larvae, which cause vomit, nausea, abdominal pain, diarrhoea and even potential allergic reactions that can vary from an allergic reaction to anaphylactic shock. Even after heat treatment, the proteins of *Anisakis* larvae maintain their allergenic potential, and therefore, the potential risk of allergy for consumers sensitized to the allergens of this parasite should be considered.[2]

Raw fishery product should undergo a previous temperature treatment to kill viable parasites before consumption, accordingly to the European Union legislation which establishes that the temperature in all parts of certain fishery products must be lowered to at least -20 °C for not less than 24 hours or -35 °C for not less than 15 hours, in order to kill viable parasites. (Commission Reg. EC No 1276/2011)

It is through the dissemination of knowledge and raising awareness on the issue of parasites in fishery products to health inspectors, economic agents and consumers that infection prevention must be carried out.[3]

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Impact of pH on food preservation by hyperbaric storage and potential of a novel food pasteurization process

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Proper storage conditions are necessary to preserve food products, to avoid or delay microbial deterioration or quality issues. Cold storage is the most common strategy to preserve foods and is used worldwide but it causes great energetic consumptions and emissions of global greenhouse gases, which are environmentally unsustainable.
Recently, hyperbaric storage (HS), a novel food storage under mild pressures, has been proposed as an alternative to the conventional refrigeration that can be used at room temperature. This is a major advantage, since it allows for *quasi* no energetic costs and much lower green-house gas emissions and was shown to preserve foods products equally or even better than refrigeration.[1]
Watermelon juice is known for having an interesting nutritional composition, associated to health benefits, namely vitamins (A, B, C and E), minerals (K, Mg, Ca and Fe), functionally important amino acids (citrulline and arginine) and antioxidants like carotenoids and phenolic compounds. However, watermelon juice is highly perishable, since it has a high pH (5.2–6.7) and high water activity (0.97–0.99), being prone to microbial growth and enzymatic activity, resulting in a short shelf-life.[2]
For the first time, the impact of pH on a food (watermelon juice) preserved by HS at room temperature was studied, focusing on the behaviour of *Listeria monocytogenes* (a pathogenic microorganism) inoculated in the juice, adjusted to different pH levels (4.0 and 6.5), and stored at pressures up to 100 MPa. The results showed that the pH and the storage pressure level used affected the growth and inactivation of the bacteria: the most acidic juice requires a lower storage pressure level for microbial growth and achieves a quicker inactivation of, at least, 4.7 logs in 24 hours at 100 MPa.
Taking these findings into consideration, variables such as the pH of the juice and the storage pressure level should be further studied in order to better select the best hyperbaric storage food preservation conditions. Additionally, it was noted that while the juice is preserved, not only the growth of the pathogenic *Listeria monocytogenes* can be controlled but its inactivation is also a reality at the level of pasteurization (≥ 5 logs), revealing that hyperbaric storage can also have a great potential as a novel nonthermal pasteurization technique at room temperature with *quasi* no energetic costs.

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Validation of critical control points for non-thermally processed egg-based desserts

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In recent years there has been a change in consumer eating habits with increasing demand for less processed foods, this growing popularity of unprocessed homemade foods containing raw eggs such as mayonnaises, certain sauces, and raw egg-based desserts have increased the risk of salmonellosis.[1]
In Portugal, some traditional desserts use raw or insufficiently cooked eggs in their preparation, which may result in a *Salmonella* contaminated final product. Therefore, it is important to develop methods to eliminate or reduce the risk, making them safe products for the final consumer.
This research work aimed to develop a methodology based on the Hazard Analysis and Critical Control Points (HACCP) system, applicable to the production process of two desserts: Chocolate Mousse (CM) and Vinegar Pudding (VP), through the identification of possible Critical Control Points (CCP's).
Experimental inoculation tests were performed with *Salmonella enteritidis* in an inoculum concentration of 10^8 cfu/g, where the effect of heat treatment at two different temperatures (60° C and 65° C) for 5 minutes was evaluated. *Salmonella enteritidis* counts were performed in double layer medium composed of Xylose Lysine Deoxycholate (XLD) and Tryptone Soy Agar (TSA), incubated at 37º C for 18h.
For all tests performed for both the chocolate mousse dessert and the vinegar candy, an absence (<10 cfu/g) resulted for all samples (n=20). These results demonstrate that the applied heat treatment was effective in bacterial destruction.
Through the study, 1 CCP's were identified for each preparation, namely in the egg whipping stage, added as time/temperature controls to reduce/eliminate microorganisms.

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Identification of Critical Control Points of Ceviche production

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Ceviche is a South American seafood dish original from Peru, typically made from raw fish cured in citrus juices namely lemon and lime. In addition to consumption in their country of origin, ceviche and other typical culinary preparations have gained a lot of popularity in several other countries, such as Portugal, contributing to the increasing consumption of raw fish over the last years. At the same time, emergence diseases associated with consumption of raw fish including sushi and Ceviche has been reported.[1] Considering these facts, it is crucial to develop culinary methods that assure the reduction or elimination of the risk to acceptable levels for this type of products, making them safe for the consumer. The aim of this work was the identification of critical control points of ceviche production. For this, an experimental inoculation test was carried out in raw fish with the pathogens *Vibrio parahaemolyticus* (VP) in an inoculum concentration of 10^5 cfu/g. The effect of both pH and maceration time were evaluated in two different formulations of ceviche. Both preparations of Ceviche with lime juice (traditional) and ceviche with a solution of lime juice plus cider vinegar were submitted to three maceration times (10, 30 and 120 min.). The results showed that bacterial destruction was effective, and usually slightly higher than in traditional Ceviche. The effect of the maceration time also generated positive results in the inactivation of bacterial agent. At 120 min of maceration in traditional Ceviche, bacterial destruction was ~4-log cfu/g for *V. parahaemolyticus*. Finally, 2 critical control points were established, and the respective critical limits, control measures, and corrective actions, for the consequent production of a safe food product. This work indicated that is important monitorisation of maceration step.

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Multi-mycotoxins assessment: occurrence in infant and children food

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A good nutrition is crucial for children healthy development. Regardless the recommendation of consumption of various types of foods, some of them can be also a source of toxic natural contaminants such as mycotoxins, potent secondary metabolites produced by filamentous fungi.[1] Owing to the high toxicity of mycotoxins, some of them being already classified on their carcinogenicity[2], legislative limits in food and feed continue to be set and updated worldwide. EU Commission Regulation 1881/2006 respecting to mycotoxins contents regulate processed cereal-based foods and baby foods for infants and young children[3], for the regular mycotoxins, but not yet for the emerging ones, which constitutes a worrying gap that must be fulfilled. Emerging mycotoxins have been revealed increasing interest in the last years due to their prevalence in most foodstuffs, particularly in cereals and cereal-based products. Multi-mycotoxins exposure arguably induces higher toxicity than single-exposure, which is concerning especially in products primarily marketed for children.

In the present work, the occurrence of 24 mycotoxins of different chemical classes was assessed in 39 breakfast cereal samples (12 biologic, 12 branded and 15 white label). The analytical method used was based on modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure followed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis. Seventeen out of twenty four mycotoxins (71%) under study were found in the samples under analysis (**Figure 1A**). Thirty six out of thirty nine samples analysed (92%) were contaminated with at least one mycotoxin (**Figure 1B**). Two samples were contaminated with five mycotoxins.

Overall, aflatoxin B1 (AFB1) and aflatoxin G2 (AFG2) were the compounds more commonly detected between regulated mycotoxins while enniatin B (ENNB), sterigmatocystin (STG), and enniatin A1 (ENNA1) were the mostly found of the emerging ones.

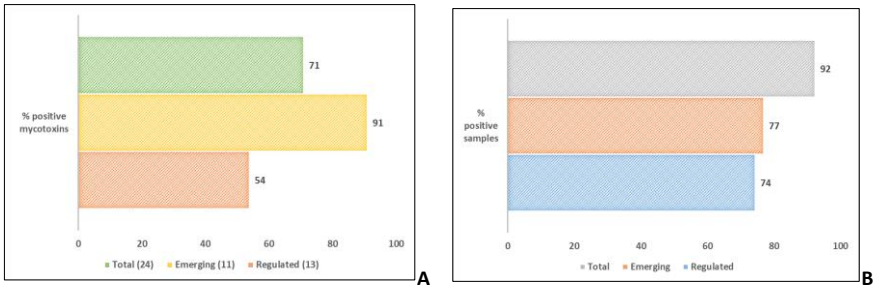

Figure 1: A- Percentage of mycotoxins detected; B- Percentage of positive samples.

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Assessment of six phthalates and one adipate in commercial beer samples

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Plastic is one of the most common materials used, transversal to all industries, leading to a rising exposure to the toxicity of its components such as phthalates and adipates that are compounds added to plastic in order to increase its plasticity and workability. However, because these compounds are not chemically bonded with plastic, there is the possibility of leaching and migration to the environment.[1] Various studies have documented the detrimental health effects of these compounds, mainly endocrine disruption in humans.[2]
Beer is one of the most consumed beverages worldwide, and due to its production process and different types of packaging, it can be a source of phthalate exposure in humans.[3] The aim of this study was to validate a method based on dispersive liquid-liquid microextraction (DLLME) coupled to gas chromatography with tandem mass spectrometry (GC-MS/MS) analysis for the simultaneous determination of six phthalates and one adipate in commercial beer samples (n=66).
Our method showed good linearity (r >0.96), with low limits of detection (0.3-1.5 µg/L) and quantification (1-5 µg/L) and good intraday (<12%) and interday (<13%) precision. Five out of six phthalates and the adipate were found in the commercial beer samples, with concentrations ranging from 1.77 to 205.40 µg/L. The results demonstrated that the adipate was the most common plasticizer in the samples (**figure 1**). Moreover, some correlations between the presence of the analytes and alcohol content, type of packaging and production origin were observed. Alcoholic samples, packed in aluminium cans from industrial production presented higher levels of contamination.

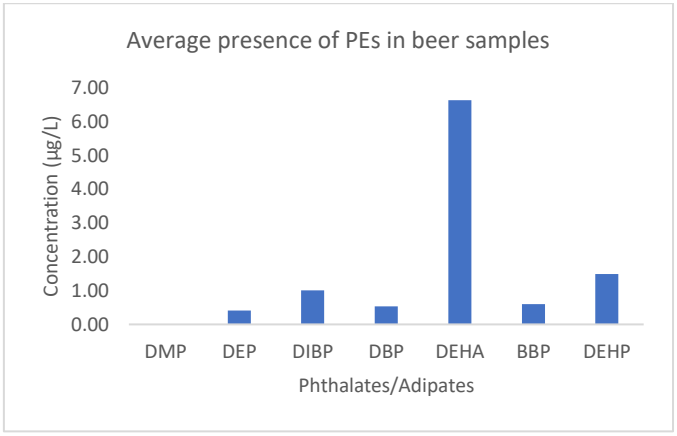

Figure 1: Average concentration of phthalates (DMP, DEP, DIBP, DBP, BBP and DEHP) and one adipate (DEHA) in commercial beer samples (n=66).

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Levels of contaminant metals in three species of holothurians from Portugal: Seasonal, sex and tissues variations

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Metals such as cadmium (Cd), lead (Pb) and mercury (Hg) are classified as non-essential because there are no known metabolic or biological functions. These metals are deleterious in several respects and therefore are listed in the top 20 hazardous substances by the US Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry (ATSDR) (Xiong et al., 2017; Rai, 2019). In aquatic ecosystems, sediments are the main source of metals, serving as the largest reservoir for the storage of toxic metals. Most marine animals can accumulate these metals in their bodies mainly due to the environmental conditions in which they thrive and their trophic level. Most sea cucumbers are not traditionally consumed as food in Europe, except for *Parastichopus regalis*, which is caught and consumed in NE Spain (Cataluña, Baleares islands and Castellón) having its internal muscle bands (espardeneyes) a high economical value (130-200 euros/kg). In recent decades, there has been a significant increase in the number of sea cucumber catches along the Mediterranean and Northeast Atlantic coasts for exporting to Asian countries, mainly China. To date, there are few studies on the elemental profile, including non-essential elements in sea cucumbers off the coast of the Northeast Atlantic. The main goal of this study was to investigate the seasonal contaminants changes of three sea cucumber species caught from Northeast Atlantic. The risk associated with the consumption of these target species taking into account the consumers age group was also evaluated. For this, was analysed the concentration of Cd, Pb and Hg during spring, summer, autumn and winter in two different tissues (body wall and muscle band) of female and male. Average concentration of the Cd, Pb and Hg is different between species, being significant differences were also observed in the sex and tissue patterns, unlike the seasons, which do not seem to interfere in the concentration of these elements. In general, *Holothuria arguinensis* (Cd <0.027; Pb <1.20; Hg <0.029), *Holothuria forskali* (Cd <0.031; Pb <LoD; Hg <0.023) and *Holothuria mammata* (Cd <0.04; Pb <0.56; Hg <0.047) showed levels lower than to European Union regulated. The risk associated with the consumption of these species is low or non-existent, since the amount that can be consumed by adults, based on the maximum concentrations of each element, is high. As it is scarce or does not exist, these same data may serve as a basis for future construction of regulations that limit the maximum concentrations of metals in the consumption of echinoderms.

	H. arguinensis	***H. forskali***	***H. mammata***
Cd	0.009±0.007^{c}	0.012±0.004^{b}	0.021±0.006^{a}
Pb	0.301±0.290^{a}	0.03±0.001^{c}	0.181±0.130^{b}
Hg	0.009±0.006^{b}	0.006±0.004^{b}	0.016±0.013^{a}

Table 1: Total of trace elements in *H. arguinensis*, *H. forskali* and *H. mammata*

Species/ Metal	Mean concentration (mg/kg)	RfD (mg/kg bw/day)[a]	CR_{lim} (gr/day)	
			Children[b]	Adults[c]
H. arguinensis				
Hg	0.15	5.7×10−4	60	300
Pb	0.573	d 6.3×10−4 / e 1.5×10−3 / f 5×10−4	[f]10	[d]210-880[e]
Cd	0.024	0.001	630	3330
H. forskali				
Hg	0.05	5.7×10−4	170	910
Pb	0.269	d 6.3×10−4 / e 1.5×10−3 / f 5×10−4	[f]30	[d]190-450[e]
Cd	0.053	0.001	280	1510
H. mammata				
Hg	0.17	5.7×10−4	50	270
Pb	0.872	d 6.3×10−4 / e 1.5×10−3 / f 5×10−4	10	[d]60-140[e]
Cd	0.056	0.001	270	1430

Table 2: Maximum allowable daily consumption rate (CRlim) of *H. arguinensis*, *H. forskali* and *H. mammata* by different age groups.

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Bioaccumulation of heavy metals in the liver of marine fish species from differenttrophic levels caught off Portuguese Exclusive Economic Zone (EEZ)

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INTRODUCTION: Fish is regarded as a source of healthy protein and important vitamins and minerals. However, the consumption of fish contaminated with heavy metals nullifies their beneficial effects. Heavy metals are transferred from the abiotic environment to marine organisms at different trophic levels, and thus contaminate the marine food webs. Trophic transfer, bioaccumulation, and biomagnification of hazardous heavy metals in food chains have important implications on human health[1]. Heavy metals can be allocated in essential and non-essential heavy metals[2]. Essential heavy metals include Manganese (Mn), Iron(Fe), Copper (Cu), Chromium (Cr) and Zinc (Zn), they are regarded as essential metals, but they can become toxic due to the narrow window between their essentiality and toxicity[2]. Contrariwise, the non-essential heavy metals, include cadmium (Cd), lead (Pb) and mercury (Hg). The arsenic (As) is traditionally included in this group, because of its toxicity, despite its classification as a metalloid, rather than heavy metal[2].

AIM: Assess the bioaccumulation of heavy metals in the liver of marine fish species from different trophic levels harvested inthe Portuguese EEZ.

METHODOLOGY: Eight marine fish species were selected and their livers collected by fishmongers from traditional markets andsupermarkets. The marine fish species selected included:

- 4 TOP carnivorous' species, all with preference for fish and cephalopods (trophic level between 4.15 - 4.55), namely, the blackscabbardfish (*Aphanopus carbo*; n=15), common dentex (*Dentex dentex*; n=20), john dory (*Zeus faber*; n=18) and european hake (*Merluccius merluccius*; n=20);
- 2 LOWER carnivorous' species with a preference for fish and decapods (trophic level between 3.65 – 4.15), namely the lesserspotted dogfish (*Scyliorhinus canícula*; n=12) and gilthead seabream (*Sparus aurata*; n=20*);*
- 1 TOP omnivorous' specie with a preference for foods of animal origin (trophic level between 2.95 -3.65), namely the whiteseabream (*Diplodus sargus*; n=20);
- 1 PURE herbivorous specie (trophic level below 2.05), namely the salema (*Sarpa salpa*; n=16);

The taxonomic classification (Order, Family), the scientific and common names, the habitat and depth range and the trophic level for all the marine fish species used in this study are displayed in Table 1.

The quantification of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni) and zinc (Zn) was made by ICP-AES (Inductively coupled plasma atomic emission spectroscopy, Thermo Scientific iCAP7200). Total mercury (Hg) was analyzed by atomic absorption spectrometry using an automatic Hg analyzer (LECO apparatus AMA 254, USA).

RESULTS: The study results are presented in mg/kg of liver (Table 2). The black scabbardfish revealed significant higher contentsin As, Cd, Cr, Cu, Pb, Mn, Ni and Zn than all the other species in comparison. On the other hand, the black scabbardfish liver Hg content did not differed significantly from the common dentex liver content ($P>0.05$), but was significantly higher than all other species. Regarding Cd, Cr, Pb, Mn and Zn contents, similar values were observed in the livers of seven of the eight species studied (exception made for the black scabbardfish). Regarding As, Co, and Zn, a greater variability was observed between fish species in comparison.

The effect of trophic level on liver heavy metal bioaccumulation was not confirmed in this study, as no significant differences for Cd, Cr, Pb, Mn, Hg and Ni were observed between salema (a strict herbivorous species) and three TOP carnivorous species, namely Common dentex, European hake and john dory. Additionally, salema (an herbivorous strict) bioaccumulate high contents of Cr and Pb, while the white seabream (an omnivorous specie) bioaccumulates high contents of Cd, Cu and Zn.

Moreover, the results showed a considerable variability within fish species, suggesting that some habitats possess highercontamination in heavy metals than others.

A different analysis of the results is the total amount of heavy metals accumulated in the fish liver. The species with higher bioaccumulation were by descending order: the black scabbard fish (681.5 mg/kg of liver), the white seabream (101.5 mg/kg ofliver) and the gilthead seabream (83.4 mg/kg of liver), while the european hake (42.2 mg/kg of liver) and the lesser spotted dogfish (26.4 mg/kg of liver) were the species with the lowest liver bioaccumulation of heavy metals

CONCLUSION: The results of the study do not confirm the influence of the trophic level on liver bioaccumulation of heavymetals among the species in study.

Table 1 – The phylogenetic Classification (Order, family and species) of the marine fish species included in this study, their common names, habitat, depth range trophic level and samples (n)

Order	Perciformes	Perciformes	Zeiformes	Gadiformes
Family	Sparidae	Trichiuridae	Zeidae	Merlucciidae
Scientific name	*Dentex dentex*	*Aphanopus carbo*	*Zeus faber*	*Merluccius merluccius*
Common name	**Common dentex**	**Black scabbardfish**	**John dory**	**European hake**
Habitat	Benthopelagic	Bathypelagic	Benthopelagic	Demersal
Depth range	15 - 50	700 - 1300	50 - 150	70 - 400
Trophic level	4.50±0.50	4.50±0.77	4.5±0.80	4.40±0.10
n	20	15	18	20
Order	Carcharhiniformes	Perciformes	Perciformes	Perciformes
Family	Scyliorhinidae	Sparidae	Sparidae	Sparidae
Scientific name	*Scyliorhinus canícula*	*Sparus auratus*	*Diplodus sargus*	*Sarpa salpa*
Common name	**Lesser spotted dogfish**	**Gilthead seabream**	**White seabream**	**Salema**
Habitat	Demersal	Demersal	Demersal	Benthopelagic
Depth range (m)	80 -100	1 - 30	0 - 50	5 - 70
Trophic levels	3.80±0.30	3.7±0.00	3.4±0.10	2.00±0.00
n	12	20	20	16

Table 2 – Heavy metal contents (wet weight) in the liver of marine fish species from different trophic levels (expressed as mg/kg of liver)

Species	Arsenic	Cadmium	Chromium	Copper	Lead	Manganese	Mercury	Nickel	Zinc
Salema	0.65±0.5^{d}	1.96±1.02^{b}	0.65±0.06^{b}	7.87±1.75^{c,d}	0.32±0.17^{b}	1.84±0.20^{b}	0.04±0.25^{b}	1.13±3.42^{b}	29.2±5.78^{c,d}
White seabream	2.32±0.4^{c,d}	13.9±13.2^{b}	0.37±0.06^{b,c}	21.7±1.57^{b}	0.16±0.15^{b}	1.93±0.18^{b}	0.43±0.23^{b}	0.84±3.06^{b}	59.8±5.17^{b}
Gilthead seabream	1.35±0.4^{c,d}	4.08±8.83^{b}	0.47±0.06^{b,c}	15.3±1.57^{b,c}	0.21±0.15^{b}	2.38±0.18^{b}	0.48±0.25^{b}	0.84±3.06^{b}	58.3±5.17^{b}
Lesser spotted dogfish	5.10±0.5^{b}	0.62±0.28^{b}	0.46±0.07^{b,c}	7.85±2.02^{c,d}	0.18±0.19^{b}	1.49±0.23^{b}	0.08±0.25^{b}	1.25±3.95^{b}	9.40±6.67^{d}
European hake	0.89±0.4^{d}	0.10±0.06^{b}	0.39±0.06^{b,c}	6.20±1.57^{d}	0.15±0.14^{b}	2.22±0.18^{b}	0.07±0.25^{b}	0.83±3.06^{b}	31.3±5.17^{c,d}
John dory	0.84±0.4^{d}	0.33±0.70^{b}	0.52±0.06^{b,c}	9.12±1.65^{c,d}	0.35±0.17^{b}	1.78±0.19^{b}	0.22±0.25^{b}	2.17±3.22^{b}	30.5±5.44^{c,d}
Common dentex	2.87±0.4^{c}	5.28±5.61^{b}	0.45±0.06^{b,c}	10.1±1.56^{c,d}	0.17±0.15^{b}	1.61±0.18^{b}	1.03±0.24^{a,b}	0.50±3.06^{b}	38.3±5.17^{b,c}
Black scabbard fish	20.0±0.5^{a}	330±89.3^{a}	1.04±0.07^{a}	30.4±1.81^{a}	3.58±0.17^{a}	9.01±0.21^{a}	1.69±0.25^{a}	29.9±3.53^{a}	192.9±5.96^{a}
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Different superscripts in the same column are associated with significant differences (P<0.05)

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Contaminant metals in bivalve molluscs from Portugal

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Bivalve molluscs play an important role in the Portuguese socio-economic context and are highly appreciated due to their sensory attributes. However, as these organisms feed by filtration, they can concentrate various chemical contaminants in their tissues, which could pose a risk to the health of the consumer. According to the legislation, these species can only be collected in authorized production areas, which are monitored to ensure compliance with chemical and microbiological criteria. As part of IPMA's regular activities, bivalve molluscs from the various production areas in Portugal have been regularly monitored for mercury (Hg), lead (Pb) and cadmium (Cd) levels. Thus, the main objective of this work was to present the results obtained during the last five years on the levels of these metals in bivalve molluscs from different production areas.

Mercury, Pb and Cd were determined by atomic absorption spectrometry, the first according to the method 7473 EPA (2007)[1] and the last ones by the standard NP EN 14084 (2003).[2]

The levels of Hg, Cd and Pb in almost bivalve species in the different production areas did not attained the limits established by the EU, 0.50, 1.0 and 1.5 mg/kg, respectively.[3] Only samples of *Scrobicularia plana* and *Crassostrea angulata* from the Tagus and Sado estuaries, respectively, presented higher levels of Pb and Cd than those limits.

This study shows that bivalve molluscs produced in Portugal, in what concerns the studied metals, do not represent a hazard for human consumption. However, the capture of *Scrobicularia plana* from Tagus estuary and *Crassostrea angulata* from one of the areas of the Sado estuary are prohibited due to high concentrations of Pb and Cd, respectively.

Funding: This work was financially supported by the project SNMB Monitor IV - Operational Program Mar2020.

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Uncertainty of chemical and microbiological quantitative methods: scenarios for conformity assessment of live bivalves

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Evaluation of measurement uncertainty (MU) is a requirement for laboratories operating ISO/IEC 17025 accreditation systems and this includes those carrying out chemical and microbiological quantitative essays in foodstuffs. Measurement uncertainty can de defined as a parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand. The main sources of uncertainty must be identified, which are usually associated with the main stages of the methodologies.
The estimation of MU for chemical methods applied in seafood is generally based in Guide Eurachem (2012),[1] whereas for microbiological methods of the food chain, including bivalves, is calculated according to ISO 19036 (2019).[2]
When performing official control of live bivalve molluscs, including monitoring of production areas, there is no guidance on the use of uncertainty for the conformity assessment of primary production and/or live bivalves placed on the market.[3]
In this study, several scenarios are presented concerning results in bivalves obtained from chemical methods used for the quantification of Cd, Hg and Pb contaminants and regulated marine biotoxins groups [3]. Moreover, different scenarios are also shown for the assessment of the level of faecal contamination in live bivalves by the quantification of *E. coli* using the most probable number (MPN) technique.
Results suggest the need of establishing a decision rule for the above parameters, in particular for chemical contaminants when their concentrations are near the EU regulatory limits. In what concerns *E. coli* levels, the distributional uncertainty associated with the MPN value of 230 can be equal to 700. This latter value is the maximum allowable in 20% of samples or sample units collected from production areas with the best sanitary status (class A) or live products placed in the market.
As a consequence, the use of MU in conformity assessment of bivalves from primary production can have a relevant impact in the management of production areas, such as the prohibition and/or reclassification of an area, with great implications in both aquaculture and fishing activities. Therefore, the rule that describes how measurement uncertainty is accounted for stating conformity of bivalves from production areas with the specified requirements should be harmonized at European level.

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Water quality on small ruminants' dairy farms in Castelo Branco region

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The importance of providing quality water for cleaning milking machines and other equipment is perhaps one of the most overlooked factors in ensuring milk quality on most dairy farms. Water for cleaning in the dairy barn is used for different situations, including for the process of milking, which requires cleaning and disinfection of the milking equipment, the milking parlour, and the milk cooling tank. Many factors on dairy farms can contribute to contamination of the raw milk, and one of the major factors responsible for this contamination is the water used to clean the milking and storage equipment.[1]

The objective of the present study was to quantify the quality of water and identify the bacterial and chemical risk milk contamination linked to water quality used in several small ruminants' dairy farms in Castelo Branco region. So, between 2019 and 2021, A total of 50 water samples were collected from 39 farms in the region under study and analysed for various physical-chemical and microbiological parameters, according to the standard methods.[2] The water supply includes public water supply, but mostly, it was found that the source of water used in the farms studied came from wells and boreholes. The water samples were collected as "point-of-use" samples, meaning that each sample was taken from a tap or water hose. Water used for this purpose should meet the requirements of at least drinking water quality, so the results were compared with the prescribed limit of the current legislation for drinking water (Decree-law 152/2017 of December 7). There were variations in the quality of water from different sources, and from similar sources, but the results obtained showed that, regardless of origin, the physicochemical parameters met the quality criteria required by Portugal standard. However, only 40% of the samples proved to have microbiological quality, which suggests the need for improvements in obtaining water that will not be a source of microbiological contamination of raw milk.

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Pilot survey of Nitrates, Nitrites, and histamine in raw Tuna in Sashimi

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Portugal is the second largest consumer of fish in the European Union (EU), with 59.91 kg per capita consumed annually in 2020.[1] According to a recent study, about 10% (~6.3 kg) of all fish consumed corresponds to raw fish, mostly in sushi and sashimi dishes, namely salmon, trout, tuna, and cod.[2] Sashimi, a traditional Southwest Asian specialty, dating back to the 7th century, is made up of raw only marine fish or seafood products.[3]

When fresh, immediately after being caught, tuna has a reddish color which, over time, because of oxidation processes, changes to a brownish color. The treatment, by injection or immersion in solutions based on nitrites, allows the restitution of the reddish color, misleading the consumer who considers the tuna as fresh. It is therefore considered a fraudulent practice to use additives such as nitrates/nitrites to change the color of tuna. In the legal framework in force in the EU, nitrates can be used as an additive "only in canned herring and sprats in vinegar" at a maximum level of 500 mg/kg. Consequently, the addition of nitrates and nitrites to fish and fishery products under conditions other than this exception is not authorized (Regulation EC 1333/2008; Regulation EC 1129/2011). However, it is estimated that 80% of fresh tuna traded in the EU is a source of illegal practices (EC, 2017).

In addition to a legal violation, this practice represents a Public Health concern, as nitrites are precursors of N-nitrosamines and other known carcinogenic compounds. For this reason, according to the IARC (International Agency for Research on Cancer) classification, ingested nitrates and nitrites are classified as probably carcinogenic to humans (Group 2A) under conditions that allow endogenous nitrosation reactions (Group 2A IARC 2012). In the EU, the European Commission's former Scientific Committee for Food (SCF, 1997) set the acceptable daily intake (ADI) of nitrates as 3.7 mg/kg body weight/day for nitrates and 0.06 mg/kg bw/day for nitrites.

Additionally, it can represent exposure to high levels of histamine, and development of the scombroid syndrome, a type of food poisoning that occurs when improperly refrigerated fish, specially from Scombroidae and Scomberesocidae families (e.g. tuna, characterized by the high histidine levels), allows bacteria present in the fish to convert histidine to histamine.

The present survey aimed to analyze fresh tuna in terms of the presence or absence of nitrates, nitrites and histamine in tuna sashimi prepared in restaurants in Coimbra. The tuna sashimi samples were acquired in 2021, during the months of May and June, in 13 Take Away establishments of Japanese cuisine, belonging to the municipality of Coimbra. In most establishments, 3 doses were collected, with each dose including 4 ready-to-eat pieces, that is, 12 samples per establishment. In four establishments, only 1 dose of 4 pieces was collected. The different doses from each restaurant were crushed, homogenized, and mixed to give rise to 13 composite samples, each corresponding to a restaurant.

For analytical determination of nitrates/nitrites, the Ultra Efficiency Liquid Chromatography (UPLC) method associated with a photodiode array detector (UPLC - ACQUITY® PDA) was used, with the following limits of quantification: Nitrites: 15 mg/Kg and Nitrates: 30 mg/kg. For histamine determination, the commercial RIDASCREEN® Histamine-enzymatic assay (R1605, r-biopharm, Germany) was used. The detection limit was 0.75 mg/kg and the limit of quantification was 2 mg/kg.

None of the 13 analyzed tuna sashimi samples presented detectable levels of nitrates, nitrites, or histamine This pilot survey reports, for the first time, an analysis of nitrates, nitrites, and histamine in tuna sashimi in Portugal. Further studies should follow, given that according to the 2021 annual report of the Alert and Cooperation Network (EC, 2022), 39% of the fraud notifications on fishery products involved suspicions of illegal treatment of tuna (carbon monoxide, nitrates, and nitrites), abuse of additives (ascorbic and citric acid) and labelling deficiencies. A foodborne outbreak was reported in RASFF with 12 persons poisoned after consuming tuna illegally treated with a high dose of nitrites. Furthermore, in 2021, 4 foodborne outbreaks were linked to histamine poisoning (EC, 2022).

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Determination of Aflatoxin M1 in milk and cheese from the Azores

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Aflatoxins (AF) are secondary metabolites produced by fungi of the *Aspergillus* genus characterized by their high toxicity to animals and humans. When ruminants ingest AFB1-contaminated feed, it undergoes metabolization mainly in the liver, originating other metabolites, namely AFM1, which is excreted in milk.[1] AFB1, according to the International Agency for Research on Cancer (IARC), are included in group 1 as a human carcinogen. AFB1, the mycotoxin with the greatest known carcinogenicity, has been shown to be associated with the occurrence of hepatocellular carcinoma, besides the evidence of its synergistic action with the hepatitis B virus (HBV).[2] Since it is impossible to eliminate the presence of mycotoxins, it is essential to ensure the implementation of strategies that reduce their concentration in products intended for human and animal consumption, as well as control the levels present in food.

The aim of the study was to evaluate the occurrence of AFM1 in bovine and goat milk produced in the Azores, intended for the manufacture of fresh cheese. It was also intended to determine the content of AFM1 in the resulting cheese and whey, to assess the exposure and risk of consumers. For this purpose, 14 sets of samples of bovine milk, corresponding fresh cheese, and whey and 6 sets of goat milk samples, corresponding cheese and whey were collected, making a total of 60 samples. These were collected between March and April 2021 and analyzed using a competitive immunoassay method (Ridascreen, R-biopharm).

It was found that, considering all analyzed samples, the frequency of contamination and the AFM1 content was higher in cheese samples (65%; 159.3 ng/L) compared to milk samples (15%; 6.9 ng /L) and whey (5%; 6.9 ng/L). In the case of goat cheese, the frequency of contamination was higher and the AFM1 content was lower (83.3 %; 116 ng/L) compared to cow's cheese (57.1%; 186.3 ng/L). These results are like those reported in previous similar studies that describe an increase in the concentration of AFM1 in cheese, although there is no maximum limit applicable in this food in the European Union (EU).

In the evaluation of human exposure to AFM1 through the consumption of fresh cheese, it was found that the consumption of fresh bovine cheese represents a greater contribution than the consumption of goat cheese. The estimated risk in the case of children was higher than that of adults, which is why they are identified as more vulnerable.

The estimated concentration of AFB1 in animal feed for cattle was 0.35 µg/kg, while in goats it was 3.99 µg/kg, and therefore below the current Maximum Limit (5 µg/kg) in force in the EU.

Additional studies should be carried out to ensure continuous monitoring and reduction of the risk associated with human exposure to AFM1, in particular of the most vulnerable groups such as children.

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Influence of wood type on polycyclic aromatic hydrocarbons profile in a Portuguese traditional fermented sausage

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Consumers are exposed to complexes cocktails of substances through the diet, some of which have negative health impacts. Among potential dietary chemical hazards, polycyclic aromatic hydrocarbons (PAHs) occur naturally in foods or as a consequence of their processing. Smoking is widely used in Portuguese traditional dry-fermented sausage to achieve aroma, external appearance, taste and preservation characteristics. The smoke generated from wood combustion under low oxygen environment, contains considerable amounts of PAHs, some of them having carcinogenic and mutagenic properties. The composition and concentration of PAH profiles, contaminating smoked meat products, is affected by many factors (type of wood, its moisture content and age and temperature associated to smoke generation). The wood characteristics and combustion environmental conditions also have a paramount effect on deposition rate and evolutionary dynamic of carcinogenic PAHs in meat products, along the smoking stage of processing and storage phase.[1,2,3]
Otherwise, wood species, smoke generation techniques and the ventilation rate evolving at the smoking area would also be different affecting the composition of the smoke itself as well as its deposition rate on products surface. The wood nature has an important influence on the amount and type of PAH found out in smoked meat products. These authors reported that the samples smoked with apple-tree and alder contain the smaller contents of individual and total PAH. The samples smoked with spruce generally have the highest values of individual and total PAH contents. The choice of wood type for smoking is one of the critical parameters to be controlled in order to reduce the contamination level of smoked food products. The current European regulation establishes 2 μg kg^{-1} as the maximum level of BaP permitted in smoked meats and meat products and 12 μg kg^{-1} in relation to the sum of benzo(a)pyrene, benzo(a)anthracene, chrysene and benzo(b)fluoranthene, known as PAH4 marker.
The aim of our study was to investigate the effect of using olive tree (*Olea europaea*), cork oak (Quercus suber), holm oak (Quercus ilex) and their mixtures on PAH contamination of a Portuguese traditional dry fermented sausage produced in central region of Portugal through artisanal processing means.
A total of 18 samples of "*Chouriço de Carne*" were taken from distinct home producers smoked by the combustion of *Olea europea* (olive tree), *Quercus suber*, *Quercus ilex* or a mixture of these two species. The duration of the smoking/drying phase differed between producers, varying between 5 to 20 days. PAHs were determined using phase reverse-HPLC method. After saponification with potassium hydroxide, water and methanol mixture and the saponified was extracted with n-hexane 4 times. The resulting fractions containing PAHs were combined and evaporated to dryness. The final residue was redissolved in acetonitrile and an aliquot injected into HPLC system.
Products smoked with *Olea europea* showed lower mean total PAHs level (837.9 μg kg^{-1}) while products processed with the mixture of *Quercus suber* and *Olea europea* presented the highest value (2414.16 μg kg^{-1}). BaP content has received particular attention due to its higher contribution to cancer in humans, being used as a marker for the occurrence and effect of carcinogenic PAHs in food. In our study, only the products smoked with *Olea europea* showed a BaP content not overpasses the limit of 2.0 μg.kg^{-1} established by UE legislation for this type of products. All products treated with smoke produced from *Quercus suber and the mixtures of Quercus suber/*Quercus ilex and *Quercus suber/Olea europea* overpass that limit (10.8 μg kg^{-1}, 2.11 μg kg^{-1} and 7.97 μg kg^{-1} respectively). In relation to the PAH4 marker, all tested wood types originated products with PAH4 levels above 12 μg.kg^{-1}, with the products smoked with *Olea europea* showing the lower mean content (14.6 μg kg^{-1}) (p=0.024). The incorporation of *Quercus ilex* to the wood mixture used in the combustion, reduced PAHs contamination of products in practically all indicators (BaP, carcinogenic PAHs and PAH4).

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Production of egg white protein films for food packaging applications

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Most of the food packaging is made of plastic materials, but these materials are harmful to the environment since they take a long time to decompose in nature, leading to severe environmental impacts. In this context, natural and sustainable materials are valued because they are more environmentally friendly and can be easily broken down. Several raw materials, such as polysaccharides, proteins, and lipids, are commonly used in edible films production. The edible films composed of such materials have some beneficial properties, such as better food preservation, and an improved performance in preventing moisture from evaporating and excluding oxygen. It is known that egg white is a natural endogenous protein and has been used widely in agro-food sectors due to its low cost, high nutritional quality, and excellent functional properties, such as foaming, gelling and emulsification. The most abundant proteins in egg white are ovalbumin, lysozyme, ovotransferrin and ovomucin. Ovalbumin is a phosphoglycoprotein with 385 amino acids. It plays a significant role in immunological and nutritional studies. Lysozyme contains five α-helical regions and five β-sheets regions that are linked by β-turns and random coils. Egg white is a rich natural source of lysozyme. The World Health Organization and many countries regard lysozyme as a preservative material in foods.
Cymbopogon martinii are perennial grasses grown and used in the fragrance industry, which produce many secondary metabolites, for example essential oils. The majority share of production of this essential oil is estimated at around 100 tons/year and is divided between India and Brazil. *C. martinii* essential oil is extracted by steam distillation of leaves; it has been widely used in aromatherapy as a skin tonic due to its antibacterial, antiviral, and anti-inflammatory properties.
The main goal of this work was to produce and characterize egg white protein films incorporating *C. martinii* essential oil as a new food packaging material. The optical, mechanical, and barrier properties of the developed films were assessed. Furthermore, the antioxidant and antibacterial activities of the egg white protein films were additionally evaluated. The chemical composition of the essential oil was studied by GC-MS, being geraniol its major compound (82.04%). The essential oil demonstrated to possess significant antioxidant activity measured by DPPH free radical scavenging assay and β-carotene bleaching method, which evaluates the inhibition of lipid peroxidation. *C. martinii* essential oil presented antibacterial activity against several strains of Gram-positive and Gram-negative foodborne pathogens. The produced egg white films incorporating the essential oil presented better mechanical properties (peak elongation, tensile index, and elastic modulus) than those of the films without the essential oil. The films were transparent (91.81%) and almost colorless. Concerning the water barrier properties, the incorporation of the essential oil in the films reduced the water permeation, probably due to its hydrophobic nature. The antioxidant and antibacterial properties of the essential oil were maintained when it was incorporated in the films, which shown to be antioxidant and active against the growth of the tested foodborne pathogens. The incorporation of the essential oil affected the thermal behavior of the egg white films, as the DSC and TGA results shown.

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State of the art of non-conventional treatments for insect control in rice storage

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Rice (*Oryza sativa* L.) is a staple food for half of the world's population. Consequently, the search of solutions to prevent rice losses by insect infestation is a priority subject for the rice value chain.
The rice weevil and the rice moth are key insect pests causing indoor infestations, as part of a set of other insect species, which chew and bore the rice grains. The most relevant species are *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Sitotroga cerealella*. To reduce insect infestations, chemicals are usually applied during storage, fumigations being the most used. However, those chemicals produced residues as contaminants which may compromise the quality of rice and its derivates. With the climate change scenarios, it is expected an increase of insect infestations, due to temperature and moisture changes, but also an increase on resistance issues caused by excessive use of fumigation. Furthermore, public regulators are also setting lower residue limits for synthetic pesticides, setting precise targets for reducing pesticide use in plant production, and directly encourage the use of alternatives.
The risk for human health, the emergence of insects resistant to the chemical insecticides[1,2], the imposed regulations and the growing consumer concerns on food residues, compromise and all the rice chain and promote the development of alternative solutions to control insect infestations in stored grains.
The practical solutions already available can be applied at industrial scale includes the atmosphere control and it is important to eliminate oxygen, since without oxygen there is no development of insects. The packages must be hermetic to prevent air entry, and vacuum packaging can be used, or packaging with inert gas such as nitrogen and carbon dioxide. These atmospheres are safe and ecologically accepted for insect control and have been proposed as viable alternative treatments. Also, the application of radiofrequency waves can be used in the disinfestation of insect pests for a short period of time, being able to eliminate the offspring and maintain the quality of the rice. Biopesticides can be an alternative to insecticides. They generally have high selectivity against pests, low toxicity, and are biodegradable. Another alternative to the use of pesticides could be ozone gas. There were already studies about the use of this gas for the removal of toxic substances in food[3] but is important to test the effectiveness of this method in controlling insect infestations. Finally, essential oils or plant extracts can be another alternative to be explored since some essential oils have antifungal and anti-insect properties.
Therefore, several non-chemical alternative treatments to prevent rice infestation are in literature, but further studies are needed to assess the economic viability of applying these solutions at industrial scale.

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Red-legged partridge (*Alectoris rufa*) hunting with lead projectiles results in lead contamination of meat portions, even after the removal of all visible lead projectiles

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INTRODUCTION: Lead is a naturally occurring metal in the Earth's crust, and it is also the most important toxic heavy metal in the environment.[1] Lead physical-chemical properties, such as softness, malleability, ductility, poor conductibility and resistance to corrosion, rendered it a broad use in many different anthropogenic activities, throughout the last 5000 years.[2] Lead continuous use by humans and its non-biodegradable nature resulted in its ubiquitous occurrence in the environment and in the food chain, being for that reason regarded as a hazard to public health.[1,2] Lead is a highly poisonous element, affecting almost every organ in the body, but the central nervous system is the main target organ for lead toxicity.[3] In adults lead-associated neurotoxicity results in cognitive impairment and may even cause psychiatric symptoms. However, children are more vulnerable to lead neurotoxicity, resulting in stronger and broader negative consequences, including cognitive loss, conditioning behavior problems, learning deficits and lowered IQ[1,3]. Moreover, in adults, several other diseases have been associated with lead toxicity, namely, chronic kidney disease, reduced fertility, anemia, hypertension and even cancer[1].

AIM: Evaluate the invisible lead contamination of red-legged partridge meat, as consequence of hunting with lead projectiles.

METHODOLOGY: A total of 40 red-legged partridges (*Alectoris rufa*) obtained from a single driven shooting hunting were gathered for the study. The red-legged partridges were plucked and eviscerated, then they were individually subjected to double X-rays imaging evaluation (lateral and dorsoventral positioning). Thereafter, all the lead projectiles previously identified by X-ray imaging were removed, using a scalpel and tweezers. Skinless meat samples from the breast and leg portions meat were obtained from each specimen, which were frozen and lyophilized. Lead quantification in red-legged partridge meat samples was performed by inductively coupled plasma optical emission spectroscopy (ICP-OES). The lead contents in breast and leg meat portions were subjected to a statistical descriptive analysis using Excel (**Figure 1**).

RESULTS: The average lead content in leg meat portion was 1172% higher in relation to breast meat portion (422.3 versus 33.2 mg/kg of fresh meat). The leg meat portion revealed a much larger standard deviation than observed on breast meat portion (1356.2 versus 109.9). The study results showed that the lead content in the leg meat portion could range between 0.06 and 7680 mg/kg of fresh meat, while in breast the lead content ranged between 0.02 and 580.2 mg/kg of fresh meat (**Figure 1**).

The European Union Scientific Committee on Food recommends that the provisional tolerable weekly intake (PTWI) should not exceed 25 µg/kg of body weight, as previously proposed by the WHO in 1986[1]. Therefore, considering an adult average weight of 70 kg, the week ingestion of lead should not exceed 1.75 mg of lead. If we consider the average lead content in breast and leg meat portions, the weekly ingestion of red-legged partridge should not overdo the 4.15 g of leg meat or 52.7 g of breast meat. However, the high lead contamination level of 3 leg meat portions reveals that the ingestion of a single gram of leg meat was enough to surpass the PTWI levels.

The wide range of lead contents in red-legged partridge observed in this study were obtained after the removal of all visible lead projectiles from the red-legged partridge, a procedure that was only possible after the X-ray imaging performed in all specimens. This procedure is not available to the common consumer.

CONCLUSION: Game meat contamination with variable amounts of lead is a consequence of using lead projectiles, even when all visible projectiles are removed from the carcass, such situation is probably the consequence of lead projectiles micro-fragmentation. This reality represents a hazard to hunters and their families, and could be solved by replacing lead projectiles by other type of projectiles, made out of nontoxic elements.

	Leg	Breast
Mean	422,30	33,20
Standard Deviation	1356,20	109,90
Minimum	0,06	0,02
Maximum	7680,06	580,30

Lead contents expressed as mg/kg of fresh meat

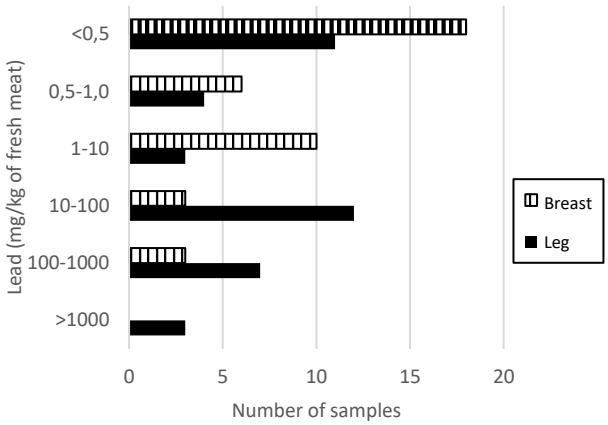

Figure 1: Lead contamination of red-legged partridge breast and leg meat portions and breast and leg meat samples in different lead contamination intervals

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Evaluation of the impact of innovative and sustainable crystal glass bottles during accelerated storage of Port wine

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Port wine is a fortified wine, produced in the Douro Appellation (Portugal), under specific conditions, in order to ensure its high quality and unique features. Port wine is commonly commercialized in glass bottles, nonetheless, specific vintages can be commercialized and stored in lead crystal glass bottles. The sonic and optical characteristics of this material are highly appreciated and valued by the consumers. Taking this into account, there is an opportunity for the development of innovative crystal glass bottles (lead-free), which guarantee the food safety principles, also contribute for environmental sustainability, due to use of sustainable mineral elements in their formulation; and that preserve the crystal glass characteristics valued by consumers. One crucial step in the product development of these new crystal glass bottles is to evaluate the impact of the packaging on the organoleptic and physicochemical characteristics of beverages. Nevertheless, it is expected that the storage of Port wines on bottles may be long, which depend on several factors related to the commercialization path and consumer habits. Thus, the development of new types of bottles is conditioned to the estimation of their impact for more or less long periods of storage of the wine in the bottle. Thus, to overcome this drawback, the aim of this work was to developed prediction models to evaluate the impact of innovative and sustainable crystal glass bottles during the storage of Port wine, based on the data obtained from an accelerated storage assay. This assay was performed at 40 °C, and several physico-chemical parameters were evaluated in Port wines along storage time, namely volatile composition (using headspace solid-phase microextraction combined with gas chromatography-mass spectrometry – HS-SPME/GC-MS), sugars contents (°Brix), potential alcoholic strength (% v/v), total phenolic compounds, total acidity, pH and color. The developed predictive models will foresee the impact of the innovative and sustainable crystal glass bottles on Port wine in a short time (few months), that may correspond to several storage years in bottle. This is an important feature for the producers once these developed models will help them in the decision support during the product development: by one side that the new bottles have sonic and optical characteristics of crystal glass, and on other side that is guarantee the food safety of Port wine, as well as its organoleptic and physicochemical characteristics are preserved.

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An Innovate and Rapid Separation Methodology Based on µSPEed/UHPLC-PDA for the Determination of Eight Pesticides in Water Samples

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Pesticides are ubiquitously used to protect crops from different aggressions and obtain higher yields in less time.[1] However, their incorrect, or excessive use can cause adverse effects on human health and the environment. Thus, control of pesticides presence in food and environmental samples (vegetables, fruits, water, and soil samples) are of great importance for consumers´ safety and human health[1]. µSPEed is a novel and more advantageous extraction approach than conventional extraction techniques because it miniaturizes and fastens the extraction process, requiring very small solvent and sample volumes and time.[2]

In this work, µSPEed procedure, operated by a semiautomatic electronic syringe, digiVol®, was optimized for the simultaneous extraction of eight pesticides (paraquat, thiabendazole, asulam, picloram, ametryn, atrazine, linuron, and cymoxanil) from residual waters. C_{18} was shown to be the best sorbent among the eight different sorbents assayed, and minor solvent and sample volumes (250µL methanol activation, water equilibration and sample loading and 2×50µL methanol elution) were required to complete the extraction in less than 5 min. This was coupled to a 7.5 min chromatographic separation using a 1.8 µm ACQUITY UPLC HSS column, at 40 °C, an acidified acetonitrile gradient and PDA detection. The good analytical performance achieved for the target analytes, combined with the simple, semiautomatic, and fast extraction and analysis, confers to the µSPEed/UPLC-PDA method here proposed a great potential for pesticides analysis in residual waters.

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Alimentos Funcionais

Freeze-dried aqueous extract from sea buckthorn (Hippophae rhamnoides) leaves: phenolic composition and application in functional ice creams

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Sea buckthorn (*Hippophae rhamnoides*) produces orange-yellow berries, which have been used over centuries as food. The berry contains high vitamin C and carotenoid contents, but the food company does not usually use the leaves for product development. Thus, this study aimed to recover the phenolic compounds from sea buckthorn leaves (SBL), characterise its phenolic composition via liquid-chromatography and spectrophotometric assays, and assess the effects of SBL freeze-dried aqueous extract on the quality traits of Italian-type ice cream. SBL were extracted via maceration (1:10 w/v, 50 ºC, 60 min) and freeze-dried (FD-SBL). FD-SBL presented 200 mg/g of total phenolics, 93 mg/g of proanthocyanidins, 31mg/g of ellagitannins, and 3000 µmol Trolox equivalent/g of antioxidant activity (ORAC assay). The main phenolic compounds were pinitol (302 mg/g), quercetin (21 mg/g), quinic acid (15 mg/g), and 3,4,5-trihydroxy benzoic acid (14 mg/g). Italian-type ice creams added with sea buckthorn fruit pulp were prepared with (F1) or without (F2) the addition of 0.5% of FD-SBL and compared with vanilla ice cream (blank). Total phenolic content was 30 and 50 mg/100 g for F2 and F1, respectively. The antioxidant activity measured followed the same trend: FRAP (F1 = 82 mg ascorbic acid equivalent, AAE/100 g; F2 = 18 mg AAE/100 g), and DPPH (F1 = 60 mg AAE/100 g; F2 = 13 mg AAE/100 g). The hedonic test (**Figure 1**) with potential consumers showed that there was no difference ($p>0.05$) in the degree of liking of taste, odour, and overall acceptance, whereby the mean sensory scores were above 7 (like moderately) on a 9-point structured scale. However, the F1 formulation had lower ($p<0.05$) mean values for colour (F1=6, F2=8.1). Overall, SBL was shown to be a valuable source of antioxidants to be incorporated in functional ice creams.

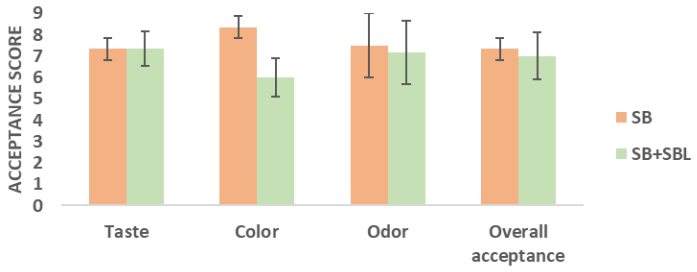

Figure 1: Sensory attributes of ice cream samples made with sea buckthorn pulp (SB) and/or sea buckthorn pulp and freeze-dried SB leaf extract (SBL, 0.5% w/w).

Functionality assessment of *Scolymus hispanicus* (golden thistle) for its daily-basis incorporation in the Mediterranean diet

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Golden thistle (*Scolymus hispanicus* L.) is naturally distributed in the Mediterranean region. Its roots and fresh rosettes are traditionally consumed in soups and special meals, and have been reintroduced in some European countries since the consumption of native species is an integral and crucial part of the so-called Mediterranean diet; long associated with several beneficial health effects against diseases spread worldwide[1]. Although most of these native species are traditionally collected in the wild by the local communities, the increasing demand for such edible plants has created a market niche for the commercial exploitation of Wild Edible Plants (WEPs). This practice may fulfill consumer demands for product availability throughout the year, as well it prevents the risk for genetic erosion due to irrational gathering. There are reports, available in the literature, for cultivation practices of native species and how these practices may affect its nutritional profile, chemical composition, and bioactive compounds content[2]. The aim of the present study was to evaluate the effect of fertilization with nutrient solutions that contained different ratios of nitrogen (N), phosphorus (P), and potassium (K) on the nutritional profile (AOAC methods) of *S. hispanicus* edible plant parts. The mineral content was determined by atomic absorption spectrophotometry. Energy was calculated according to the equation: energy (kcal per 100 g) = 4 x (g protein + g carbohydrate) + 2 x (g total dietary fiber) + 9 x (g fat). The sample fertilized with 200:200:200 ppm of N:P:K (S222) stood out for its fiber content (40.7±0.2 g/100 g dry weight), followed by the sample S211 (fertilized with 200:100:100 ppm of N:P:K) that also showed promising crude protein values (10.8±0.3 g/100 g of dw); however, the crude protein content showed no significant differences between this sample (S211) and samples fertilized with 100:100:100 N:P:K (S111) and S222, respectively. The total dietary fiber content was different among the seven experimental treatments, which suggests the influence of the nutrient solution on this parameter. Sample S111 showed similar fat content to the control sample. The control sample (without fertilization) showed the highest levels in the majority of the studied parameters, except for fiber content, carbohydrates, and energy. The sample fertilized with 300 ppm of nitrogen had the lowest values in relation to fat, crude protein, and fiber contents. The energy calculation ranged from 301 to 285 kcal/100g of dry weight, while the sample with the highest energy value had the highest carbohydrate content. Mineral composition was affected by fertilization treatments for most of the minerals evaluated in the present study. Sample S311 (fertilized with 300:100:100 ppm of N:P:K) showed the highest amount of sodium, calcium, and magnesium and the lowest content of potassium and zinc. On the other hand, the control sample had the lowest amounts of sodium, magnesium, manganese and copper and the highest levels of potassium, iron, and zinc. Iron and other micro minerals are an essential part of many compounds in the oxygen transport and storage system and function as cofactors for enzymes[3]. It was possible to verify that the concentration of nitrogen, phosphorus and potassium in nutrient solution may directly affect the nutritional value and mineral content of the plant under study, with high concentrations of nitrogen presenting a negative impact on the protein content, indicating the low response of the species to increasing nitrogen fertilization rates. With the results obtained, it is possible to select the appropriate nutrient solution to obtain golden thistle with a promising nutritional profile and high mineral contents, as well as to promote its incorporation into commercial farming systems and the exploitation in a more sustainable manner through tailor-made fertilization regimes.

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Characterization of different fractions of fruit products obtained from the SUMOL+COMPAL pilot line to obtain Clean label fruit derivatives

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Currently, food waste is a critical global issue, since approximately 1/3 of all food produced in the world is wasted somewhere in the food supply chain from initial agricultural production down to final household consumption. Despite an estimated 1.3 billion tonnes of food wasted each year, approximately 700 million people are still affected by hunger and 3 billion people cannot afford a healthy diet. Furthermore, the degradation of food waste contributes to more than 3.3 billion tons of CO_2, consuming about 250 km of blue water and nearly 1.4 billion hectares of land occupied (FAO, 2011).

There is currently a huge demand for "clean label" food products in Europe because people are becoming more heed than ever to their health and wellbeing (*The Clean Label Guide to Europe*, 2014) to offer solutions to these issues, the "cLabel+: Innovative food", "clean label" natural, nutritious, and consumer-oriented project was developed. Led by SUMOL+COMPAL and promoted by a consortium that brings together twenty entities, it aims to tackle the problems related to circular economy and promote nutritional value through macromolecules enhancement, with reduction of salt-and sugar, the replacement of additives with more natural and healthier alternatives, to ensure food safety and to redefine the products with clearer and more intuitive labelling information. The solution to food waste reduction in the food value chain, which means the transition from a linear to a circular food economy to phase out this concept and contribute to a more sustainable world.

For this work, SUMOL+COMPAL within the cLabel+ project provided apple by-products resulting from the processing of fruit juices at the pilot plant. In the first instance, these fractions were characterized at the nutritional, physicochemical and rheology levels, (**Figure 1**) to deeply characterise these products. In addition, as it was already done for the use of pear by-products (Lomba-Viana *et al.*, 2022), the study focused on the development of a satiating snack, incorporating the most interesting by-products previously evaluated, namely the "pomace" for its dietary fiber content, a component that has increased interest for the consumer benefit. The snack also incorporates inulin to obtain the "Food Fiber Source" – "Nutrition Claim" - and a consortium of microalgae since they have been recognized as a source of various phytochemicals with outstanding functional activity and significant positive health impact, and they are also a sustainable food ingredient that can be extremely useful in terms of climate change and food scarcity.

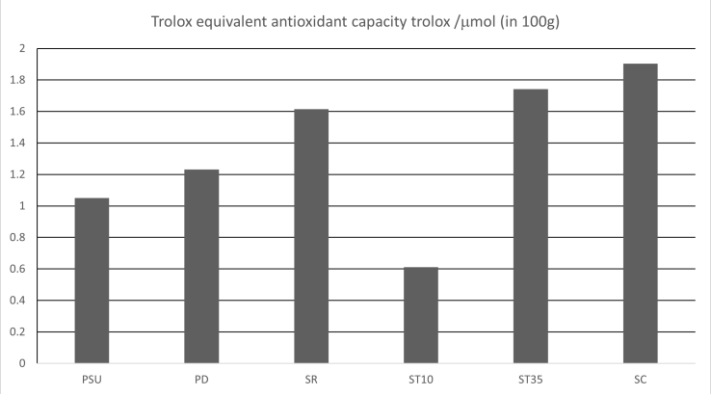

Figure 10:Trolox equivalent antioxidant capacity of the apple fractions

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Nutraceutical value of *Camellia sinensis* blend with aromatic medicinal plants: Comparison of antioxidant properties, phenolic and flavonoids contents

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Aromatic plants have been used in the Middle East since approximately 5000 BC for their preservative and medicinal properties, and for improving the aroma and flavor of foods.[1] Herbal preparations have been used as traditional medicines to ameliorate several diseases, acting as, antioxidant, antiseptic, anti-inflammatory, antimicrobial, calming effects, among others and they also may reduce the risk of cancer and/or cardiovascular diseases.[2] There is increasing interest for industry and scientific research in aromatic herbs mainly for their antioxidant and antimicrobial properties. These properties are due to many active phytochemicals such as, vitamins, flavonoids, polyphenols, terpenoids, carotenoids and curcumins. Generally, the bioactive components in the aromatic plants have an important role in the oxidative stress protection, because their ability to protect the body from damage caused by free radicals.[2,3] *Camellia sinensis* is also a plant with scientifically proven beneficial effects on health and the blend with aromatic plants can add value for human health. Taking to this in account, the main objective of this study was to compare the antiradical capacity, phenolics and flavonoids contents from five aromatic plants: *Aloysia citrodora, Cymbopogon citratos, Mentha pulegium, Pimpinella anisum, Stevia rebaudiana* and *Camellia sinensis* green tea. The determination of free radical scavenging activity – FRSA, ferric reducing antioxidant power – FRAP, Ferric Ion-Chelating – FIC, the total phenolic – TPC and total flavonoid – TFC contents were determined by colorimetric methods. The results presented in **Table 1** revealed that *C. sinensis* presented, higher values for FRSA, FRAP and TPC. However the aromatic plants also presented significant results in terms of FRSA and FRAP, in particular, *A. citrodora*, *M. pulegium* and *S. rebaudiana*. For FIC, the highest value was found in *C. citratus* (80.60%). *M. pulegium* and *A. citrodora* revealed a significant value for TPC, but lower than *C. sinensis*. For TFC, the highest value was shown in *A. citrodora*. This study revealed the importance of some aromatic plants in terms of biological activities and that the combination with green tea is a perfect blend for added value with beneficial effects on human health.

Table 1. Antioxidant activities (free radical scavenging activity – FRSA, ferric reducing antioxidant power – FRAP and ferrous - FIC), total phenolic - TPC and flavonoid – TFC contents in aromatic plants and *Camellia sinensis*[a].

	FRSA (EC_{50}, µg/mL)	FRAP (EC_{50}, µg/mL)	FIC (%)	TPC (mg GAE/g DE)	TFC (mg RE/g DE)
Aloysia citrodora	14.14 ± 0.14	11.42 ± 0.18	51.90 ± 0.88	187.15 ± 1.11	204.64 ± 1.47
Cymbopogon citratus	35.30 ± 0.61	39.50 ± 0.05	80.60 ± 1.43	82.85 ± 2.19	49.74 ± 1.46
Mentha pulegium	14.22 ± 0.23	12.07 ± 0.14	53.71 ± 0.42	199.15 ± 1.23	92.84 ± 1.11
Pimpinella anisum	94.48 ± 0.17	75.00 ± 0.14	36.71 ± 2.77	47.37 ± 1.15	28.08 ± 1.03
Stevia rebaudiana	20.40 ± 0.91	15.10 ± 0.14	42.80 ± 2.51	163.66 ± 2.55	74.55 ± 1.42
Camellia sinensis (green tea)	4.13 ± 0.06	6.40 ± 0.11	76.18 ± 1.18	325.14 ± 0.88	51.85 ± 0.88

[a] Values are mean ± SD (*n = 3*). EC_{50}, Half-maximal effective concentration. GAE, Gallic acid equivalents. RE, Rutin equivalents. DE, dry extract.

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Strategies for reducing the allergenicity of hen egg by treatment with phenolic compounds

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Food allergies are caused by abnormal immune responses to food components (allergens), namely proteins. Hen's egg allergy has increased worldwide due to the ubiquity of eggs. Ovalbumin (OVA) is the most abundant protein in egg white and can cause IgE mediated allergy, especially in children and juvenile.[1] The processes used to decrease the allergenicity of egg proteins have not been effective, because the proteins' epitopes have high stability and resistance. Phenolic compounds (PC) are recognized for their antioxidant action and also for forming complexes with proteins, leading to alteration of their native structure and a potential decrease of their allergenicity. [2] Therefore, the goal of this work was to develop a process to reduce the allergenicity of hen egg proteins through the action of natural antioxidants, such as PC. Thus, OVA solutions prepared in 50 mM phosphate buffer pH 7.4 were incubated for 10 minutes, at different temperatures, with the following phenolic compounds: gallic, caffeic, ferulic, chlorogenic and tannic acids, resveratrol, and quercetin. OVA conformational changes in consequence of the interaction with PC were analysed through fluorescence, circular dichroism (CD), and Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR).[3] The CD and ATR-FTIR results showed changes in the secondary structure of OVA, while the fluorescence spectra demonstrated that PC quenched intrinsic protein fluorescence. Moreover, fluorescence data suggests changes in OVA tertiary structure and the formation of OVA-PC complexes. Fluorescence derived thermodynamics indicate that these complexes are thermodynamically favored (ΔG<0). Molecular docking with SwissDock (www.swissdock.ch) and Chimera (http://www.cgl.ucsf.edu/chimera/) showed that the PC can interact directly with OVA epitopes or in its neighborhood, preventing IgE binding (**Figure 1**). Therefore, the application of PC for treatment of egg proteins appears to be a potential alternative to the current methods used to decrease its allergenicity.

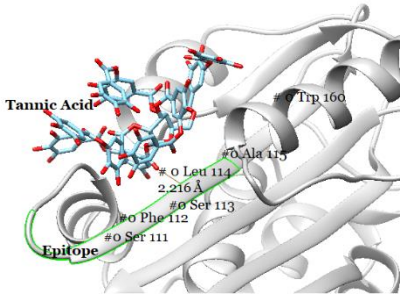

Figure 1: Tannic Acid (TA) binding to OVA epitope with a Hydrogen bond between residues of Ser111 and TA.

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Can avocado oil enriched fresh cheese modulate the obesity-related metabolism?

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Currently, obesity is one of the most important public health problems. Strategies for its prevention involve modifications in the diet, and in this way, functional foods have an important contribution. In this regard, virgin avocado oils present an interesting nutritional profile due to the presence of high levels of oleic acid and other bioactive compounds, such as alpha-tocopherol and beta-sitosterol. This composition enables them to be used as a functional ingredient in the management of several health conditions such as hypercholesterolemia, diabetes, and fatty liver diseas.[1,2] In this work avocado oil structured as a bigel was used to develop a fresh cheese rich in oleic acid. After gastrointestinal tract simulation, the oleic acid permeability was evaluated using the intestinal Caco-2/HT29-MTX co-culture model. The impact of permeated fatty acid in obesity-related metabolism was evaluated in terms of hepatic lipid accumulation, adipolysis, and adipokines secretion. In addition, inflammation biomarkers were monitored in 3T3-L1 and RAW cell lines. The results showed that avocado oil fresh cheese reduce by 22% the accumulation of triglycerides in differentiated adipocytes and reduce the adipokines secretions. Moreover, the presence of these samples was responsible for the reduction in pro-inflammatory cytokines secretion (IL-1α, TNF-α, and IL-6) in macrophages and a reduction in TNF-α, IL1-β, IL-6, and INF-γ secretion in differentiated adipocytes.
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Extrusion cooking effect on antioxidant activity and total phenolic content in novel gluten-free flours enriched with Fibersol and Passion fruit

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In recent years, there has been growing interest in the use of gluten-free flours by the food industry in order to satisfy the demands of the celiac population. This has led to the use of rice or pulses flours for the development of ready-to-eat products, most of them produced by extrusion cooking[1]. Pulses are an important part of the Mediterranean diet as they present a rich composition in proteins, complex carbohydrates, dietary fibre, vitamins and minerals[2]. The aim of this study was to evaluate the influence of the extrusion process in different gluten-free formulations, based on rice and chickpea which were enriched with Fibersol (0 –10%) and passion fruit (0 –20%).

Antioxidant activity was determined by three different *in vitro* methodologies (Folin-Ciocalteu, FRAP, and DPPH), in addition the total phenolic content (TPC, Fast Blue BB method) and hydroxybenzoic acid (HBA) content were also measured. All the determinations were carried out through QUENCHER (QUick, Easy, New, CHEap and Reproductible) methodology, which allows to avoid extraction steps, as it is based in the direct contact of the homogenized solid sample with the corresponding reagents of each determination[3]. In this way, the activity of soluble and insoluble compounds is quantified allowing to obtain more reliable results.

The highest values of TPC and HBA were found in the samples with a 12.5% of passion fruit (7.84 mg GAE/g and 5.35 mg GAE/g), as well as the highest activity measured by FRAP assay (6.93 mg TE/g). On the other hand, sample with a 20% of passion fruit presented the highest antioxidant activity measured by DPPH and Folin-Ciocalteu assays (8.73 mg TE/g and 8.54 mg GAE/g, respectively). Regarding the influence of the extrusion process, it could not be evidenced a specific behaviour, however, it could be notices that several of the samples with the lowest amounts of passion fruit, presented representative losses of TPC and antioxidant activity after extrusion cooking. Nonetheless, most of the samples with the highest amounts of passion fruit (12.5 and 20%) maintained or even slight increase their TPC and antioxidant activity.

It was evidenced that the addition of passion fruit in the analysed gluten-free flours, not only increased its amount of phenolic compounds and antioxidant activity, but also avoid the loses of this compound that generally occur by the use of extrusion cooking. Therefore, it can be concluded that the addition of fruits such as passion fruit can be a resourceful strategy to enhance the antioxidant activity as well as the bioactive composition of pulses flours aimed to produce ready to eat snacks.

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Biosorption studies of iodine by brewer`s spent yeast to be used as natural iodine-food-carrier

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Iodine Deficiency Disease (IDD) affects one-third of the global population and is highly prevalent in children and pregnant women.[1] The Recommended Dietary Allowance (RDA) for iodine in nonpregnant adults is 150 µg/day, for pregnant women the RDA is 250 µg/day. Although the universal salt iodization has been the common strategy to prevent/control IDD, it presents chemical disadvantages. Hence, the development of efficient natural iodine-food-carriers is crucial. This work is the first attempt to evaluate the potential of brewer´s spent yeast (BSY), the second major by-product of the brewing industry, to biosorb potassium iodate from aqueous solutions.

For such purpose, kinetic and equilibrium experiments were performed using non-living (lyophilized) BSY biomass. Considering a previous study of the effect of pH solution on the potassium iodate biosorption capacity of BSY biomass, the experiments were carried out at pH 4, at room temperature and at predefined initial concentrations of potassium iodate and mass of BSY. The quantification of potassium iodate was made by the Sandell-Kolthoff method.[2] The BSY, before and after potassium iodate uptake, was characterized by the determination of the point of zero charge (pH_{PZC}), Fourier transform infrared (FT-IR) analysis, and scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS).

The Elovich's, pseudo-first-order's, and pseudo-second-order's models were fitted to the experimental kinetic results. The kinetics of potassium iodate was better described by the pseudo-2nd order model. The equilibrium was achieved in 5 min and the kinetic constant determined was 0.40 ± 0.04 $g_{BSY}/(\mu g_{iodate} \cdot min)$. The Freundlich's, Langmuir's, Langmuir-Freundlich's (Sips), Redlich-Peterson´s, and Tóth's models were fitted to the experimental equilibrium results. The system followed the Sips isotherm model. The maximum biosorption capacity of potassium iodate was 13 ± 6 $\mu g_{iodate}/g_{BSY}$.

This work demonstrates the great potential of BSY biomass for the development of a nutraceutical for the treatment/prevention of iodine deficiency. The ingestion of 6.0 g/day of iodine-enriched BSY could satisfy around 50% of RDA for adults and around 30% of RDA for pregnant women.

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Continuous Lipase-catalyzed synthesis of MLM-type structured lipids containing capric acid, in packed-bed bioreactors, using crude olive pomace oil as raw material

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The health benefits of a Mediterranean diet are associated with the consumption of specific products, which can be considered functional foods, namely virgin olive oil. Olive oil is extracted only by mechanical methods from the olive fruit (*Olea europea* L.). Then, a solid byproduct (olive pomace) having 3-4 % of oil (wet basis), is obtained. This oil can be recovered using solvent extraction. The olive pomace oil (OPO), presents similar composition to that of olive oil, and once refined, is employed in human consumption. In order to increase the OPO valorization, several studies have been implemented [1].

The aim of the present work was the valorization of OPO with high acidity for the synthesis of structured lipids (SLs) with reduced caloric value (c.a. 4.5 kcal/g instead 9 kcal/g for natural lipids). These SLs are triacylglycerols (TAGs) with improved nutritional properties, which contain medium-chain fatty acids (M) at positions *sn*-1 and *sn*-3 and long-chain fatty acids (L) at position *sn*-3 (MLM)[2]. They can be produced either by (i) interesterification with ethyl esters or by (ii) acidolysis with free fatty acids, using *sn*-1,3 regioselective immobilized enzymes. In the present study, a commercial immobilized lipase from *Thermomyces lanuginosus* (Lipozyme TL IM), kindly donated by Novozymes, was used as biocatalysts, in a continuous packed-bed bioreactor, in solvent-free systems. In each reaction, the continuous column bioreactor operated from 67 to 122 h.

Three crude OPO from different batches were used after centrifugation. The OPO acidity was 12-30 %, and all samples presented high values of oxidation products and chlorophyll pigments. All the reactions were developed with molar ratio between OPO and ethyl ester/acid forms of 1:2. Along continuous reactions, the yield of new TAGs, conversion of TAGs and ethyl esters or medium-chain fatty acids (MCFAs) were determined. The biocatalyst showed high operational stability along continuous bioreactor operation. In interesterification reactions with C10 ethyl, no deactivation of Lipozyme TL IM was observed, while a first order deactivation, with a half-life time of 290 h, was observed in acidolysis with C10:0.

This study demonstrated the feasibility of producing MLM structured lipids in continuous bioreactors, using crude acidic oils and hence lowering oil refining process costs, valorizing OPO, and contributing for process sustainability.

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Bioactive properties of *Sorbus aria* (L.) Crantz fruits: antioxidant and antimicrobial activities

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In Spain there is a great diversity of wild fruits that have traditionally been used for therapeutic and culinary purposes. Its uses for health and its role in food have been studied in many nutritional studies (Petkova et al., 2020). Moreover, synthetic fungicides are widely used in the protection of crops (Maggi et al., 2019). The Farm to Fork strategy announced the need to reduce by 50% the use and risk of chemical pesticides by 2030 (European Commission, 2020). These Mediterranean wild fruits could be an interesting source of antioxidants for functional foods and food supplements, as well as source of natural pesticides due to its nutritional value and biological activity, such as antioxidant and antimicrobial activities. *Sorbus aria* (L.) Crantz or whitebeam is a member of subfamily *Maloideae, Rosaceae* family, occurring in almost all mountainous regions of southern and central Europe, from the Iberian Peninsula and southern Italy to the Balkans (Petkova et al., 2020). The fruits are red or brown, when ripe in autumn, globose, less than 1 cm and edible. This work is aimed to characterize the antioxidant, antibacterial, and antifungal activities in the epicarp of the fruits of *S. aria*.

The samples were identified and gathered in their natural habitat, and optimal ripening status between September and October 2021, with collection permits Ref. PN-NC_032021 National and Ref. ABSCH-IRCC-ES-257749-1 issued by the Ministry of Agriculture, Fisheries and Food, Government of Spain. The fruits were collected in two different Spanish locations (Zarzuela de Galve- Valverde de los Arroyos in Guadalajara; and Puerto de la Quesera in Segovia) to obtain a representative sample. The fruits were peeled and processed to be freeze-dried, being crushed to obtain a fine powder.

The antioxidant activity of dried plant material from *S. aria* has been assessed *in vitro* using QUENCHER methodology (Del Pino et al., 2015). The Q-Folin method was an adaptation of the FC trial developed by Slinkard & Singleton (1977). The antioxidant capacity by the Q-DPPH method was determined following the methodology proposed by Del pino et al. (2015). The Q-FRAP methodology described by Benzie & Strain (1996) was used for the determination of the Fe (III) reduction activity by Q-FRAP analysis, with modifications from Del Pino et al. (2015), at 595 nm.

Table 1: *In vitro* antioxidant activity by Quencher of *Sorbus aria* (L.) Crantz frutis. Values expressed as mean ± standard deviation (SD), dw (dry weight), TE (Trolox equivalent), GAE (gallic acid equivalent).

***Sorbus aria* (L.) Crantz (whitebeam)**			
	Q-Folin (mg GAE·g^{-1} dw)	Q-DDPH (mg TE·g^{-1} dw)	Q-FRAP (mg TE·g^{-1} dw)
Site 1	*8.98 ± 0.28*	8.77 *± 0.37*	68.23 *± 1.21*
Site 2	8.22 *± 0.22*	*8.70 ± 0.37*	*53.48 ± 3.36*
Average	**8.60**	**8.74**	**60.86**

The antibacterial activity of the dried plant material was carried out testing the extracts against five Gram-negative bacteria, and three Gram-positive bacteria. The Minimum Inhibitory Concentration (MIC) / Minimum Bactericidal Concentration (MBC) were determined using colorimetric assay according to described by Pires et al. (2018). The antifungal activity was performed according to described by Heleno et al. (2013), testing the extracts against *Aspergillus fumigatus* (ATCC 204305) and *Aspergillus brasiliensis* (ATCC 16404). The determinations of minimum inhibitory concentration (MIC) / Minimum fungicidal concentration (MFC) were made by a serial dilution technique using 96-well microplate until determining the minimum concentration that inhibits the growth of the fungus. The commercial fungicide ketoconazole (1 mg/mL) was used as a positive control and the culture medium Malt Extract was used as a negative control.

In the current study, fruits of the common whitebeam (*S. aria*) were characterized as a natural source of antioxidants. These antioxidant properties of the fruit demonstrated their potential for the preparation of foods with potential beneficial effects for a healthy nutrition. About the antimicrobial activity, on the one hand, the antibacterial activity of *S. aria* is moderate, especially against Gram negative bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa* and, in particular, against *Salmonella enterocolitica*. On the other hand, the extracts showed antifungal activity, inhibiting the growth of *A. brasiliensis,* however no inhibition of fungal growth by the extracts of the fruits analysed was detected for *A. fumigatus*. These data reveal the existing antifungal potential presented by natural sources as red wild fruits and open new fields for research.

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Crataegus monogyna Jacq.: source of antioxidants for functional foods and antifungal activity for natural pesticides

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Crataegus monogyna Jacq. is widely distributed and considered native to Europe, belonging to the *Rosaceae* family, which fruits are the hawthorn berries. There are many studies that have been carried out of the bioactivities of *C. monogyna*, in which its antioxidant and antimicrobial activities are proven (Garcia-Oliveira et al., 2020).[1] These activities are related to the content of phenolic compounds, such as phenolic acids and flavonoids, which is variable depending in the analysed tissue. All these molecules are mostly found in the epicarp so it would be interesting to use whole fruits or their peel as a source of antioxidants and antimicrobial compounds for the formulation of foods with functional properties as well as for the formulation of natural pesticides for crops. For all this, the present work is aimed to characterize the antioxidant, antibacterial, and antifungal activities in the epicarp of the fruits of *Crataegus monogyna* Jacq. using QUENCHER methodology for the determination of the *in vitro* antioxidant activity, as well as the determination of antimicrobial activity against different bacteria and fungus using broth microdilution assay.

The samples were identified and gathered in their natural habitat and optimal ripening status between September and October 2021, with collection permits Ref. PN-NC_032021 National and Ref. ABSCH-IRCC-ES-257749-1 issued by the Ministry of Agriculture, Fisheries and Food, Government of Spain. The fruits were collected in two different Spanish locations (El Encín, Alcalá de Henares; and Valdelatas, Madrid), from different trees and shrubs, to obtain a representative sample. Once in the laboratory, they were peeled and processed to be frozen and then freeze-dried, being crushed as soon as possible to obtain a fine powder.

The antioxidant activity of dried plant material from *C. monogyna* has been demonstrated in vitro using three methodologies coupled by QUENCHER technique: Q-DPPH, Q-FRAP and Q-Folin. The QUENCHER methodology (Del pino et al., 2015)[2] was used to carry out the tests, allowing a direct reaction, using very little sample, generating less waste. The antioxidant capacity by the Q-DPPH method in dry plant material was determined following the methodology proposed by Del pino et al. (2015).[2] The Q-Folin method was an adaptation of the FC trial developed by Slinkard, & Singleton (1977). The Q-FRAP methodology described by Benzie and Strain (1996) was used for the determination of the Fe (III) reduction activity by Q-FRAP analysis, with modifications from Del Pino et al. (2015),[2] at 595 nm.

Regarding the antimicrobial activity, on the one hand, the antibacterial activity of the dried plant material was carried out according to described by Heleno et al. (2013),[3] testing the extracts against five Gram-negative bacteria and three Gram-positive bacteria. On the other hand, the antifungal activity was performed according to described by Heleno et al. (2013),[3] testing the extracts against *Aspergillus fumigatus* (ATCC 204305) and *Aspergillus brasiliensis* (ATCC 16404). The assays were conducted to determine the Minimum Inhibitory Concentration (MIC) / Minimum Bactericidal Concentration (MBC) for the determination of the antibacterial activity, and the determination of the Minimum inhibitory concentration (MIC) / Minimum fungicidal concentration (MFC) for the antifungal activity. Two negative controls were prepared, one with TSB or Malt Extract and another one with the extract. Two positive controls were prepared with TSB/Malt Extract and each inoculum and another with medium, antibiotics/antifungal, and bacteria.

It was observed that the epicarp of *C. monogyna* present antioxidant activity. This activity may be associated with a cardioprotective effect related to the traditional uses of these fruits reported by Garcia-Oliveira et al. (2020)[1] and can be due to the limitation of free radical formation, as well as reducing the deposition of cholesterol in the arteries and possible damage to the heart. About the antimicrobial activity, the antibacterial activity of *C. monogyna* is moderate, especially against Gram negative bacteria, in particular, against *Salmonella enterocolitica*. On the other hand, fungistatic activity has been observed against *Aspergillus brasiliensis* and *Aspergillus fumigatus*, both common contaminant in food and crops. These activities can support the use of hawthorn berries or extracts as ingredients for functional foods or natural pesticides.

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Influence of different cultivation conditions on the biomass growth and protein content of *Chlorella vulgaris*

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Predictions suggest that the global population will reach nearly 10 billion, by 2050[1,2]. This phenomenon, along with the extreme dependency on animal protein, leads to an unsustainable demand, compromising protein needs at a global scale.

Seeking for new protein alternatives represents an urgent priority. Microalgae have arisen as excellent sustainable protein sources since they entail several advantages when compared with animal-based and vegetal protein sources. They can grow using non-arable land and in wastewater or even seawater, with greater photosynthetic and growth rates, as well as improved ability to capture CO_2, when compared to protein vegetal sources. In addition, some microalgae attain a high protein content – up to 70% of dry weight -, and produce a wide range of bioactive compounds with application in food, cosmetic, and nutraceutical fields.[1] During the cultivation of *Chlorella vulgaris*, using a standard growth medium, it was possible to obtain biomass containing a protein content of 45.7 % of dry weight, which agrees with other published studies.[2] Nonetheless, high cultivation costs and low productivity yields remain major bottlenecks towards scale up and economic feasibility. Given the ability of microalgae to change their biomass composition and metabolic patterns under stress conditions, manipulating growth conditions to induce the production and accumulation of protein is essential to harness the potential of microalgae as a protein source for food applications.[3]

Nitrogen and phosphorus content in the culture medium, as well as light intensity, were some of the variables that show to have significant effects on the final protein content[1]. For instance, consensual results seem to point that nitrogen concentration in the medium is directly correlated to the protein content, until a certain point.

This work provides some insights about how the cultivation conditions of *Chlorella vulgaris* can be tuned for the accumulation of proteins within the produced biomass, envisaging food applications.

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Study of the antiproliferative and antitumour effects of chia (*Salvia hispanica* L.) seed

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Currently, it is widely recognized that one's risk of developing many types of cancers can be influenced by diet, and estimates suggest that roughly one-third of all human cancers can be attributed to diet, therefore dietary changes could significantly reduce the incidence of certain cancers. The regular consumption of bioactive compounds present in foods has been a protective measure against the development of cancer. These bioactive compounds can interfere in specific physiological targets, such as redox status, inflammation and mutagenic processes, which are related to several diseases, and therefore are essential for health maintenance. Studies have shown that bioactive compounds, specifically phenolic compounds, act as positive anticancer regulators through modulation of the oxidative stress response, inhibiting cancer cell proliferation and modulating autophagic signalling.[1]

Chronic inflammation plays a vital role in both the initiation and development of carcinogenesis, and chronic inflammation has been reported to mediate various key steps involved in tumorigenesis, including cellular transformation, survival, proliferation, invasion, angiogenesis, and metastasis.[2] Within the tumour microenvironment, inflammation generally contributes to favourable conditions that promote both the development of genomic aberrations as well as the progression towards a malignant state, as inflammation has the potential to cause mutations due to the production of reactive oxygen species (ROS) by inflammatory cells.[2] Studies with extracts derived from plants and seeds, or using fruits and edible seeds containing high content of the bioactive compounds, have focused on the evaluation of their antitumour effects, due to their anti-inflammatory and antioxidant actions.[1]

In this context, Chia (*Salvia hispanica* L.) is an herbaceous plant that belongs to the *Lamiaceae* family, native from southern Mexico and northern Guatemala, and its seeds are source of the phenolic compounds, such as caffeic acid, danshensu, rosmarinic acid and ferulic acid.[3] Thus, new preventive agents, especially those of easy access such as agents incorporated in diets, are necessary, and chia seeds and its products represent a promising alternative. Firstly, cell-based assay with a human colorectal cancer (CRC) cell line (HT29 cells) was performed to explore the effect of phenolic compounds from chia seed in inhibiting cancer cell growth and targeting cancer stem cells using monolayer cultures. Second, this study also evaluated the effects of the regular consumption of chia seed incorporated into the diet in the prevention of tumour growth in mice using the Ehrlich solid tumour model, an aggressive and fast-growing murine breast adenocarcinoma, characterized by the generation of local inflammatory response. Thirty male Balb/C mice were randomized into five groups (n = 6). Control group (vehicle/negative control) and the doxorubicin group (positive control) were fed with the American Institute of Nutrition (AIN-93M) diet, and other groups were fed with their respective AIN-93 diets added with 10%, 15% e 20% of chia seed, during a period of 56 days.

The result corresponding to the antiproliferative effect of extract from chia seed using HT29 cell monolayers is shown in Figure 1. The extract had ability to impair cell proliferation in dose-dependent manner in a 2D cell model of CRC after exposition for 72h with concentrations that varied from 0.3125 to 20 mg/mL (**Figure 1A**). The presence of phenolic acids (caffeic, rosmarinic and ferulic acids) in the extract, in particular rosmarinic acid could partly explain the antiproliferative effect observed on HT29 cancer cells, as stated in other studies.[1] Regarding the antitumour assay, the groups that consumed 10%, 15% and 20% of chia seed presented smaller tumours (**Figure 1B**), with a slight reduction in tumour mass compared to the control group (24%, $p>0.05$). Thus, it can be concluded that the regular consumption of chia seeds in the diet may confer a preventive action against tumour development. Certainly, phenolic acids of chia seed have great potential to be explored and future studies will be carried out to confirm its potential preventive action in chronic models of carcinogenesis.

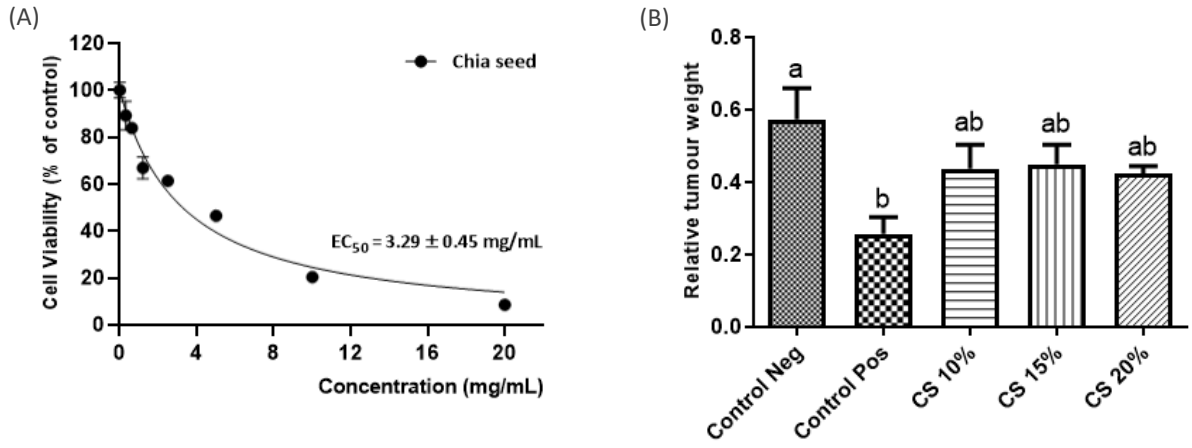

Figure 1: Antiproliferative and antitumour effects of the chia seed (A) Dose-response curves of the antiproliferative effect induced by extract of chia seed in HT29 cell monolayers after 72h (EC_{50} = 3.29 ± 0.45 mg/mL). Results shown means of at least 3 independent experiments performed in triplicate ± SD. (B) Relative tumour weight of the Ehrlich solid tumour from the different experimental groups. Control neg: AIN-93M diet; Control pos: AIN-93M diet plus treatment with doxorubicin (3 mg/kg); CS10%: 10 % (w/w) chia seed; CS15%: 15 % (w/w) chia seed and CS20: 20 % (w/w) chia seed. Bar graphs were expressed as mean ± SEM (standard error of mean ((n= 6/group)). Different letters (a-b) indicate significant differences detected by one-way analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$).

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Phytochemical profile and physicochemical characteristics of Fundão sweet cherries

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Portugal produces thousands of tons of sweet cherries per year, being in the northeast of the country, comprising Fundão, Covilhã and Belmonte municipalities, where their cultivation is more practiced and favourable, since the low humidity levels and temperatures around 25.0 °C, promote the development of attractive organoleptic characteristics and enhancement of phytochemicals.[1] Actually, and regarding consumers' acceptability and market prices, physical attributes, as well as sweetness (total soluble solids-TSS) and sourness (titratable acidity-TA) and their maturity index (TSS/TA ratio) and colour, assume a major role, providing valuable information to facilitate cultivar selection to meet market demand.[2] In this context and given the economic importance of sweet cherries in this region, we decided to evaluate the physicochemical characteristics, the phenolic profile by HPLC-DAD-ESI/MS^n and the volatile compounds by SPME/GC–MS of 23 sweet cherries from Fundão region. The analysis of the physicochemical properties of the selected cultivars indicated that length and width ranged between 1.85-2.59 and 2.08-2.8 cm, respectively; weight varied between 4.65 to 11.75 g; firmness ranged from 7.28 to 20.05 N; moisture and ash contents ranged from 75.08 to 88.56% and 0.44 to 2.88%, respectively. The darkest cultivars were Santina and Cristalina, while Sunburst and Sweetheart were the clearest ones, showing also low TSS and maturity index values. Additionally, a total of 46 phenolics were identified, including 19 hydroxycinnamic acids, 2 hydroxybenzoic acids, 13 flavonols, 5 flavan-3-ols, 2 flavanones, 1 flavanonol and 4 anthocyanins.[2] As expectable, cyanidin 3-*O*-rutinoside and chlorogenic acids and were the predominant ones.[1] Regarding the volatile composition, there were detected and quantified 66 compounds, namely 16 aldehydes, 23 alcohols, 6 ketones, 6 esters, 8 monoterpenes, 3 norisoprenoids, 2 hydrocarbons and 2 acids. Among compounds, benzaldehyde, hexanal, nonanal, benzyl alcohol, (E)-2-hexen-1-ol, 1-hexanol, (Z)-2-hexen-1-ol, 2-ethyl-1-hexanol, linalool, α-terpineol and α-ionone were the main ones.[3] The obtained data revealed that sweet cherries represent a supply of high-value bioactive compounds, which are usually associated with the health benefits of their consumption, namely antioxidant, anti-inflammatory and antimicrobial properties. Their characteristics are mainly influenced by genotype. Additionally, these results also will aid in the selection of superior and more desirable cherry cultivars that meet the consumers' expectations, and incentive their incorporation into new pharmaceutical products, smart foods and nutraceuticals.

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Olive oil-based spread functionalized with an olive pomace extract: antioxidant advantages

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Two-phase olive oil production is increasing, leading to the obtention of higher amounts of olive pomace (OP), the major by-product of this process. The high organic load and phytotoxic compounds of OP represent an environmental hazard and new approaches must be implemented to valorise this by-product and reduce environmental constraints. Green methodologies to obtain extracts rich in healthy compounds, assessing their viability to be active ingredients in novel foodstuffs, are strategies that will help to upcycle OP, close the loop, and achieve a real circular economy.[1]
In this work, OP from Trás-os-Montes and Alentejo (the two major Portuguese olive oil production regions) were pooled and processed by combination of physical parameters (time and pressure) to separate the OP liquid phase (EP patent application PCT/IB2018/060111; US patent application 6954060), which can be regarded as an OP functional ingredient (OFI).
OFI can be an advantageous ingredient for food products as a nutritional profile enhancer, since it comprises specific lipid and hydrophilic bioactive compounds, usually not present in other plant extracts, such as hydroxytyrosol (HT). It was, thus, used to functionalize a previously developed olive oil-based spread (containing over 70% of olive oil).
To develop the olive oil-based spread, the lipidic ingredients were first solubilized in the lipid phase and the OFI was mixed with the water-soluble ingredients. The phases were blended to form an emulsion and cooled.
Two different formulations were developed and analysed (A and B). Both presented a similar composition (70% olive oil and OFI), but sample B also contained coconut oil. Also, three commercially available vegetable creams (C1, C2, and C3) were selected and submitted to the same chemical analysis for comparison and evaluation of the potential nutritional advantages of the olive oil-based spread functionalized with OFI.
The extracts were prepared according to Capannesi *et al*.[2], with slight modifications, and the analysis included total phenolics content (TPC)[3], antioxidant activity (ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH$^{\bullet}$))[3] and HT quantification by HPLC-DAD-FLD[1].
Sample B showed a higher TPC (33 mg gallic acid equivalents (GAE)/100 g) in comparison to sample A (28 mg GAE/100 g). Both presented higher TPC than the commercial creams (2-4 mg GAE/100 g). Regarding the HT content, A and B formulations contained higher amounts of this phenolic compound (2.2-2.4 mg/100 g) in comparison to C2 (0.02 mg/100 g), probably due to the extra amount provided by both olive oil and the OFI addition. Moreover, the other two commercial creams did not present HT.
Concerning the antioxidant activity, sample B presented the highest FRAP result (33 mg ferrous sulphate equivalents (FSE)/100 g), followed by sample A (26 mg FSE/100 g), then C3, C2, and C1 (22 > 16 > 12 mg FSE/100 g, respectively). In the DPPH$^{\bullet}$ assay, the formulation B and C3 presented the highest values (25-27 mg trolox equivalents (TE)/100 g), followed by A, C1, and C2 (18 > 12 > 8 mg TE/100 g, respectively).
Besides antioxidant properties, HT is also known for its antimicrobial activity. Several mechanisms of action have been proposed, particularly: i) capability of chelating transition metals, reducing the reactivity of iron and copper by forming an inert metal-ligand complex, which decreases the bioavailability for bacterial growth; ii) reduction of intracellular ATP concentrations; iii) cell membrane depolarization; and iv) reduction of the bacterial protein content.[2] These properties might not only provide beneficial health effects, but also help to extend the shelf-life of the developed spreads.
In conclusion, olive oil-based formulations with OFI incorporation presented significant nutritional advantages compared to the commercial vegetable creams, regarding their antioxidant profile. Moreover, their richness in a healthy fat such as olive oil may also provide interesting amounts of oleic acid (C18:1*n*9*c*), which is associated to cardiovascular health protection.
In addition, the developed spreads are different from those already available in this food market segment because i) the bioactive compounds present (from olive oil and olive pomace) have synergistic beneficial effects on health; ii) their main ingredients are obtained naturally, without the use of solvents; iii) are healthier alternatives, and iv) reduces the environmental impact of the increasing olive oil production.

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Cherry stem infusion: a potential beverage to restrain intestinal glucose uptake

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Cherry stem infusions (CSI) are traditionally consumed worldwide, especially to prevent and manage recurrent urinary tract infections. However, other beneficial effects have been described in literature such as draining, sedative, anti-inflammatory, antioxidant, anti-hemolytic, and antidiabetic.[1,2] Nevertheless, more studies are still needed to better understand the concrete biological properties of this infusion and its potential use as a functional beverage. In this work, the antidiabetic potential of CSI was better explored by studying their effects on intestinal glucose uptake.

For that, dried cherry stems intended to be consumed in the infusion form were purchased in an herbalist shop. Infusions were prepared, in triplicate, according to the manufacturer's recommendation, thus mimicking the way they are commonly prepared and consumed at home.

In order to ascertain the effects of CSI on intestinal glucose uptake, human intestinal epithelial (Caco-2) cells were incubated (37 °C, 24h) with CSI (25%, v/v) or H_2O (25%, v/v) (control). Then, glucose apical uptake was measured by incubating the cells (37 °C, 6 min) with 10 nM ^{3}H-deoxy-D-glucose (^{3}H-DG). In addition, the effect on the apical-to-basolateral transepithelial transport (apparent permeability (P_{app})) to ^{3}H-DG (10 nM) was studied using Caco-2 monolayers grown in a Transwells® system (polyester (PET) microporous filters, 0.4 µm of pore size). Finally, quantification of the mRNA levels of the intestinal glucose transporters GLUT2 and SGLT1 was also performed by RT-qPCR.

The results showed that CSI markedly reduced ^{3}H-DG apical uptake (~55%, p<0.05). Furthermore, a tendency for a reduction in the apical-to-basolateral P_{app} to ^{3}H-DG was also observed (~25%, p>0.05). In these experiments, the amount of ^{3}H-DG present in the cells was significantly reduced in the presence of CSI (~36%, p<0.05), thus corroborating the former results. Finally, these effects seemed to be related to a reduction in the expression levels of both GLUT2 and SGLT1, as expressive and significant reductions in their mRNA expression levels were found (GLUT2: ~75%; SGLT1: ~63%, p<0.05). Of note that all these effects were not related to a cytotoxic effect of CSI on Caco-2 cells, as determined through the LDH assay.

In conclusion, this work showed that inhibition of intestinal glucose absorption constitutes a potential target of this commonly consumed infusion to act in the prevention/management of type II diabetes mellitus and other metabolic-related disorders. So, these results highlight the possible use of CSI as a functional beverage.

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Cucumis melo L. peel flour: Potential as food ingredient

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Food sustainability is a current and extremely important concept, as it ensures sufficient, safe and nutritious food for the world's population and future generations. However, there are some problems that can compromise its implementation, such as the increase in food waste. On the other hand, the modern societies are facing two quite different situations: the developed countries that are dealing with obesity and over-consumption of food, while the population of some developing countries is undernourished and starving. Therefore, the valorisation of these food residues, for example, to develop and formulate new food products with beneficial properties for health can be an advantageous approach. *Cucumis melo* L. is usually consumed as a fruit, but peels and seeds are discarded. However, it has excellent flavor qualities and nutritional composition, being an excellent source of bioactive compounds for humans. [1,2,3]

The aim of this study was to evaluate the nutritional composition, the total phenolic content and antioxidant potential of the *C. melo* L. peel flour.

In 2021, the *C. melo* L. samples were collected from melon production and distribution companies (Frutas A. R. Santos, Torres Vedras, Portugal and Planície Verde, Rio Maior, Portugal). Nutritional composition was analytically determined; the energy value and available carbohydrates were calculated. Antioxidant activity of *C. melo* L. peel flour was determined using two different methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and ferric reducing antioxidant power (FRAP). Total phenolic content was also assessed by spectrophotometry. The determination of total amino acids was performed by UHPLC with diode array detection.

According to the obtained results, dietary fibre and available carbohydrates are the main constituents of the *C. melo* L. peel flour, 50 and 24 g/100 g, respectively. Regarding total phenolic compounds, the *C. melo* L. peel flour presented a content of 249 mg gallic acid equivalents/100 g. For DPPH$^{\bullet}$, the value obtained was 26 mg trolox equivalents/100 g, while for FRAP, 863 mg trolox equivalents/100 g were observed. The main amino acids present in *C. melo* L. peel flour is glutamic acid (891 mg/100 g), aspartic acid (653 mg/100 g) and alanine (423 mg/100 g). Regarding essential amino acids, the *C. melo* L. peel flour presented a content of 668 mg/100 g.

In conclusion, *C. melo* L. peel flour can be considered a good source of dietary fiber and total phenolic compounds and can be a sustainable option to enrich/develop new food products with beneficial properties for the health of the population, also contributing to the reduction of food waste.

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Olive pâté incorporating olive pomace: a sustainable and functional food product

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Olive oil production generates high amounts of olive pomace (OP), a biomass composed of olive skin, pulp, and stones. This is a promising food ingredient owing its high content in bioactive compounds. However, for food applications, the pieces of OP stones must be removed, leading to the olive pomace paste (OPP). This work aimed to valorize heat-treated OPP (HT-OPP) by incorporation into an olive pâté.

OP was obtained from an olive mill (Trás-os-Montes) and the remaining pits were manually removed using a stainless-steel sieve leading. The obtained OPP was heat-treated at 88 °C for 15 sec, using a Vorwerk Thermomix TM 31®. Next, three olive pâtés were produced with increasing percentages of HT-OPP (0, 20 and 25%) and processed by high pressure processing (HPP) at 550 MPa/3 min, at 10 °C, in a pilot-scale equipment with a 55 L vessel (Hiperbaric 55, Burgos, Spain) to assure microbial safety by pasteurization. A commercial olive pâté was also analyzed to compare the commercial potential of the pâtés with HT-OPP.

The nutritional composition [1] and hydroxytyrosol content (HPLC-DAD-FLD) of all the olive pâtés were assessed. A sensory analysis (CATA test, acceptability, and purchase intention) was also carried out.

The olive pâtés with HT-OPP showed interesting chemical attributes. Indeed, the nutritional composition analysis showed that both (with 20 and 25% HT-OPP) had significant high-fibre contents. More important, these pâtés showed to be an excellent source of hydroxytyrosol.

The sensorial tests (CATA, acceptability, and purchase intention) highlighted differences between the pâtés and showed that those incorporating HT-OPP could be considered potential commercial products. In fact, in the CATA test, negligible differences were found between the pâtés incorporating 20 and 25% HT-OPP concerning the typicity attributes. The acceptability test showed that consumers liked the pâtés incorporating HT-OPP, and more importantly, both registered a considerably higher acceptability score than the commercial pâté. In the same way, the purchase intention test showed that consumers would more likely buy the pâtés incorporating HT-OPP than the commercial pâté.

In conclusion, the pâtés incorporating HT-OPP are promising commercial products that can meet current food trends: health concerns, sustainability, and convenience. Additionally, the one incorporating 25% of HT-OPP was regarded as the best commercial option considering the circular economy of the Portuguese olive oil production chain.

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Cladodes flour and coffee silverskin powder as ingredients for low-carbohydrate diets: is the use of these by-products feasible?

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The search for sustainable and economically adequate alternatives for human nutrition is extremely important in view of the growing demand for food around the world. In this sense, many answers can be linked to the use of unconventional foods and residues from the agricultural food industry, topics on which the number of scientific research grows everyday. Added to this, the concern with nutritional quality of these foods, especially regarding the level of macronutrients, in which a significant reduction of carbohydrates in food formulations starts to serve a large portion of the population that has adhered to the so-called *lowcarb* and *ketogenic* diets, where wheat and sugar are practically abolished.

Opuntia ficus-indica (L.) production has an economic importance since the fruit is eaten raw and used in foodstuffs and seeds are used for oil extraction. In many countries cladodes are still not consumed in regular diets as they are considered a by-product. Due to the invasive behavior of this plant, the pruned cladodes constitute an environmental concern, being the valorization of this biomass a necessity and an opportunity. A similar situation occurs with coffee silverskin, a by-product from coffee roaster industry with very important nutritional and biological properties already demonstrated in the scientific literature.[1]

The objective of the present work was to develop *lowcarb* bread formulations using the replacement of ingredients from a traditional *lowcarb* bread formulation by cladode flour (formulation 1) and by cladode flour and coffee silverskin powder (formulation 2).[2] In addition to these substitutions, aquafaba was chosen instead of eggs, used in the traditional formulation, making the formulations more economically suitable. The developed formulations also meet the growing demand of the population for vegan foods, becoming vegan alternatives.

To meet the main objective of the study, preliminary nutritional analyses of the proposed formulations were performed [3] and the results treated using R software (version 4.1.1). To perform the statistical analysis, one-way ANOVA for independent variables and Tukey's post-hoc test was used. To reinforce the analysis of the ANOVA results, MANOVA was used for a multivariable comparison, to identify in this first moment of the study if there is interference of the independent variables on the dependent ones.

The results showed that macronutrients content (protein, carbohydrate, and fat) of formulations 1 (F1) and 2 (F2) did not show significant differences ($p > 0.05$). The analysis of the dependent variables (protein, carbohydrates, fat, and ash) showed differences between F1 and F2 and the traditional *lowcarb* bread formulation (TF), the latter showing higher levels of fat and protein, result suitable for a formulation with eggs as ingredients. On the other hand, F1 and F2 showed lower levels of carbohydrates and higher levels of ash, indicating that the use of by-products such as cladode flour and coffee silverskin powder may represent nutritional alternatives and low-cost ingredients for the food industry. These approaches can be significant answers to the replacement of wheat flour in bakery products, for example. Furthermore, the salt content in the traditional *lowcarb* bread formulation, also used in the two formulations proposed by the study, is above the EU recommended levels for bakery products (1.4%), suggesting that salt addition must be reduced in these products.

To conclude, regarding their nutritional composition, cladodes flour and coffee silverskin powder are promising and sustainable ingredients for food formulations, namely in bakery. Also, they can be gluten-free and *lowcarb* alternatives to be used in special diets.

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Can coffee silverskin be as promising as green coffee on type II diabetes prevention?

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Several studies document the potential benefits of green coffee (GC) on the modulation of glucose metabolism and, consequently, its potential to prevent or modulate some metabolic disorders, such as type II diabetes (DM2).[1] This work aimed to ascertain the effects of coffee silverskin (CS), the major by-product of coffee roasting, on intestinal glucose uptake in comparison with green coffee and, hence, the possibility of valorizing it.

For that, GC and CS were used to prepare extracts by a green extraction method (ultrasound-assisted extraction, using only water as solvent). The lyophilized extracts were chemically characterized by RP-HPLC-DAD regarding caffeine content and chlorogenic acids profile (CGA), using a Tracer-Excel ODSA column (5 µm; 250 x 4 mm) and a gradient solvent system: A) 0.2% acetic acid in water and B) methanol. Caffeine was monitored at 274 nm and CGA at 320 nm.[2]

To ascertain the effects of extracts on intestinal glucose uptake, Caco-2 cells were incubated (37 °C, 24h) with extracts (1 mg/mL) and, then, glucose uptake was evaluated by incubating the cells (37 °C, 6 min) with 10 nM ^{3}H-deoxy-D-glucose (^{3}H-DG).[3] Finally, a quantification of the mRNA levels of the intestinal glucose transporters (GLUT2 and SGLT1) was also performed after incubating Caco-2 cells (37 °C, 24h) with the extracts.

As expected, the GC extract presented significantly higher amounts of CGA compared to the CS extract (~253.1 mg/g *vs.* ~8.1 mg/g, respectively) and, in both, 5-caffeoylquinic acid (5-CQA) was the major CGA (~129.8 mg/g in GC extract and ~3.5 mg/g in CS extract), followed by 5-feruloylquinic acid (5-FQA, ~52.6 mg/g and ~2.6 mg/g in GC and CS extracts, respectively). In addition, other CGA (4-CQA, 3-CQA, and 4-FQA) were also detected in both extracts, although in minor amounts. Caffeine was the major compound in the CS extract (~32.8 mg/g), but even though the GC extract presented higher amounts (~51.7 mg/g).

Considering the results above, it would be expected that CS would have a significantly lower ability to inhibit intestinal glucose uptake compared to GC. Nonetheless, no significant differences (p>0.05) were found between the two extracts on the effects on ^{3}H-DG uptake and both extracts presented high and significant reductions (p<0.05, GC: ~37% of reduction; CS: ~28%). Finally, they both significantly reduced the mRNA levels of the GLUT2 transporter (GC: ~67% of reduction; CS: ~64%, p<0.05), but only the GC extract reduced the mRNA levels of the SGLT1 transporter at a significant level (~36%, p<0.05).

These results are quite promising as they show that despite the CS extract was not as rich as the GC extract in caffeine and CGA, it was able to similar and effectively reduce the ^{3}H-DG uptake at a significant level, suggesting that other compounds rather than caffeine and 5-CQA also contribute to ^{3}H-DG uptake reduction. Finally, for both extracts, the effects on ^{3}H-DG uptake seemed to be mainly related to a reduction of the mRNA expression levels of the GLUT2 transporter, although reductions (significant for GC extract) of the mRNA expression levels of the SGLT1 transporter have also been found.

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Wild endogenous mushroom species: ethanolic and aqueous extracts for biological studies

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Since ancient times, mushrooms have been increasingly appreciated in the traditional cuisine due to their value organoleptic and nutritional characteristics. Furthermore, mushrooms are considered as functional foods, since they present in their chemical composition several compounds [1], such as polysaccharides, proteins, lipids, which have bioactive properties, such as anticancer, neuroprotective or hypocholesterolemic properties[2].

In this work, four edible Portuguese endogenous mushroom species with gastronomic importance were studied- *Hydnum* spp ("pés-de-carneiro"), *Lactarius* spp ("sanchas"), *Macrolepiota* spp ("frades") and *Tricholoma* spp ("míscaro amarelo"), as well as the medicinal *Ganoderma* spp mushrooms ("pipa"). The ethanolic extracts from all mushrooms are rich in lipophilic compounds, determined by FTIR, and free sugars, determined by gas chromatography. The mushrooms with gastronomic importance were rich in mannose, mainly the *Macrolepiota* spp, possibly resultant from mannitol[3]. The aqueous extracts presented polysaccharides, rich in glucose, and proteins.

The ethanolic extracts obtained from *Ganoderma* spp of four different colors (brown, red, yellow and white) presented similar FTIR spectra to the edible mushrooms. The sugar composition of all extracts and residues showed that glucose was the major sugar, mainly in *Ganoderma* spp.

The ethanolic and aqueous extraction allowed to selectively extract several types of compounds, obtaining extracts that could be used in studies of the biological activity of these compounds, such as hypocholesterolemic activity.

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Quimiometria na Ciência dos Alimentos

Critical study of moisture determination in fish samples using microwave radiation

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The lyophilization process of seafood matrices allows the determination of chemical elements using spectroanalytical techniques obtaining advantages such as quantifying low levels of analytes due to the preconcentration step and facilitating the sample mineralization step. For the mercury determination using Direct Method Analyze (DMA), the lyophilization step is mandatory[1]. However, analytical methods developed using lyophilized samples require the moisture determination of the samples in order to express the results of the analyses as wet mass.

On the other hand, the determination of moisture in marine food matrices is not an easy task. Procedures using infrared radiation, conventional oven heating, microwave radiation[2], and the Karl Fisher method have been proposed. Methods using microwave radiation have been the most employed, although the uneven power distribution during the application of the MW radiation causes excessive localized heating, resulting in sample deterioration.

This work reports the critical evaluation of moisture determination in fish samples by microwave radiation using multivariate experimental design techniques for optimization[3]. The tests were carried out using minimal amounts of samples to avoid the inconvenience of uneven distribution during the radiation. The fish species investigated were: *Argyrosomus regius* (corvina), *Caranx latus* (Guaricema), *Sardinella janeiro* (sardinha), Oreochromis niloticus (tilápia), *Merluccius merluccius* (pescada branca).

Two-level full factorial designs were performed with the experimental dominions varying from 600 to 1000 W for microwave radiation power and 30 and 120 sec. for the radiation time. All the tests demonstrated no significant interaction between these two factors.

General procedure

Weigh a mass of fish sample from 1.40 to 1.60g, place under microwave radiation at 800W power for 75 seconds, then cool in a desiccator and weigh again. Repeat this cycle until constant weight.

The precision expressed as relative standard deviation (RSD) was determined for a *Sardinella Janeiro sample with a moisture content average of 74.9% being 0.14% for an observation number of 5.* The procedure was applied for the moisture determination, and the results are *Argyrosomus Regius (81.4%), Caranx Latus* (76.4%), *Sardinella Janeiro (74.9%), Oreochromis Niloticus* (78.7%) and *Merluccius Merluccius (81.1%). These results* agree with other data reported in the literature.

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