BOOK OF ABSTRACTS

XXI EUROFOODCHEM

22-24 November 2021

On-line conference

EuChemS
European Chemical Society
Division of Food Chemistry

Sociedade Portuguesa de Química

Serbian Chemical Society

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XXI EUROFOODCHEM CONFERENCE

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Conference organized under the auspices of the Food Chemistry Division of the European Chemical Society (FCD-EuChemS), the Portuguese Chemical Society (SPQ) and the Serbian Chemical Society.

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22-24 November 2021

Scientific Program

(Timetable is set for CET)

22th November	
14:30-14:45	Opening Ceremony
14:45-15:20	**PL1. Richard Stadler** Virtual Room 1 – "Real and not-so-real problems of chemical food safety"
15:20-15:50	**Oral and flash presentations** Virtual Room 1 – OC1, Flash 1-3 Virtual Room 2 – OC2, Flash 4-6
15:50-16:40	Poster session 1 – Wonder platform
16:40-17:15	**PL2. Michele Suman** Virtual Room 1 – "Untargeted analysis with GC-Orbitrap as powerful tool for the authentication of spices and herbs: focus on oregano"
17:15-18:00	**Oral presentations** Virtual Room 1 – OC3-5 Virtual Room 2 – OC6-8

23th November	
14:30-15:05	**PL3. Lenka Kouřimská** Virtual Room 1 – "Insects as sustainable alternative food and feed protein source"
15:05-15:50	**Oral presentations** Virtual Room 1 – OC9-10, Flash 7-9 Virtual Room 2 – OC11-12, Flash 10-12
15:50-16:40	Poster session 2 – Wonder platform
16:40-17:15	**PL4. Victor Freitas** Virtual Room 1 – "Molecular approaches for understanding and modulating food taste to make food healthier and more tasty "
17:15-18:00	**Oral presentations** Virtual Room 1 – OC13-15 Virtual Room 2 – OC16-18

24th November	
14:30-15:05	**PL5. Yiğit Altay** Virtual Room 1 – "The analysis of the future of food: What, why and how? "
15:05-15:50	**Oral presentations** Virtual Room 1 – OC19-20, Flash 13-15 Virtual Room 2 – OC11-12, Flash 16-18
15:50-16:30	Poster session 3 – Wonder platform
16:30-17:05	**PL6. Robert Wolff** Virtual Room 1 – "Total utilisation of marine biomass – How can Norwegian seafood industry create more value and bring healthy products to the marked? "
17:05-17:50	**Oral presentations** Virtual Room 1 – OC23-25

Detailed Program

22th November (CET)

14:30-14:45	Opening Ceremony
Plenary session 1	
Virtual Room 1 – Chairs: **Tanja Cirković Veličković** and **Michael Murkovic**	
14:45-15:20	**Richard Stadler** - Real and not-so-real problems of chemical food safety
Virtual Room 1 – Chairs: **Tanja Cirković Veličković** and **Michael Murkovic**	
15:20-15:35	OC01 – David Moreno González – A worldwide study of pesticide residues in fruit-based soft drinks using liquid chromatography/tandem mass spectrometry
15:35-15:40	F01 – Maria da Luz Galante Maia – Synthetic musks in shrimp and seawater samples from the NW Portuguese coast
15:40-15:45	F02 – Cátia Sofia Faria Martins – Extension of shelf-life of lager beer can be a solution to prevent beer wastage resulting from its reduced consumption during the SARS-Cov-2 pandemic?
15:45-15:50	F03 – Lukas Bodenbender – Development of a prototype GC-(ion trap)MS/MS-IMS-system
Virtual Room 2 – Chairs: **Slavica Ražić** and **Maja Natić**	
15:20-15:35	OC02 – Margita Utczás – Analysis of WADA prohibited substances in ecdysterone-containing dietary supplements
15:35-15:40	F04 – Bram Miserez – Food fingerprinting techniques for the authentication of oregano
15:40-15:45	F05 – Philipp Weller – Non-targeted VOC profiling by GC-IMS and machine learning - principles and applications
15:45-15:50	F06 – Mónica Honrado – DNA-based methods as a powerful tool for the entomological authentication of honey
15:50-16:40	***Poster session*** – Wonder platform
Plenary session 2	
Virtual Room 1 – Chairs: **Joana Amaral** and **Manuel Coimbra**	
16:40-17:15	**Michele Suman** – Untargeted analysis with GC-Orbitrap as powerful tool for the authentication of spices and herbs: focus on oregano
Virtual Room 1 – Chairs: **Joana Amaral** and **Manuel Coimbra**	
17:15-17:30	OC03 – Leslie Valeria Simon – Deep-learning assisted data augmentation of spectral data for the authentication and quality analysis of food products
17:30-17:45	OC04 – Charlotte Capitain – Optimized headspace gas chromatography-ion mobility spectrometry (HS-GC- IMS) and non-negative matrix factorization (NNMF) for non-targeted VOC profiling of fermented dairy

17:45-18:00	OC05 – Helmut K. Mayer – Extended shelf life (ESL) milk displaces pasteurized fresh milk from Austrian market – boon or bane?
Virtual Room 2 – Chairs: **Zuzana Ciesarová** and **Michael Granvogl**	
17:15-17:30	OC06 – João Siopa – Development of a fast method for prediction acrylamide formation in industrially produced biscuits with and without the use of asparaginase
17:30-17:45	OC07 – Lucía González Mulero – Acrylamide content in common Spanish culinary preparations and exposure from household, catering and industrial settings
17:45-18:00	OC08 – Rosa Pilolli – In-house validation of a prototype reference method for six allergens detection in chocolate by HPLC-MS/MS analysis

23th November (CET)

Plenary session 3

Virtual Room 1 – Chairs: **Cristina Todasca** and **Livia Simon Sarkadi**

14:30-15:05	**Lenka Kouřimská** – Insects as suitable alternative for food and feed source
Virtual Room 1 – Chairs: **Cristina Todasca** and **Livia Simon Sarkadi**	
15:05-15:20	OC09 – Cláudia Pereira Passos – Raspberry fruit drying stabilization and application in the development of muffins
15:20-15:35	OC10 – Filipa Fernandes – Nutritional enrichment of "Económicos" through the incorporation of chestnut flour
15:35-15:40	F07 – Joscha Christmann – Monitoring of fermentation processes by gas chromatography-ion mobility spectrometry (GC-IMS) and machine learning
15:40-15:45	F08 – Ricardo Moura Ferreira – Production of *Opuntia ficus-indica* fermented beverage: The effects of fermentation time and pasteurization methods on the physicochemical proprieties
15:45-15:50	F09 – Mareike Krell – Determination of benzyl isothiocyanate-protein conjugates in a vegetable- enriched bread with different cress genera
Virtual Room 2 – Chairs: **Matthias Wüst** and **Beatriz Oliveira**	
15:05-15:20	OC11 – Bianca Rodrigues de Albuquerque – Chemical composition and bioactivities of the *Nephelium lappaceum* L. epicarp
15:20-15:35	OC12 – Eleomar Pires Júnior – *Tradescantia zebrina* Bosse: study of the phenolic composition and bioactive properties of a potential natural coloring ingredient
15:35-15:40	F10 – Ana Luísa Pires Fernandes – Recovery of polyphenols and polysaccharide-polyphenols conjugates from grape pomace. Application for type II diabetes mellitus prevention
15:40-15:45	F11 – Rafael Mascoloti Sprea – Volatile composition and bioactive properties of lemon verbena (*Aloysia citrodora* Palau) essential oil: comparison of two extraction methods
15:45-15:50	F12 – Ana Barros – Preliminary study of winery by-products from Dão Region: Phytochemical potential to fight multidrug bacteria resistance

15:50-16:40 ***Poster session*** – Wonder platform

Plenary session 4

Virtual Room 1 – Chairs: **Małgorzata Starowicz** and **Celestino Santos-Buelga**

16:40-17:15 **Victor de Freitas** – Molecular approaches for understanding and modulating food taste to make food healthier and more tasty

Virtual Room 1 – Chairs: **Małgorzata Starowicz** and **Celestino Santos-Buelga**

17:15-17:30 OC13 – Bartosz Fotschki – Stimulation of intestinal microbiota with fructooligosaccharides favourably enhances the effects of raspberry polyphenols in rats

17:30-17:45 OC14 – Marcelo Dias Catarino – Impact of *Fucus vesiculosus* phlorotannins on the human gastrointestinal tract

17:45-18:00 OC15 – Gregorio Peron – A multi-target strategy to prevent urinary tract infections: the dual mechanism of action on intestinal barrier and urinary epithelium of a novel nutraceutical combining cranberry extracts, D-mannose and ascorbic acid

Virtual Room 2 – Chairs: **Marija Stojadinovic** and **Vieno Piironen**

17:15-17:30 OC16 – Helena Kieserling - Structure analysis of proteins at interfaces

17:30-17:45 OC17 – Inês Filipa Mourão Ferreira - Oxidative stability of beer assessed by electron paramagnetic resonance (EPR) spectroscopy

17:45-18:00 OC18 – Marta Malheiro Leite - Validation of an analytical methodology for mycotoxin determination by UHPLC-MS/MS in the maize value chain

24th November (CET)

Plenary session 5

Virtual Room 1 – Chairs: **Marco Arlorio** and **Karel Cejpek**

14:30-15:05 **Yiğit Altay** – The analysis of the future of food: What, why and how?

Virtual Room 1 – Chairs: **Marco Arlorio** and **Karel Cejpek**

15:05-15:20 OC19 – Zuzana Ciesarová – Innovation in puffed breads production: asparaginase application to acrylamide diminishing

15:20-15:35 OC20 –Laura Alessandroni – Organic chicken meat in a compostable biopackaging solution: a comparative shelf-life study

15:35-15:40 F13 – Lukáš Kolarič – The production of low-cholesterol milk products

15:40-15:45 F14 – Kamgang Nzekoue Astride Franks – Vitamin D from edible mushroom wastes: a new sustainable approach

15:45-15:50 F15 – Sara Alexandra Cunha – Microalgae hydrolysates as functional ingredients: antihypertensive potential

Virtual Room 2 – Chairs: **Wiesław Wiczkowski** and **Maria J. Cantalejo**

15:05-15:20	OC21 – Haizhou Wu – Lipid oxidation in sorted herring (*Clupea harengus*) filleting co-products and its relationship to composition
15:20-15:35	OC22 – Paula Albendea – Effect of feeding olive pomace and soybean acid oils on European seabass fillet quality
15:35-15:40	F16 – Filipa Rego Pinto – Seasonal variation of mineral content in the muscle of fish species with no or low commercial value in Portugal
15:40-15:45	F17 – Tania Körber – Influence of a magnesium sulfate application on the content of sulfolipids in green multi-leaf lettuce
15:45-15:50	F18 – Leon Valentin Bork – Melanoidin formation based on aldol reactions of norfuraneol and short- chain Maillard intermediates

15:50-16:40 ***Poster session*** – Wonder platform

Plenary session 6

Virtual Room 1 – Chairs: **Irena Vovk** and **Lillian Barros**

16:30-17:05	**Robert Wollf** – Total utilisation of marine biomass – How can Norwegian seafood industry create more value and bring healthy products to the marked?

Virtual Room 1 – Chairs: **Irena Vovk** and **Lillian Barros**

17:05-17:20	OC23 – Petras Rimantas Venskutonis – Biorefining platform for the recovery of health beneficial fractions from fruit processing by-productsenhances the effects of raspberry polyphenols in rats
17:20-17:35	OC24 – Custódio Roriz– Betacyanins from *Gomphrena globosa* L. as natural food colorants: application in different foodstuff
17:35-17:50	OC25 – Neda Ahmadiani – Characterization and quantification of apple pomace's phenolic compounds extracted using conventional and pressurized liquid solvent extraction techniques
17:50-18:00	***Closing ceremony and awards***

LIST OF POSTERS

No.	Title	Presenting author
1	Nutritional and antioxidant characterisation of the peel of 10 species of coloured potatoes	Izamara de Oliveira
2	Olive leaves as a source of biophenols: extraction, quantification, and antioxidant activity evaluation in Portuguese olive trees	Carolina L. Ronca
3	Analytical tools to support the development of new protein ingredients – chemical analyses and nutritional quality	Minna Rotola-Pukkila
4	Analytical tools to support the development of new protein ingredients – sensory analyses	Nora Logrén
5	Analytical tools to support the development of new protein ingredients – technological properties	Joni Viitala
6	Chemical characterization and bioactive properties of different winemaking residues towards their valorization	Cristina N. Duarte
7	Multi-response optimization of enzyme-assisted extraction of bilberry (*Vaccinium myrtillus* L.) pomace	Michail Syrpas
8	Effect of supplemental red grape pomace on proportion of valuable meat parts of Ross 308 broiler chickens	Matej Čech
9	Microwave-assisted extraction of phenolic compounds from pine nut skin	Soraia P. Silva
10	Ultrasound-assisted extraction of bioactive compounds of olive seeds from three cultivars with valuable antineurodegenerative properties	Irene Gouvinhas
11	Determination of enzymes activity in mango (*Mangifera indica* L.) peel extracts	Nika Kučuk
12	Extraction of essential oils from the residues of two shrub species aiming for their revalorization: chemical characterization and antioxidant, antimicrobial and cytotoxic activities	Virginie Xavier
13	Extraction of phenolic compounds with antioxidant activity from cherry seeds: preliminary study	Raquel Guiné
14	High hydrostatic pressure, a green processing for apple by-product valorisation	Rocío De la Peña-Armada
15	How the extraction method affects the bioactive and antimicrobial properties of pomegranate peel and seed extracts	Lara Campos
16	Physicochemical characterization and bioactive potential of Cocfee silverskin	Pedro Esperanço
17	NMR a tool for unicity evaluation of Feteasca Neagra traditional Romanian wine	Cristina Todasca
18	Optimization and validation of HS-SPME GC-MS method for the analysis of volatile organic compounds (VOCs) in dry-cured ham	Katja Babič
19	Authenticity of coffee: Arabica or robusta?	Jana Kvirencova
20	Fiber and low molecular weight carbohydrate composition in new industrial milling fractions of rye varieties	Marietta Szentmiklóssy
21	An innovative control strategy based on machine learning and miniature near infrared spectroscopy to assure the geo-traceability of cephalopods	Maria Olga Varrà
22	Mineral profile of sea cucumber caught off Northeast Atlantic (Portugal)	Helena Maria Lourenço
23	Physicochemical properties of inulin isolated from dandelion (*Taraxacum officinale*) roots by “green” extraction	Ivanka Hambarliyska
24	Analytical tools to identify authenticity markers of PDO “Pera Rocha do Oeste” and PGI Alcobaça apple var. golden delicious	Ana M.S. Costa
25	Interactions of apocarotenoids with β-lactoglobulin	Maximilian Martin
26	Amino acid composition of Rugova cheese	Kaltrina Berisha
27	Microwave-assisted extraction: an eco-friendly alternative for extraction of antioxidant compounds from blueberry	Débora G. Bortolini
28	UHPLC-PDA-MS analysis of vitamin B12 and its pseudo-form in nutritional supplements based on microalgae	Sabrina Van den Oever
29	Structure and antioxidant activity relationships of dipeptides derived from foods	Damir Mogut
30	The profile of polyphenolic compounds, total phenolics and flavonoids contents, anti-oxidant and anti-microbiological properties of bee products	Małgorzata Starowicz
31	Comparison of total antioxidant capacity and total content of polyphenols in green coffea arabica from South and Central America	Katarína Poláková
32	Micro- and macroalgae amino acid profile and protein content	Elisa Costa
33	Barley (*Hordeum vulgare* L.) grain as a source of antioxidant peptides	Justyna Bucholska
34	Geographical origin authentication of roasted coffea arabica using volatiles profile and linear discriminant analysis	Alžbeta Demianová
35	Phenolic compounds of blackthorn (*Prunus spinosa* L.) fruits originated from Serbia	Nenad Mićanović

36	Differentiation of bee pollen samples according to their intact glucosinolate content	José Bernal
37	Study of the nutritional profile of *Cichorium spinosum* L. after fertilization with different nutritional solutions	Beatriz Paschoalinotto
38	Breads enriched with different flours: a new solution for healthier diets	Liege Aguiar Pascoalino
39	Influence of the maturation stage on the chemical composition and bioactive properties of *Cynara cardunculus* L. var. altilis seeds	Filipa Mandim
40	Oxidative changes in potatoes caused by deep-frying process with sunflower oil and omega 3 sunflower oil: A food modelling study	Birsen Yilmaz
41	Kynurenic acid in honey from various botanical species	Anže Pavlin
42	Molecular level depiction of how stearic acid enhances β-carotene solubilization in dietary mixed micelles	Esra Tunçer
43	Comparison of nutritional properties and in-vitro antioxidant activity of organically grown garlic and its fermented product	Mihajlo V. Jakanovski
44	Identified Saccharomyces cerevisiae strains from wine fermentation	Mariangie Castillo
45	Assessment of gamma-aminobutyric acid contents in brown rice and bran: comparison of HPLC and colorimetric methods	Cristiana Pereira
46	In vitro antioxidant activity and FTIR characterization of polyphenolic extracts from carob kibbles upon roasting	Anna Marina Grigoriou
47	Valorisation of Roman chamomile (*Chamaemelum nobile* L.) herb for the development of flavourings and natural antioxidants	Renata Baranauskienė
48	Biobased food packaging with electrical conductivity for in-pack treatment by pulse electric field	Cláudia Nunes
49	Extrusion cooking effect on arabinoxylans content in novel gluten-free flours based on rice and chickpea	María Ciudad-Mulero
50	Water desorption kinetic curves as a tool for quality and history of products analysis	J.M. Rocha
51	Extrusion process effect on resistant and total starch in corn and lentils enriched flours with grape skin (*Vitis vinifera*) by-product	M. Cotacallapa-Sucapuca
52	Selecting ingredients and processing methods to increase carotenoid contents of carrot chips	Amy Schmiedeskamp
53	Influence of yeast strain and vessel type on aroma profile of Chardonnay white wine	Ivana Ivić
54	Functional properties and chemical profile of aged carioca beans (*Phaseolus vulgaris* L.) cooked under the steam of autoclave	Suélen Caroline Frantz
55	Colourful carrot snacks manufacturing by applying osmotic dehydration, convective drying and vacuum microwave drying	Emel H. Yusuf
56	Blue Honeysuckle (*Lonicera caerulea* var. *caerulea*) extract as potentional natural antioxidant for raw-cooked meat products	Lukáš Jurčaga
57	Multi-step recovery of antioxidant-rich fractions from *Hierochloe odorata*	Kiran Subbarayadu
58	Recovery of valuable constituents from hop residues with pressurized solvents: Process optimization and extract characterization	Nóra Emilia Nagybákay
59	Valorization of cranberry pomace by using supercritical fluid and pressurized liquid extraction processes	Laura Tamkutė
60	Evaluating applicability of wood hemicelluloses as potential wall materials for spray dried microencapsulation of berry juice	Abedalghani Halahlah
61	High-resolution mass spectrometry analysis of melanoidins: The role of methylglyoxal in the formation of Maillard colorants	Clemens Kanzler
62	Association between ultra-processed breakfast cereals and acrylamide	Francisco J. Morales
63	The reaction of thioglucose and isothiocyanates lead to new transformation products during cooking	Holger Hoffmann
64	Regulation of enzyme activity in spelt flour for breadmaking	Gordana Hojnik Podrepšek
65	How stable are anthocyanins? A study with elderberry juice	Cláudia M. B. Neves
66	Extraction and quantification of tropomyosin in selected samples of shellfish	Mirjana Radomirović
67	Herbal teas with Cannabis: Assessment of potential consumers exposure to THC	Petra Peukertová
68	Effect of pH on the kinetics of the reaction of gallic acid with methylglyoxal	Charia Hadjipakkou
69	Bio-based pH indicator films for intelligent food packaging applications	Bîtcan Ioan
70	Effect of sodium nitrite dose on lipid oxidation and colour changes during the shelf-life of refrigerated pork liver pâtés packed in MAP	Martine Carlier
71	Miniaturized, green salting-out liquid-liquid microextraction coupled with GC-MS used to evaluate biogenic amines in wine samples	Magdalena Fabjanowicz

72	The use of salting-out assisted liquid-liquid microextraction and gas chromatography-mass spectrometry for the determination of biogenic amines in fruit juices	Anna Różańska
73	Determination of organic pollutants in bivalve samples from South Korean markets	Vesna Jovanović
74	Allergenicity assessment of Cor a 8 from raw and roasted hazelnut upon oral-gastric digestion phase of INFOGEST protocol	Tanja Cirkovic Velickovic
75	Investigating the Influence of Brewing Parameters on Coffee Furan & Alkyl-Furan Exposure	Anja Rahn
76	Oxidative stability and protein degradation in Vietnamese pig meat during storage	Peter Herc
77	Impact of heat treatment of non-wheat flour on acrylamide presence	Kristína Kukurová
78	Investigation on heat-induced chemical indexes in traditional and reformulated biscuits	Cristina Delgado-Andrade
79	Consumers' decisions on the selection of the end-point in a controlled potato frying process influence the exposure to acrylamide.	Marta Mesias
80	Determination of etoxazole in the plum by gas chromatography-mass spectrometry	Tasic Aleksandra
81	Development of novel colorimetric pyranoflavylium-biopolymer hybrid conjugates	Ana Sofia Pires
82	Physico-chemical properties and antioxidant activity of rose (*Rosa dama*scena Mill.) petals extracts encapsulated in four hydrocolloids	Nadezhda Petkova
83	Halophytes – Future food? Introducing alternative crops for food production	Maria Fitzner
84	Awareness among Croatian university students about the use, nutritional potential and risks associated with the consumption of hempseed oil	Tonka Žgela
85	Biochemical characterization of protein fractions extracted from three edible insect species	Marija Stojadinovic
86	Probing the stability of the food colourant R-phycoerythrin from dried Nori flakes	Simeon Minic
87	Fermented and non-fermented Spirulina water and ethanol extract treatment effect on yeast at a proteome level	Jasmina Masten Rutar
88	Effect of chitosan on encapsulation of chokeberry polyphenols and volatiles in alginate-based hydrogel beads	Ina Ćorković
89	Complexation of quercetin with apple and citrus fibres: Study of quercetin affinities in model systems	Ivana Buljeta
90	Technological changes of wheat-based breads enriched with hemp seed press cakes and hemp seed grit	Verena Wiedemair
91	Encapsulation efficiency of spray-dried juniper berry (*Juniperus communis* L.) essential oil microcapsules prepared with different wall materials	Jelena Bajac
92	Elderberry wine as a new potential product of functional food	Milena Vujanović
93	Investigation of the effect of a new feed additive formulation on the egg nutritional properties	Zsuzsa Jókai
94	Drying of halophyte plants: Effect on the antioxidant activity	Aida Moreira da Silva
95	Essential oils from *Thymus vulgaris* and *Thymus* x *citriodorus* dose-dependently reduce nitric oxide release from LPS-stimulated macrophages	Tânia L. Silva
96	Easy-to-swallow functional food with the potential neuroprotective properties designed for the elderly	Andreia Lopes
97	The NUTRIBOX project: a healthy & smart eathinking e-commerce platform for vulnerable consumers	Antonella Lamonaca
98	Microwave-assisted extraction of phlorotannins from *Fucus vesiculosus*	Susana M. Cardoso
99	Neglected wild edible fruits as potential dietary sources of antioxidants in the Mediterranean Area	Erika N. Vega
100	Bioavailability of Mg and Zn in grain of plant *Amaranthus cruentus* after simulated gastrointestinal digestion	Maja Krstić Ristivojević
101	The effects of gastrointestinal digestion on antioxidant and anti-inflammatory abilities of phlorotannins from *Himanthalia elongata* and *Laminaria digitata*	Ana R. Circuncisão
102	Anti-Inflammatory effects of oleacein and its metabolites	Fátima Paiva-Martins
103	Antioxidant protection of endangered *Thymus* spp. aqueous extracts on t-BHP-induced oxidative damage in an intestinal cell model	Carlos Martins-Gomes
104	Plant matrix induces thermal degradation of glucosinolates in Brassica vegetable broth	Matthias Renz
105	A simple high-performance liquid chromatographic method for the determination of glucosamine used in the treatment of periodontal disease	Daniela Alexandra Scurtu
106	Development of high-protein and low saturated fat bread formulations enriched with microalgae	Tatiana Pereira
107	Optimization of microwave-assisted digestion prior plasma-based spectrometric techniques for the tissue distribution of gold	Oana Cadar

108	A novel high pressure liquid chromatographic method for the determination of vitamin D3 in simulated human gastrointestinal tract	Anca Becze
109	Seasonal variations in the fatty acid profile of unexploited and low commercial value fish species from the Portuguese coast	Sónia Barroso
110	Potential use of edible flowers as substitutes of synthetic antioxidants	Cristiana Breda
111	Assessment of the possibility of enclosing chokeberry powder inside a polysaccharide capsule using microencapsulation by extrusion	Kamil Haładyn
112	Interaction of SARS-CoV-2 Spike protein with phycocyanobilin	Ana Simovic
113	CBD-based food supplements: Are phytocannabinoids transferred to the brain? (case study)	Zuzana Binova
114	Potential of natural lavonoids in skin photoprotection	Tiago E. Coutinho
115	Insight into the interaction of NaCl with sugars from computer simulations	Paulo E. Abreu
116	Determination of mineral content in extracts of *Centaurium erythraea*	Valentina G. Nikolic
117	Impact of antifogging food packaging material on phenolic compounds in green and red lettuce cultivars	Vanessa Harbart
118	Fatty acid composition of mother milk	Miaomiao Zhang
119	Enzymatic synthesis and biological evaluation of crude fructooligosaccharide preparations	Meda Bytautaitė
120	Production of fructooligosaccharides by Aspergillus welwitschiae inulinase enzyme complex, obtained on natural substrate	Sanja Stojanović
121	Resveratrol interaction with starch: implications on digestibility?	Andreia F.R. Silva
122	Impact of phenolic acid derivatives on β-lactoglobulin stabilized oil-water-interfaces	Alina Bock
123	Application of raw and defatted hempseed press-cake and sweetgrass antioxidant extract in pork burger patties	Kristi Kerner
124	Influence of thickness on mechanical properties of composite biopolymer films based on sunflower oil cake	Jovana Ugarković
125	Formation of epithionitriles in vegetables and metabolism in humans	Franziska S. Hanschen
126	Binding affinity ovalbumin on different type of microplastics using Langmuir isotherm	Tamara Mutić
127	Toxicological and anti-tumoral potential of pomegranate (*Punica granutum* L.) leaf infusion in HPV16-transgenic mouse model	Manyou Yu
128	Methylglyoxal trapping ability of phenolic compounds from *Sambucus nigra* L.	Sandrine dos Santos Ferreira
129	Optimization of the extraction method for determination of total phenolic content (TPC) of bee pollen	Rita Végh

PLENARY LECTURES

Real and not-so-real problems of chemical food safety

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The contamination of food and food raw materials/ingredients can occur at any stage of the supply chain. In the case of chemicals, these encompass a plethora of substances such as pesticides, mycotoxins, veterinary drugs, heavy metals, packaging and environmental toxicants. Literally thousands of substances need consideration to ensure compliance and safety of the foods that are produced. However, not all chemicals are of equal concern, determined by their toxicity, occurrence in foods and the amounts of food typically eaten. Ranking substances and materials according to their severity versus the likelihood to cause harm is one approach explained in this presentation that enables prioritization, and the outcome is of importance for HACCP studies, establishing contaminant surveillance programs, and setting purchasing specifications for vendors.

A more recent addition to the list of unwanted substances in foods that require assessment are processing contaminants, formed for example when we heat foods, either in the home or in an industrial setting. Acrylamide, discovered nearly 2 decades ago in many cooked foods such as baked/fried potatoes, bakery wares and coffee, has been intensively studied across all relevant scientific disciplines. The food industry addressed this issue early on, establishing a “Toolbox” of measures in the pertinent food categories that enable food business operators (FBOs) to mitigate this contaminant following the ALARA (As Low As Reasonably Achievable) principle. In 2017 the European Commission published Regulation (EU) 2017/2158 that ensures that FBOs apply the necessary tools to achieve so-called “benchmark” levels. However, the EC is now contemplating strengthening the rather recent Regulation by proposing maximum levels for certain foods. Similar trends in risk management are evident for MCPD esters/glycidyl esters, as well as for furan and its alkylated congeners in different composite foods.

Another subject that may lead to issues in foods are endocrine active substances. The presentation will highlight an example of a commercial research organization (CRO) that provided data on the endocrine activity of commercial breakfast cereals, claiming potential health issues based on the results of *in vitro* tests. Our research revealed that extracting endocrine active substances from foods and applying the extracts to biological assays is highly challenging, with the potential to create erroneous data. In this context there is an urgent need for standardised method protocols.

Finally, a recent incident related to the presence of the fumigant ethylene oxide (ETO) in locust bean gum and consequently ice cream will be reviewed, with emphasis on the Europe-wide recall/withdrawal of ice creams mid 2021. Latest research on the stability of ETO in ice cream during typical processing conditions is presented, as well as thoughts related to the risk assessment of the major metabolite 2-choroethanol.

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Untargeted analysis with GC-Orbitrap: a powerful tool for the authentication of spices and herbs: focus on oregano

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Oregano is widely used as an ingredient in food and beverage products and as a flavouring ingredient for culinary purposes due to its organoleptic properties. Adulteration of oregano can be accidental or intentional with the latter driven by price and demand. Leaves from other plants (e.g., olive, thyme, marjoram, sumac, myrtle, and hazelnut) are frequently used as adulterants as they are difficult to detect by visual inspection. Consequently, food manufacturers must check regularly for the quality and purity of oregano outsourced from various suppliers to ensure the quality and consistency of the end product. Oregano is a complex matrix containing essential oils, phytosterols, and pigments; its aroma derives from a complex mixture of volatiles, mainly monoterpenes and sesquiterpenes, which can be easily extracted and concentrated in one single step using the headspace solid-phase microextraction (HS-SPME) technique. This allows for minimal sample preparation, a critical point in non-targeted analysis since every manipulation could alter the sample composition. The fingerprint of the oregano aroma constituents can be investigated with a multiplatform approach using isotope ratio, liquid or gas chromatography coupled with mass spectrometry (LC or GC–MS), or high-resolution mass spectrometry (LC or GC-HRMS), and in combination with software tools for data reprocessing and statistical analysis. The high-resolution GC-MS approach has become very popular as it offers the advantage of full-scan data acquisition combined with high sensitivity, high resolving power (up to 240,000 FWHM), and accurate mass (< 5 ppm). Moreover full-scan data acquisition allows for targeted, non-targeted, and retrospective data analysis.
In this study GC-Orbitrap technology coupled with solid-phase micro-extraction (SPME) with Arrow technology was used to assess the volatile profile of intentionally adulterated and native oregano samples. Data were acquired in full-scan electron ionization (EI) mode and analysed with Thermo Scientific™ Compound Discoverer™ software. Positive chemical ionization (PCI) was used to confirm the elemental composition of the molecular ions using accurate mass information, isotopic match (measured versus theoretical), and presence of specific adducts. Additional MS/MS data were acquired and used to explain the proposed chemical structure of the compounds identified via mass spectrum matching.

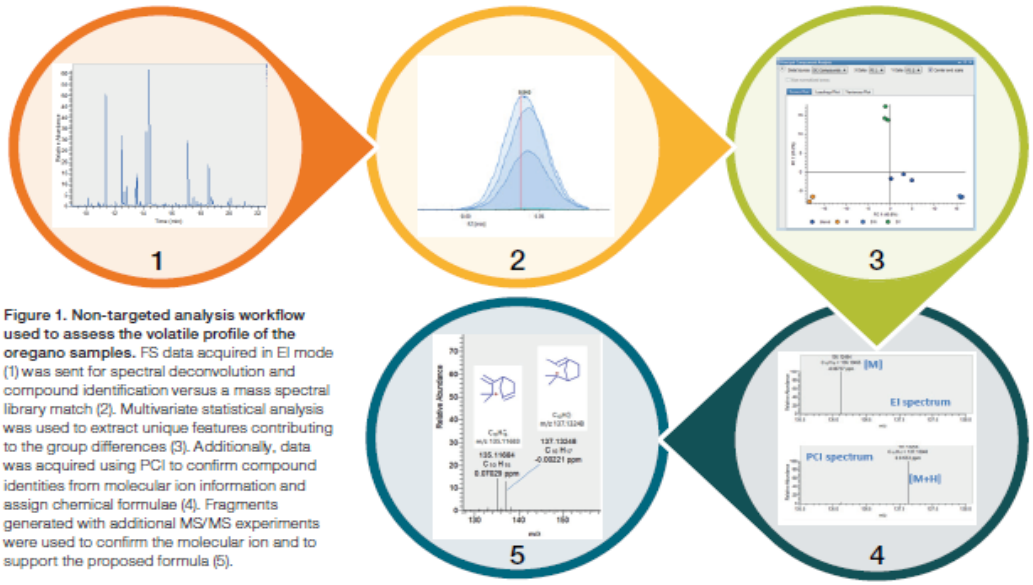

Figure 1. Non-targeted analysis workflow used to assess the volatile profile of the oregano samples. FS data acquired in EI mode (1) was sent for spectral deconvolution and compound identification versus a mass spectral library match (2). Multivariate statistical analysis was used to extract unique features contributing to the group differences (3). Additionally, data was acquired using PCI to confirm compound identities from molecular ion information and assign chemical formulae (4). Fragments generated with additional MS/MS experiments were used to confirm the molecular ion and to support the proposed formula (5).

Fig.1. Non-targeted analysis workflow used to assess the volatile profile of the oregano samples.

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Insects as suitable alternative for food and feed source

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AIM
The aim of the presentation is to describe and compare the nutritional quality and bioactive compounds present in different kinds of insects. The issue related to entomophagy and the eventual risks that should be considered when consuming insects were also discussed.

MATERIAL AND METHODS
Sustainability is becoming increasingly important in the world we live in. Edible insects belong to the possible alternative food source replacing traditional ingredients. With the growing population and increasing demands on world food production, research on this topic has intensified over the last 20 years. While 59 articles containing the keyword "edible insects" were published on the WOS in 2000, there were already 1182 of such articles in June 2020. However, there is still insufficient information on the composition of some edible insect species and on the benefits or disadvantages of their breeding, processing and use as food or feed. Therefore, the recent published data on the nutritional value and composition of insects will be compared with our experimental values and critically evaluated. Attention will be focused on basic nutrients: content and composition of proteins and lipids, as well as on micronutrients: minerals and vitamins. In particular, we compared the fatty acid profiles of selected insect species, the proportion of saturated and unsaturated fatty acids and the indices of atherogenicity and thrombogenicity. Some biologically active substances in insects will be also mentioned (e.g. purines, taurine), microbiological or chemical risks, as well as allergenicity of insects. We also carried out experiments focussed on the by-product's utilization, such as a replacement of soy protein by rapeseed cakes in the diet for insects.

RESULTS
From a nutritional point of view, insects contain an easily digestible quality protein with an optimal representation of most essential amino acids, as well as lipids containing a number of essential fatty acids. The nutritional value of insects is subject to a number of factors including breeding technology. Although insects are a nutritionally valuable and potentially ecological resource, there are still some concerns about placing insects on the European market. The biggest concerns relate to the safety and acceptance of this food source.

CONCLUSION
In the view of the growing market demand for edible insects and considering the inclusion of this new source of food, it is very important to analyse the available literature data and to increase the number of studies providing new data on the quality and safety of edible insects. There will also be challenges in legislation and the regulation of the edible insect sector.

Molecular approaches for understanding and modulating food taste to make food healthier and more tasty

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Plant-based foodstuffs are in general rich in bioactive compounds. However, some of these compounds have unpleasant taste properties such as astringency, which could interfere with consumers' choices. Modulating these untasty properties could be important to promote the intake of healthy foodstuffs while keeping consumer's approval.
Currently, astringency is recognized as a trigeminal sensation although the molecular pathway responsible for its onset has not been yet fully established. Polyphenols are usually associated with flavor, and particularly with astringency, due to their ability to complex with salivary proteins and namely basic PRPs, glycosylated PRPs, acidic PRPs, statherin/P-B peptide and cystatins [1].
There are some endogenous factors that affect astringency perception such as the physiological response, circadian rhythms, salivary flow rate and time of exposure to be elicited. Indeed, astringency is perceived as a diffuse stimulus and dynamic process in the oral cavity that requires time to be elicited. It is known that astringency increases upon successive exposures to tannins [2,3].
Different oral models have been developed in our group [4], comprising different oral epithelia (buccal mucosa (TR146) and tongue (HSC-3)) and other main oral constituents (human saliva and mucosal pellicle). These models have been used to study the interaction with different polyphenol extracts as well as with food matrices [5].
Food industry has some strategies to balance astringency and bitterness. Common practices usually lead to a decrease of nutritional properties and the removal of potential health benefits, rising concerns about food allergies, safety, and security. Polysaccharides have been an emerging natural and sustainable option to be used on taste properties modulation. In fact, polysaccharides can influence salivary protein-tannin interactions and they could be used to modulate astringency and bitterness.

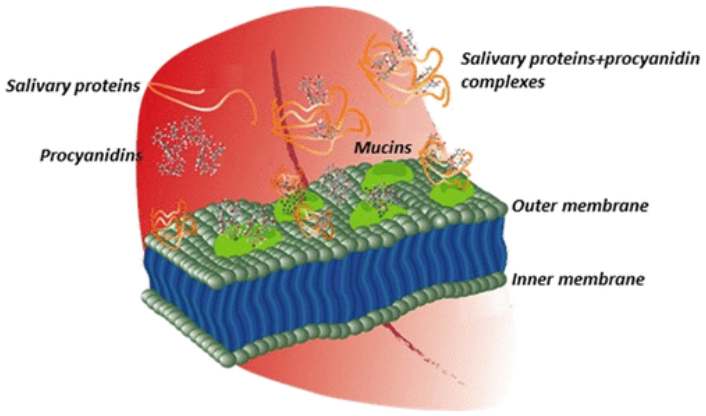

Fig 1. Oral model constituents involved in astringency perception [4].

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The analysis of the future of food: What, why, and how?

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World's population is projected to grow over twenty percent by 2050 [1]. The growing population in combination with the socio-economic changes in the developing countries leads to increased pressure on the demand for high-quality protein from animal sources. Cellular agriculture, and cell-cultured meat, in particular, offers a sustainable alternative to industrial animal agriculture which is not only a significant source of environmental stress but also a very inefficient way to meet this increasing demand. Many of these products will be considered novel foods and will therefore be subject to regulation. Assessments of novel foods for regulation so far are primarily performed on single ingredients or food additives. Applying this framework to complex foods as cell-cultured meat is uncharted territory. Nonetheless, the EU has been guiding in establishing a detailed description of assessments required to judge safety [2].

Analysis of cell-cultured meat for the evaluation of nutritional value and the assessment of safety does not only include the analysis of the end product but also requires a critical review of the entire manufacturing process and identification of potential hazards. Any safety concerning parameter should be tested during or after target tissue/cell procurement, cell preparation, biomass production, and product collection. Established methods and techniques that are used for the evaluation of conventional meat can be either directly used or can be derived from the analysis of related products in different fields such as the pharmaceutical industry. In this talk, the focus will be mainly on the analysis of the end product. An overview of what needs to be tested (quantitative and qualitative analysis on the composition, physicochemical, biochemical, and microbiological properties), why they have to be tested (food safety, toxicology, and bioavailability), and how they can be tested (contamination, residue and by-product analysis by chemical and microbiological means) [3] will be discussed.

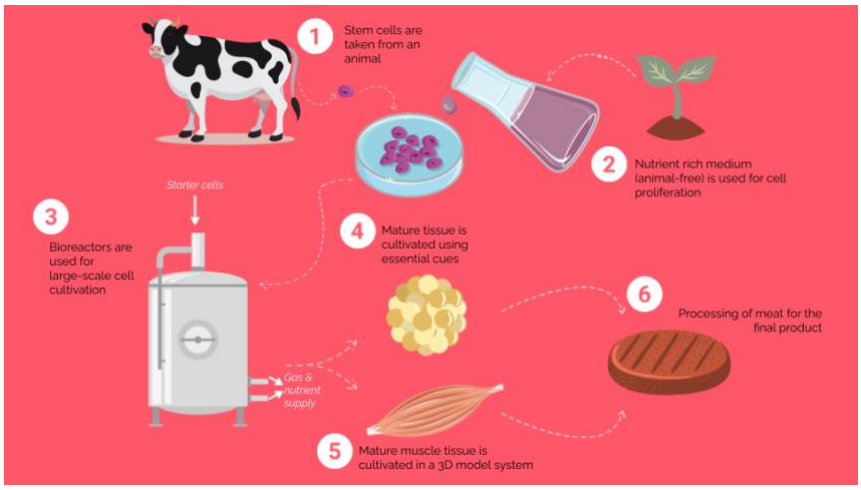

Fig.1. The manufacturing process of cell-cultured meat.

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Total utilisation of marine biomass – How can Norwegian seafood industry create more value and bring healthy products to the marked?

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The seafood industry represents one of Norway's largest export industries, with a total export value exceeding 10 billion EUR per year. Every day, all year round, approximately 36 million meals are prepared with origin from the Norwegian seafood industry. Norway exports farmed and wild fish to more than 150 countries.

Marine residual raw material (cut offs, viscera, heads, back bones etc.), constitutes an important value-creating resource generated from the Norwegian seafood industry. Today, most of the residual material are utilised. Nevertheless, there is a potential to increase rate of utilisation. Cod (*Gadus Morhua*) and other species within the cod family, are the main species harvested in Norway, but are also less exploited. Both the players in the seafood sector and the R&D environment, have an increasing focus on finding sustainable solutions to increase utilisation. Every year SINTEF conduct a survey which gives and overview of total quantities of residual raw material generated, where it arises and how it is utilised. This has become a powerful decision support tool for what is known as the marine ingredients industry in Norway. The aim is to provide an overview of the availability of, and which product flows arise from, marine residual raw materials [1].

In 2020 a total of approximately 1 mill ton of rest raw material. From this, just above 860 000 ton were exploited fully. There has been a tremendous increase over the last decade. Among products based on biomass from residual raw materials from the seafood industry are pet-food, animal feed, protein rich products and fish oil. To further increase the value creation and fully exploit the potential which lays in the biomass, a lot of R&D are conducted, specially related to products that are prepared for nutritional health marked and pharma. Among products omega-3 products should be mentioned.

This presentation will give an overview of how the Norwegian marine ingredient industry are innovating and how the industry even can increase the utilisation of unexploited biomass, to create more value.

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ORAL COMMUNICATIONS

A worldwide study of pesticide residues in fruit-based soft drinks using liquid chromatography/tandem mass spectrometry

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Soft drinks consumption has increased worldwide over the last decades, and it is expected to grow in the 2019-2025 period. In order to protect the consumers, a plethora of regulations is now available to control pesticide residues in food, feed and water intended for human consumption. Therefore, maximum residue levels (MRLs) around μg kg^{-1} has been established. Regarding the European pesticide control of food commodities from plant origin, up to 315 raw matrices, like fruits or vegetables, have been regulated. But their derived products, in which citrus-flavored soft drinks could be included, have not been considered in any country around the world. Our research group first reported the presence of pesticide residues in fruit-based soft drinks in 2008. Then, we conducted several screening studies that confirmed the presence of pesticides in fruit-based soft drinks, which were manufactured in up to 15 countries. In all of them, around 90% of the studied samples contained at least one pesticide, and MRLs for drinking water were often exceeded. Many of the detected compounds were post-harvested fungicides, which are generally used at the final production stage to prevent fruit spoilage. Accordingly, the most frequently found pesticides were carbendazim, imazalil, imazalil metabolite BTS40348, prochloraz, and thiabendazole. After more than ten years since pesticide residues were detected in citrus-flavored soft drinks, this study reveals that the situation has not changed. To our knowledge, this is the first project that monitors pesticide residues in citrus-flavored soft drinks (with or without juice in their composition) collected in 5 continents. A new sensitive multiresidue UHPLC-MS/MS method has been validated for 88 pesticides, which was used to analyze 200 samples manufactured in 67 countries, 80% corresponding to fruit-based soft drinks. The results show that 98% of the samples collected worldwide contained at least one pesticide, and 85% of them contained more than 4 pesticides. 40 out of 88 target compounds were quantified among the screened samples. The total concentration of pesticides per sample was ranged from 0.01 to 835.50 μg L^{-1}. There has not been any substantial change from the previous studies in pesticide residues in fruit-based soft drinks. Europe was the world region with the highest total amount of pesticides, probably due to the higher content of juice concentrate in samples, which may be the main source of the pesticide residues. Nevertheless, residues were also found in samples with no juice, so water quality also plays an essential role as the main ingredient of citrus-flavored soft drinks. To conclude, it would be highly recommended to change elaborating processes to reduce pesticide residues in citrus-flavored soft drinks and establish MRLs to protect consumers' health, especially for vulnerable groups, as children and teenagers who are the largest consumers of these beverages.

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Analysis of WADA prohibited substances in ecdysterone-containing dietary supplements

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The World Anti-Doping Agency (WADA) issues the prohibited list in sport annually. The prohibited list is not a static one, it changes dynamically according to the state-of-art of related studies. Since this year ecdysterone has been added to the monitoring program. The substances on monitoring program are not on the prohibited list, but they are monitored by WADA in order to detect potential patterns of misuse in sport. Ecdysterone is a plant-derived steroid hormone, mainly available from spinach leaves, which has a structure similar to anabolic androgenic steroids. Ecdysterone has several physiological effects, the most important is the anabolic effect, without any androgenic side-effect. This substance is very popular among athletes, who try to find “legal” performance enhancing products. The ecdysterone-containing supplement, however, can be contaminated with prohibited substances, thus their use can lead to a positive doping test. Therefore, the analysis of dietary supplements (DS) containing ecdysterone are very important. Consequently, our aim was to analyse ecdysterone and further prohibited compounds with suitable and innovative chromatographic techniques (liquid (LC) or gas (GC)) coupled to tandem mass spectrometry (MS/MS).
Our analytical methods were optimized according to two different scopes: exact quantitation of ecdysterone with excellent linearity and accuracy on one hand, and limit test for prohibited steroids, stimulants and narcotics with the lowest possible limit of detection, on the other, by means of LC and/or GC-MS/MS. Sample preparation was also optimized for different matrices (simple DS, complex DS with high oil or sugar or protein content, liquid DS). Totally, 7 different ecdysterone-containing DS and fresh spinach leaves were analysed. More than half of the smpales contained almost one prohibited substance. At the same time in many cases the measured ecdysterone content was not in accordance with that on the label. Except in one case, the measured and the labelled ecdysterone content was not even in the same order of magnitude. The developed methods follow continuously the changes of the WADA list, thus the analysis of the products prior to use by elite athletes can support the choice of safe DS and reduce the possibility of unintentional violation of doping rule.

Deep-learning assisted data augmentation of spectral data for the authentication and quality analysis of food products

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Significant efforts have been made to assess the authenticity of food products over the course of the past decades. This comprises methods such as the analysis of fatty acid profiles with gas chromatography [1] in the early 1980´s for olive oil authenticity assessment, which required derivatisation reactions and therefore high sample preparation efforts. Another example is the analysis of whether a honey sample meets the standard criteria for a specific botanical origin, which are often based on comprehensive organoleptic, physicochemical, and pollen data [2] which mostly are target oriented. This may be problematic, because only holistic approaches covering a wide range of chemical markers by analysis is capable of diminishing loopholes for food fraud.
In addition, most conventional analytical methods for such food products are destructive and time-consuming. Therefore, other analytical methods gradually replaced the classical techniques or at least evolved as potential alternatives. The main challenge of these profiling techniques is the vast amount of data generated and therefore. are typically combined with diverse mathematical methods, also known as machine learning or chemometrics. Commonly used techniques up to now are based on a variety of instrumental coupling techniques as well as machine learning algorithms such as k Nearest Neighbours (kNN), Support Vector Machines (SVM) and Partial Least Squares-Discriminant Analysis (PLS-DA) to identify classes of food products. However, these algorithms, often also called “static machine learning” have their limitations when it comes to year-to-year sample variabilities. In this context, deep learning is a promising alternative, as the diametrically different approach is much closer to artificial intelligence than classic machine learning.
Until now, deep learning plays a minor role when it comes to the evaluation of food products from a chemical perspective, although it is suggested that food chemistry could highly benefit from using it [3] [4]. In contrast, deep learning proves to be highly effective in a broad spectrum of fields of use, such as image and semantic analysis. The main limitation and reason for the relatively low usage of deep learning in food authentication lies in the fact that deep learning is dependent on large amounts of learning data. As the generation of analytical data is limited by costly resources such as time, sample availability, personnel and data storage capacity, the use of deep learning algorithms in food chemistry is still limited. To close the gap between deep learning and food chemistry, the augmentation of instrumental data is a promising approach. Via augmentation, high amounts of data can be generated from relatively small data sets by approximation of the data distribution and random sampling from that particular distribution.
This talk presents a proof-of-concept study which demonstrates that the use of artificial neural networks (ANN) in different architectures can be used to classify olive oil samples according to their geographical origins and honey samples according to their biological origin. It will be demonstrated how data augmentation improves the ANN-model performance and it will be discussed how the ANN model compares to the state-of-the-art machine learning methodology.

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Optimized headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) and non-negative matrix factorization (NNMF) for nontargeted VOC profiling of fermented dairy

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Fermentation of food (such as milk) is an ancient practise for the preservation of food through the production of acids (such as lactic acid) and possibly antimicrobial compounds [1]. Traditionally, many fermented foods were produced by natural fermentation processes, that involve the symbiotic fermentations of, for example, lactic acid or acetic acid bacteria and yeasts [2]. Since each microorganism produces a versatile spectrum of flavour compounds, the composition of microorganisms influences the sensory properties, such as taste and flavour. The inherent diversity of biogenic samples requires analytical methods capable of discovering unknown or non-targeted compounds from the complex sample matrices [3]. This approach, also referred to as non-targeted screening (NTS), requires comprehensive extraction and analysis of compounds of interest. Analysis of the volatile organic compounds **(VOCs)** of samples, also known as VOC profiling, allows for the detection of compounds in complex sample matrices and provides an insight into the flavour profile of a complex sample. Gas chromatography-mass spectrometry **(GC-MS)** is commonly used to obtain a characteristic VOC profile. However, due to its high sensitivity and resolving power on the one hand and its simplicity and robustness on the other, ion mobility spectrometry **(IMS)** coupled to gas chromatography **(GC-IMS)** has gained popularity for the analysis of VOCs [4]. As a result, nontargeted VOC profiling based on GC-IMS in combination with machine learning has emerged as a promising method for sample analysis.

Kefir is a fermented beverage, which is produced through natural fermentation by a consortium of microorganisms, also known as kefir grain. Due to its putatively health-promoting effects, milk kefir has attracted increased attention of dairy producers and health-conscious costumers. In this work, we analysed traditionally made kefir, commercial kefir and commercial yogurt samples using HS-GC-IMS for VOC analysis. Key compounds of fermented dairy are for example ethanol, acetic acid, ethyl acetate and diacetyl. Preliminary analysis found overlapping peaks in both GC-MS and GC-IMS analysis. By raising the drift tube temperature in an optimized prototypic HS-GC-IMS, this problem was overcome. Furthermore, a customized chemometric toolbox, based on MATLAB, was used to extract information from high-resolution 3D VOC fingerprints. Pattern recognition techniques and non-targeted screening revealed distinct differences between traditional and commercial kefir, while showing strong similarities between commercial kefir and commercial yogurt. Similarities found between traditionally made kefir and commercial kefir are, for example, higher concentrations of acetic acid. The VOC profiles of commercial kefir and yogurt samples indicate mild flavour composition, with high concentrations of buttery flavoured diacetyl and acetoin. In contrast, traditionally made kefir showed a diverse VOC profile with high amounts of ethyl acetate, isobutanol, 2-methyl butanal, 3-methyl butanal and isoamyl alcohol. The comparison of different kefir varieties revealed distinct VOC profiles for each traditionally made kefir, indicating differences in the microbial consortium.

For in depth understanding, qPCR sequencing was used to evaluate the microbial consortiums of kefir and yogurt samples, confirming the similarities between commercial kefir and yogurt samples, and reinforced the discrepancies between traditional and home-made kefir. The diverse flavour profile of traditionally made kefir primarily results from the yeast consortium, while commercial kefir and yogurt is primarily, but not exclusively, produced through bacterial fermentation. Sequencing results confirmed the presence of a variety of microorganisms in traditionally made kefirs, which indeed differ between traditionally made kefirs. A commonly used technique for pattern recognition and subsequent dimension reduction is principal component analysis (PCA), a mathematical decomposition of a matrix into independent (orthogonal) principal components (PCs). These PCs, however, lack interpretability, wherefore in this work the less well known non-negative matrix factorisation (NNMF) was explored for data analysis. NNMF decomposes samples as sums of their parts whereby components are easily interpretable by restricting sample features to be positive values. Using PCA and NNMF, the flavour profile of traditionally made kefirs was correlated to its microbial composition to obtain species specific substances. The results may be used for direct evaluation of microbial consortium using HS-GC-IMS analysis.

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Extended shelf life (ESL) milk displaces pasteurized fresh milk from Austrian market – boon or bane?

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In the last two decades, ***E**xtended **S**helf **L**ife* (ESL) milk has gained a widespread acceptance in Austria, indicated by a decreasing market share of pasteurized milk (80.5% in 2003 down to currently 14.5%), and a simultaneous increase of ESL milk consumption from 3.8% (2003) up to 61.6% (2020). Concomitantly, UHT milk also increased from 15.7 to 23.9% [1]. However, so-called ESL milk can be either produced by direct or indirect heat treatment or through a mechanical process such as filtration (micro- and deep bed filtration) or centrifugation ("bactofugation") in combination with gentle heat treatment (pasteurization) [2]. Consequently, different heat loads (time/temperature combinations) applied to liquid milk will influence the quality of the final product and may result in enormous nutritional (e.g., vitamin loss, loss of essential amino acids, protein denaturation, formation of undesirable substances such as lysinoalanine), sensorial (e.g., cooked flavour, brown colour) and chemical (e.g., *Maillard* reaction products, unfolding of proteins) modifications. Therefore, different ***T**ime-**T**emperature **I**ntegrators* (TTIs) have been established to assess and control the actual "heat load" of ESL milk products (e.g., acid-soluble β-lactoglobulin and α-lactalbumin, furosine, lactulose) [3]. It is well known that in particular indirectly heated ESL milk (processed in plate or tube heat exchangers) suffers from excessive heating ("over-processing"), which results in a much higher heat load comparable to that of UHT milk. Directly heated ESL milk usually shows a lower heat load (complying with the legal limit), while microfiltered or bactofuged ESL milk is in the same range as pasteurized milk (indicating a more gentle heat treatment comparable to pasteurization) [4].

In 2011, the *Austrian Food Codex* introduced a minimum concentration of 1.800 mg acid-soluble β-lactoglobulin per litre ESL milk as the legal limit for the maximum heat load of ESL milk. However, an excessive transition period till 2018 was granted if indirect heating processes for ESL milk production were still in use (Austrian Federal Ministry of Health, 2011). After the end of this transition period (June 2018), two different categories of ESL milk appeared on the Austrian market: 1. ESL milk *"länger frisch"* (fresh for longer) having a minimum content of 1.800 mg acid-soluble β-lactoglobulin/L (indicating a low heat load) and a maximum shelf-life of 27 days; and 2. ESL milk *"länger haltbar"* (longer shelf life) that is below the threshold level (revealing a very high heat load, where it is no longer justified to speak of "fresh milk"!). The heat load of such high-heated ESL milk (shelf-life up to 45 days!) is very similar to the heat load observed for ultra-high temperature (UHT) treated milk, which can be easily detected by analysing appropriate TTIs (e.g., acid-soluble β-lactoglobulin and α-lactalbumin, lactulose, furosine) [5].

The objective of this study was to further improve RP-UHPLC methods for the analysis of furosine and acid-soluble ß-Lg in milk, to determine the heat load of different categories of heat-treated liquid milk samples, and to check the actual effect of the end of the transition period on the heat load of so-called ESL milk in Austria. Using furosine, lactulose and α-lactalbumin, a differentiation of pasteurized, filtered/centrifuged and directly heated ESL milk from indirectly heated ESL milk as well as from UHT milk was possible, whereas β-lactoglobulin allowed the discrimination of directly heated ESL milk from filtered/centrifuged ESL milk and pasteurized milk (as well as from indirectly heated ESL milk and UHT milk) [4, 6]. Since the end of the transition period, ESL milk labelled as "länger frisch" showed (with two exceptions) in fact a lower heat load and was above the requested threshold (minimum content of 1.800 mg acid-soluble β-lactoglobulin/L as a legal limit). This is in contrast to the situation in the years before, when more than half of the ESL milk samples had an excessive heat load, which was almost comparable to that of UHT milk. As expected, ESL milk samples designated as *"länger haltbar"* showed a very low content of acid-soluble β-lactoglobulin/L (< 200 mg/L) indicating a very high heat load comparable to that of UHT milk.

As ESL milk has shown a dramatic increase in Austria recently, and has been widely accepted in many other European countries (e.g., Germany) in the meantime, the heat load (and thereby the nutritional and organoleptic quality) of this new category of liquid milk needs to be controlled in the future. The end of the transition period for the maximum heat load of indirectly heated ESL milk has stopped the unacceptable over-processing of ESL milk in Austria, which definitely does not meet consumers' expectations! In addition, the discussion about the negative effects of a higher heat load of liquid milk will encourage more people to switch back to pasteurized (i.e., 72°C for 15-20s) fresh milk ("Frischmilch") – and that could be a model for other countries in Europe and around the world.

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Development of a fast method for prediction acrylamide formation in industrially produced biscuits with and without the use of asparaginase

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Acrylamide (2-propenamide) is a Maillard reaction product (MRP) and it is mainly produced through the reaction of the carbonyl group of reducing sugars and the amine group of asparagine [1]. Acrylamide was observed in heat-processed foods for the first time in 2002, and since then, many studies were made to understand the impact of this MRP on consumer's health. Acrylamide is potentially carcinogenic and neurotoxic and it is present in a large variety of widely consumed products, such as fries and potato chips, breakfast cereal, biscuits, bread, coffee, and many others [2]. Due to its toxicity and wide distribution in the daily diet, in 2017, the European Union (EU) set maximum levels of acrylamide in a range of food products, including biscuits [3]. Therefore, it is important to monitor the acrylamide formation and to study strategies to mitigate this MRP as much as possible, without negatively impact the sensory characteristics of foods, that the industries can use in order to produce foods less harming to consumers, but equally tasty and appealing.

In this work, an accurate and precise method to quantify acrylamide was developed and optimized for its application in biscuits produced industrially by GC-SIM-MS, through the combination and optimization of methods previously described in the literature. Wheat flour and industrially produced biscuit dough were studied regarding their sugars and amino acids content. The effectiveness of asparaginase as an acrylamide mitigating agent was also tested under industrial application: samples were previously prepared with L-asparaginase, incorporating the enzyme in the dough before the heat processing, and acrylamide was quantified. The biscuits color was also evaluated, using the CIELab method, in order to understand the effects of the asparaginase treatment on the sensory properties of the final product. Statistic models were used to create the fastest and cheaper way to predict the acrylamide content of biscuits, using biscuits color and the chemical composition of doughs, such as asparagine and sugar content, in order to facilitate the process of acrylamide monitoring by the food industries.

Asparaginase showed to be extremely effective in reducing the acrylamide content, as the final products were slightly lighter in color. Asparagine content of the ingredients seemed to be the most influencing variable for acrylamide formation and, therefore, the use of ingredients with a low content of this amino acid could be another possible way to prevent the formation of large amounts of acrylamide in the final product. The evaluation of biscuit's color and the quantification of asparagine content can be used to predict the acrylamide content in biscuits produced industrially with the use and without the use asparaginase.

Fig.1. Representative illustration of acrylamide formation and its dangerous effects.

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Acrylamide content in common Spanish culinary preparations and exposure from household, catering and industrial settings

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In recent years, eating habits in developed countries have undergone significant changes due to economic and sociocultural aspects. Among other factors, new urban lifestyles, lack of time and culinary habits of the youngest generations have increased the consumption of foods obtained from caterers, company canteens, institutional catering, restaurants and other food supply services [1]. Moreover, 'fast food' options have become firmly established among certain population groups, at the expense of fresh food [2]. In many cases, these food preparations are based on a protein source combined with cereals or potato subjected to culinary operations such as roasting, toasting, baking, or frying, which can result in the development of the Maillard reaction and consequently in the formation of acrylamide [3]. Acrylamide is a chemical process contaminant whose exposure increases the risk of developing certain types of cancer in all age groups [3]. In 2017, the European Regulation 2158/2017 established mitigation measures and benchmark levels for the reduction of the presence of acrylamide in the main sources of exposure to this contaminant, including potato-based products, cereal-based products and coffee and substitutes, in addition to infant foods [4]. The large variety of foods consumed by European populations makes it necessary to consider other food matrixes as possible sources of this contaminant. In this sense, in 2019, the European Commission issued further recommendations to monitor the presence of acrylamide in other foodstuffs [5], in order to consider the adoption of possible risk management measures, complementing those already provided by the previous Regulation. On the other hand, several studies have shown that the handling and the type of cooking have a significant influence on the formation of acrylamide. This fact leads to the need to consider different culinary environments where food is prepared to establish real values of exposure to this contaminant. In view of these considerations, the aim of the present study was to establish the range of acrylamide exposure from common culinary preparations within Spanish diets, considering household, industrial and catering settings, and taking into account recent changes in Spanish patterns of food consumption.

Eleven types of processed foods commonly consumed in Spain were selected for analysis and classified according to the main food matrix, into potato-based food (French fries and Spanish omelette), cereal-based food ('torrijas' and sponge cake) and foods based on cereal mix with meat, fish or vegetables (breaded fillet, ham & cheese fillet, pizza, puff pastry pie, patties, 'migas' and croquettes). Samples of each food group were prepared and collected from three different settings (domestic, catering and food industrial) to evaluate the influence of the food preparation setting on acrylamide formation. Dishes were weighed, and the acrylamide content was analysed by Liquid Chromatography–Electrospray Ionisation–Tandem Mass Spectrometry (LC-IE-MS/MS). Exposure was estimated considering the weight of a regular portion.

Results revealed that the highest concentrations of acrylamide were observed in chips (French fries), especially those prepared at home, which corroborates this food product as the main source of acrylamide in the diet. Although at lower levels, acrylamide was also present in the other food samples considered, which underscores the need to control its content in foods not yet addressed in EU food quality regulations. The lowest acrylamide values were observed in industrially processed foods, which involved a lower contribution to acrylamide exposure, although without significant differences, in comparison to catering services and food preparation in the household. These results probably reflect the existence of stricter control of the culinary process in the industrial environment, possibly associated with the implementation of the mitigation measurements required by the European regulation concerning this sector. The highest levels recorded for households and catering services highlights the need for tighter monitoring of food cooking processes in these settings and the promotion of mitigation measures to control acrylamide formation in foods. These actions will help reduce the amounts of acrylamide in processed foods and alleviate the risk associated with exposure to this contaminant from common culinary preparations in Spain.

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In-house validation of a prototype reference method for six allergens detection in chocolate by HPLC-MS/MS analysis

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In the last ten years, mass spectrometry has been increasingly exploited to detect allergens in food thanks to the capacity of techniques for multiplexing and providing unequivocal allergen identification. This methodology offers great potentials for designing a reference method for multiple allergen analysis in food commodities that is the major goal of the European ThRAll (Thresholds and Reference method for Allergen detection) project funded by the European Food Safety Authority. The method under development refers to a prototype quantitative method for multiple detection of food allergens in incurred food matrices and target six allergenic ingredients, including cow's milk, hen's egg, peanut, soybean, hazelnut and almond to be detected in two hard-to-analyse food matrices, namely chocolate bar and broth powder [1]. Both matrices were produced in a food pilot plant, to mimic as closely as possible the actual manufacturing processes and the allergenic ingredients were incurred in order to simulate the accidental contamination of matrices along the production line. Such materials were further used for the development of a multiplex HPLC-MS/MS method monitoring highly selective and reliable peptide markers reporting for contamination from the selected allergens [2, 3].
In this communication, the advances obtained with the first incurred matrix produced, i.e. chocolate bar, will be presented. The prototype reference method was designed for absolute quantitation of allergenic ingredients by matrix matched calibration curves prepared with synthetic peptides and isotopically labelled surrogates as internal standards. The method was validated in-house by testing the main analytical performances such as linearity, sensitivity, matrix effect, specificity, precision and trueness. The response linearity was tested over two orders of magnitude (0.5-50 fmol/µL) for all the six allergens. The method provided different sensitivity depending on the specific allergen, and grounded on the detection of at least two peptide markers per allergenic ingredient at the lowest detected point except for egg white. Different analytical approaches available for the experimental calculation of detection and quantification limits (LOD/LOQ) were applied and discussed. For most of the reporting markers, the detection sensitivity resulted matrix-dependent by comparison of matrix-matched and standard calibration curves. The detection specificity was proved (i) *in-silico* by BLAST (Basic Local Alignment Search Tool) search of the peptide markers sequence against UniProtKB database and (ii) *in-vitro* by analysing blank chocolate bar. The method reproducibility (intra-day variation) and intermediate precision (inter-day variation) were evaluated on incurred samples at two concentration levels (4 and 40 ppm), resulting in CV% always below 20%. As for method trueness, due to the limited availability of proper reference materials (RMs) a dual approach was followed including (i) recovery experiments on spiked matrices and (ii) direct analysis of the only chocolate based RM currently available on the market and commercialized by LGC Standards. Finally, the method was tested by analysing incurred samples at decreasing concentration levels (40, 10, 4 and 2 ppm) proving its applicability at the lowest recommended VITAL doses for all the six allergenic ingredients.
This method could represent, in perspective, the first quantitative reference method for egg, milk, soybean, peanut hazelnut and almond detection in chocolate based matrixes as result of ThRAll project.

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Raspberry fruit drying stabilization and application 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, with a view to its stabilization and subsequent incorporation into muffin formulations. The two red raspberry varieties under study, *Pacific Deluxe* and *Versailles* had a similar colour (*CIELab* parameters) and a similar profile and content in phenolic compounds. When evaluating the impact of the 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 the 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 30ºC revealed a higher impact in the colour of the dried fruits.
The incorporation of *Versailles* raspberry fruit 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 the alkaline pH of the dough, was enhanced by the syneresis phenomenon when using fresh raspberries. Sensory analysis revealed a good acceptance of all muffin formulations, however, those containing the freeze-dried sample proved to be the most appreciated in terms of fruit appearance and sweet/acid balance. Thus, this is a promising approach for the development of a new product promoting the valorisation and sale of raspberry fruit waste under a circular economy concept.

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Nutritional enrichment of "Económicos" through the incorporation of chestnut flour

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"Económicos" are traditional Portuguese cakes from the region of Trás-os-Montes, made from inexpensive ingredients, such as flour, sugar, margarine, olive oil, eggs and brandy. Although widely consumed, the combination of ingredients does not provide any significant nutritional benefit [1]. The species *Castanea sativa* Mill. represents a valuable natural resource in Northern Portugal, however not all fruits are commercialized due to fruit calibre requirements [2]. There are several studies that demonstrate the nutritional importance of chestnuts [3], thus, increasing the nutritional value of "Económicos" with chestnut, which could be an opportunity to improve these cakes. Therefore, in the present work, "económicos were incorporated with chestnut flour and analyzed over 32 days of storage time. The nutritional profile analysis, including proteins, crude fat, moisture, ash, fibers, carbohydrates and energy were carried out following the AOAC official methods [4]. Soluble sugars were determined using HPLC-RI, organic acids by UPLC-DAD and fatty acids by GC-FID. Two batches of "Económicos" were used, one with 9% of chestnut flour and the other one without any incorporation, and a two-way analysis of variance was applied (storage time and type of flour as the main factors). This traditional Portuguese pastry product showed a relatively low moisture content (13.1±0.8 g/100 g fw), with carbohydrates being the macronutrient present in the highest quantity (57±2 g/100 g fw), followed by proteins (7.4±0.5 g/100 g fw), offering an energy value of 417±8 kcal/100 g fw. Sucrose (28±4 g/100 g fw) was the only feee sugar present, while two organic acids were identified, with oxalic acid (0.04±0.01 g/100 g fw) standing out. Fourteen fatty acids were also found, with greater abundance of butyric (C4:0 – 11.4±1.6 g/100 g fw) and linoleic (C18:2n6c – 1.9±0.4 g/100 g fw) acids. Regarding the nutritional value of "Económicos", for moisture and ash, the interaction of both factors was significant, which did not allow for an individual classification of these factors; there was a statistically higher amount of crude fat in the control samples, but lower carbohydrates, meaning that the flour significantly influences the content of these two nutrients. Regarding the content in proteins, fibers and energy, no significant differences were verified. For the sucrose content and for the individual and total content of organic acids, a significant interaction between both factors was detected, although through the estimated marginal means, a higher amount of all these molecules was detected in the cakes with chestnut flour. Finally, concerning the fatty acid composition, the interaction of the two factors was significant, not allowing an individual classification. It could be concluded that the chestnut flour does not significantly influence the composition of the traditional "Económicos" at 9% of total flour, contributing to a market diversification and valorisation of this traditional product, while also providing higher nourishment and bioactive molecules to the traditional cakes. Sensorial analysis will be performed on the developed Económicos, as well as the bioactive analysis to understand other positive effects that chestnut flour can add to the "Económicos".

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Chemical composition and bioactivities of the *Nephelium lappaceum* L. epicarp

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The industrialization of fruits is important for economic and social development, in addition to enabling the commercialization and distribution of these fruits in different parts of the world. However, this type of industry can cause environmental damage due to the large amount of organic waste generated. In recent years, several studies have suggested the recovery of bioactive molecules from fruit by-products and bioresidues for the maximum use of natural resources, as well as adding value to parts of the fruit normally not used by the traditional industry [1,2]. In this context, *Nephelium lappaceum* L., known as rambutan, is a tropical fruit native to Southeast Asia, highly appreciated for its exotic form and flavour. However, this fruit has an inedible epicarp that can correspond to ± 48% of the whole fruit, which is converted into a by-product after fruit processing [3]. To propose a valorisation of *N. lappaceum* epicarp, this study focuses on the elucidation of its chemical composition in terms of tocopherols (by HPLC-fluorescence), fatty acids (by GC-FID), organic acids (by UFLC-PDA), and non-anthocyanin and anthocyanin phenolic compounds (by HPLC-DAD/ESI-MS). The aim of this study was also to determine antioxidant activity (by TBARS and OxHLIA assays) and antimicrobial activity (by microdilution method using six bacteria – *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella Typhimurium*, and *Enterobacter cloacae* – and six microfungi – *Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus niger*, *Penicillium funiculosum*, *Penicillium verrucosum* var. *cyclopium*, and *Trichoderma viride*). As results, *N. lappaceum* epicarp showed three tocopherol isoforms (α-, γ-, and δ-tocopherols), twenty-five fatty acids, of which oleic, palmitic, and linoleic acids were the major compounds, five organic acids, with shikimic acid being the most abundant, five non-anthocyanin phenolic compounds (ellagitannin derivatives) and two anthocyanins (*O*-glycosylated delphinidin derivatives). The hydroethanolic extract of *N. lappaceum* epicarp was able to inhibit lipid oxidation and protect erythrocytes from haemolysis at low concentrations (EC_{50} values of 2.79±0.03 and 72±2 μg/mL, respectively). The antibacterial and antifungal activities of the extract were reached at concentrations similar to, or lower than, the controls used (sodium benzoate (E211) and potassium metabisulfite (E224)), and the samples also showed bactericidal and fungicidal activity on all microorganisms evaluated. *N. lappaceum* epicarp was proved to be rich in ellagitannin derivatives, also presenting other interesting bioactive molecules. This part of the fruit showed good antioxidant activity and has the potential to inhibit the growth of microorganisms. The results found reveal that this fruit by-product can be an interesting source of bioactive ingredients that can be applied in different sectors of the industry, such as in the production of functional foods, food additives (preservatives and colorants), and pharmaceutical products, among others.

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Tradescantia zebrina Bosse: study of the phenolic composition and bioactive properties of a potential natural coloring ingredient

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Tradescantia zebrina Bosse is a succulent pantropical herb, native to Central America with a high capacity to grow in various conditions [1]. This plant is popularly used in cooking and traditional medicine [2]. Some species of the *Transdecantia* genus are described as possessing exceptionally stable anthocyanic compounds in plant organs, a condition of extreme importance for application in the food industry that is currently looking for potential natural coloring ingredients [3]. In this sense, the present work aimed to explore the phenolic composition and bioactive properties of the aerial parts *T. zebrina* extract. The phenolic composition of the extract was defined by High Performance Liquid Cromatography (HPLC-DAD-ESI/MS) and allowed the identification of 11 anthocyanins glycoside derivatives. Regarding the bioactive properties, i) the antioxidant activity was tested using an oxidative hemolysis assays (OxHLIA), reducing power, and free radical scavenging assay (DPPH); ii) cytotoxicity was evaluated in human tumor cells (MCF-7, breast carcinoma; NCI-H460, lung cancer; AGS, gastric carcinoma; and CaCo, colorectal adenocarcinoma) and hepatotoxicity in non-tumor cells (PLP2, pig liver; and Vero, monkey kidney cell) by the sulphorodamine B method; iii) the antimicrobial activity was evaluated against a panel of twelve food pathogens (*S. aureus*, *B. cereus*, *L. monocytogenes*, *E. coli*, *S.* Typhimurium, *E. cloacae*, *A. fumigatus*, *A. niger*, *A. versicolor*, *P. funiculosum*, *P. verrucosum var. ciclopium* and *T. viride*) using the microdilution method. The analyzed extract presented promising antioxidant activity, with promising EC_{50} values for all the testes assays, ranging from of 0.190 ± 0.001 mg/mL (DPPH), 0.061 ± 0.002 mg/mL (reducing power), and 0.057 ± 0.001 mg/mL (OxHILIA). Furthermore, the extract did not demonstrate toxicity at the maximum tested concentration (>400 µg/mL), but a remarkable activity against gastric carcinoma (AGS) was detected. Additionally, the extract showed excellent results in antibacterial and antifungal performance against all strains tested. In conclusion, it can be observed that the extract obtained from the aerial parts of *T. zebrina* represents themselves a natural antioxidants of great value for the industry, which, in addition to conferring colour to food products due to the quantity of anthocyanins, can contribute to the preservation of food products.

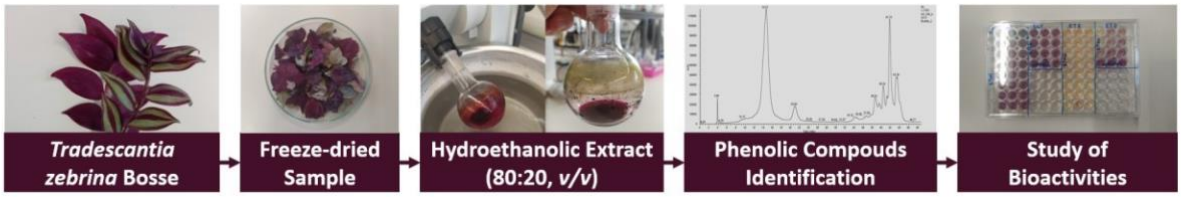

Fig.1. Study steps of the hydroethanolic extract (80:20, *v/v*) of *Tradescantia zebrina* Bosse.

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Stimulation of intestinal microbiota with fructooligosaccharides favourably enhances the effects of raspberry polyphenols in rats

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Raspberries are known as a rich source of dietary antioxidants largely due to their high level of phenolic compounds, which are primarily comprised of anthocyanins and ellagitannins [1]. In addition to strong antioxidant properties, raspberry polyphenols have also shown other beneficial bioactivities, including anti-inflammatory properties, antimicrobial activity against pathogenic intestinal bacteria, and anti-proliferative action towards human cancer cells [2,3]. However, the mechanisms linking the modulation of intestinal microbiota with metabolism of polyphenols and their effects on systemic and liver parameters are still not fully elucidated. Therefore, the aim of this experiment was to investigate whether specific stimulation of intestinal microbiota by fructooligosaccharides (FOS) may enhance the hepatic levels of bioactive compounds derived from raspberry polyphenolic preparation and how this dietary supplementation may affect liver and systemic parameters related to lipid metabolism and antioxidative status in healthy rats.

Raspberry juice was used as the raw material for the preparation of the raspberry phenolic extract. The nutritional experiment was performed using male Wistar rats allocated to 3 groups of 8 animals each with a similar initial weight. Animals were fed for 8 weeks with modified rodent diets (AIN-93G). Group C was fed a standard diet for laboratory rodents, group CP was fed a diet enriched with 0.3% raspberry polyphenols, and group CPF was fed a diet containing 0.3% raspberry polyphenols and 3% FOS. The effects of raspberry polyphenolic preparation and FOS on microbiota activity, the microbiota profile in the caecum, the hepatic level of polyphenolic compounds, the mechanisms regulating liver lipid metabolism, oxidative stress and the plasma lipid profile were analysed.

The raspberry polyphenolic extract contained 15.2 g/100 g phenolic compounds, most of which were identified as ellagitannins (7.8 g/100 g) and anthocyanins (6.3 g/100 g). After 8 weeks of feeding, there were no significant differences in values of diet intake and body weight among the experimental groups. The dietary combination of polyphenols and FOS considerably increased microbial production of short-chain fatty acids, mostly acetic and propionic acid, and favourably modulated the bacterial profile in the caecum. Microbial NGS (next-generation sequencing) analyses showed that in the CPF group, a higher abundance of bacteria responsible for the metabolism of polyphenols, e.g., *Bifidobacterium, Lactobacillus* and *Sellimonas intestinalis*, was observed. Changes in the microbiota profile indicated considerably higher levels of bioactive compounds, e.g., ellagic acid, 4-hydroxybenzoic acid, methyl-cyanidin-3-sophoroside and ellagic acid dimethyl ether glucuronide, in the livers of rats from the CPF group. Higher concentrations of these bioactive compounds might explain the observed changes in antioxidant parameters and lipid metabolism. A diet supplemented with raspberry polyphenols and FOS reduced the concentration of malondialdehyde in the liver (marker of lipid peroxidation), increased the value of the total antioxidant status in plasma and reduced the fat and triglyceride contents in the liver. In the CPF group, the hepatic mRNA expression of HIF-1α (hypoxia-inducible Factor 1α), AHR (aryl hydrocarbon receptor) and SREBP1c (sterol regulatory element binding protein 1c) was reduced. These biomarkers are involved in mechanisms of oxidative stress and liver metabolism. Moreover, hepatic mRNA expression of ANGPTL4 (angiopoietin like 4), the main inhibitor of lipoprotein lipase, was considerably elevated in rats from the CPF group. This might explain the considerably higher concentration of plasma triglycerides observed in this group.

To conclude, this nutritional experiment shows that specific stimulation of intestinal microbiota with FOS might considerably increase hepatic levels of bioactive compounds derived from a diet supplemented with polyphenolic preparation and thus enhance mechanisms associated with lipid metabolism and the antioxidative status in healthy rats.

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Impact of *Fucus vesiculosus* phlorotannins on the human gastrointestinal tract

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Seaweeds are one of the largest producers of biomass in the marine environment and a source of multiple bioactive metabolites with valuable health benefits. Among these, phlorotannins have been widely recognized for their promising bioactive properties [1,2]. However, little is known about the fate of brown seaweeds' phlorotannins when they cross the gastrointestinal tract (GIT). This work aimed to evaluate the biological effects of phlorotannins during their passage throughout the GIT, from the ability to inhibit the activity of key metabolic enzymes such as α-amylase, α-glucosidase, and lipase, to their capacity to induce cytotoxicity on different tumor cell lines from the GIT, including MKN-28 (stomach adenocarcinoma cells), HT-29 and Caco-2 (both cell lines of colon colorectal adenocarcinoma). Moreover, a simulated gastrointestinal digestion followed by a fermentation procedure using human fecal inoculates was carried out to evaluate the stability, antioxidant activity, bioaccessibility and modulatory effects of *F. vesiculosus* phlorotannins extracts on human gut microbiota and short-chain fatty acids production. Good inhibitory effects were observed on the enzymes tested, particularly against α-glucosidase on which stronger effects were observed compared to acarbose (IC_{50} values of 0.82 – 4.5 µg/mL versus 206.6 µg/mL), an anti-diabetic drug used to treat diabetes mellitus type 2 by targeting this specific enzyme. Likewise, promising cytotoxicity was found on the different tumor cell lines tested, in opposition to the normal cell line HFF-1 (human fibroblasts), suggesting that these *F. vesiculosus* phlorotannin samples present tumor selectivity. Following the simulated gastrointestinal digestion, it was found that, like land plant phenolics [3], the concentrations of phlorotannins in the extracts, as well as their antioxidant ability, tended to decrease progressively along their cross through the GIT. Moreover, from the portion that reached the small intestine intact, only a small fraction below 15% was found bioaccessible and available for absorption, indicating that most of these compounds will accumulate in the large intestine where they can interact with the gut microbiota. Indeed, the fecal fermentation of the digested *F. vesiculosus* phlorotannin extracts revealed a slight positive effect on the growth of certain commensal bacteria from the human gut, particularly on *Enterococcus* spp., and a very interesting capacity to stimulate the production of propionate and butyrate, both representing important short-chain fatty acids known for their health-promoting properties. In summary, this work provides valuable information regarding the bioactivity and behavior of *F. vesiculosus* phlorotannins along the GIT, presenting clear evidence that these compounds can positively contribute to the maintenance of a healthy gastrointestinal condition and an overall health status.

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A multi-target strategy to prevent urinary tract infections: the dual mechanism of action on intestinal barrier and urinary epithelium of a novel nutraceutical combining cranberry extracts, D-mannose and ascorbic acid

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Lower urinary-tract infections (UTIs) represent a widely diffused bacterial disease with a high risk of occurrence and recurrence, especially in women [1]. Severe UTIs are usually treated using antimicrobial drugs, however, for milder affections, alternative strategies other than pharmacological treatment have been proposed. Among these, nutraceuticals containing cranberry (*Vaccinum macrocapon* Ait.) extracts represent the most common remedies for the prevention and treatment of UTIs, due to their efficacy [2,3], scarce side effects, and high safety [4]. Nevertheless, there is still a large debate about the efficacy of cranberry in preventing UTIs, due to controversial clinical outcomes and literature data. Although several articles report the efficacy of cranberry on inhibiting the adhesion of uropathogenic bacteria to the urothelium, results from many clinical trials indicate negligible effects of this natural product compared to placebo [5]. These results could be due mainly to lack of standardization of bioactives in the preparations tested or to the inconsistency of dosages and low compliance [5,6]. Furthermore, there is still uncertainty regarding the mechanism(s) of action of cranberry, although abundant constituents belonging to the class of A-type proanthocyanidins (PAC-A) and metabolites produced by the gut microbiota from the degradation of PAC-A and excreted with urine have been proposed as co-responsible of the antiadhesive effects of cranberry [7]. More recently, positive correlations between the colonization of gut microflora by pathogenic *Escherichia* strains and the occurrence of UTIs have been reported [8], hence it has been proposed that the beneficial effects of cranberry on UTIs could also be related to its positive effects on gut microbiota composition.
In this work, a novel nutraceutical formulation (URO-F) was developed mixing a tailor-made PAC-A-rich cranberry extract with D-mannose and ascorbic acid, two ingredients already proposed as anti-adhesive agents [9,10]. URO-F was characterized by HPLC-FLD-MS to study its composition in PACs, and its protective effects on both urinary and intestinal epithelia were investigated *in vitro*. To assess the antiadhesive activity of URO-F, urine samples collected from six healthy volunteers undergoing a 2-days product consumption were studied. Their antiadhesive activity against uropathogenic *Escherichia coli* was tested on T24 cells (human bladder model), and possible molecular effectors were discovered using an untargeted metabolomics approach. Furthermore, URO-F was evaluated *in vitro* for its ability to promote gut epithelial barrier integrity and to inhibit inflammatory cytokines production on Caco2 cells, both in their basal state and under stress conditions (by treatment with H_2O_2 or extraintestinal pathogenic *E. coli*).

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Structure analysis of proteins at interfaces

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Disperse food systems such as oil/water-emulsions can be stabilized by amphiphilic food ingredients such as proteins. During interfacial stabilization, globular proteins like the whey protein β-lactoglobulin migrate towards the oil/water-interface, adsorb, structurally align, and form an interfacial protein film. The resulting partially unfolded structure of the protein at the interface is influenced, among other factors, by the protein structure in the aqueous bulk phase. Moreover, it determines the functionality of the protein as the emulsifier and thus, the long-term stability of the emulsion. However, the analytical determination of the structure of adsorbed proteins is still challenging. The reason is the superposition of the protein signal by the disperse food matrix, i.e., the signals of the water and the oil. Consequently, the aim of this work was to optimize a Fourier transform infrared (FTIR) spectroscopic method for the structural analysis of proteins at oil/water-interfaces and furthermore, the application of the FTIR method for the comparison of different protein structures of adsorbed β-lactoglobulin in dependence of its structure in the aqueous bulk solution.
First, emulsions of the model protein β-lactoglobulin and MCT-oil were prepared and measured by FTIR against pure water as background. Subsequently, the FTIR spectrum of analogously prepared sodium dodecyl sulphate (SDS)-stabilized emulsions was subtracted, as SDS is a low molecular weight emulsifier that does not absorb in the protein region. Consequently, the water and oil signals present in the emulsion could be accurately subtracted and the typical amide I and amide II bands of the adsorbed protein could be obtained *in situ* from the overall emulsion signal. Based on the second derivative, relative changes in the secondary structure elements α-helix, β-sheet, and random coil of the protein were detected at the oil/water-interface.
In a second step, the method was applied by measuring protein-stabilized emulsions made of proteins with different initial protein structures. Therefore, the structure of β-lactoglobulin was modified intentionally in the aqueous bulk solution by varying the pH and the ionic strength as well as by applying high pressure treatments. The resulting protein structures in the aqueous solution contained a charged folded monomeric β-lactoglobulin at pH 7, an electrostatically shielded partially unfolded dimeric β-lactoglobulin at pH 7 containing 100 mM NaCl, a charged folded monomeric β-lactoglobulin at pH 9, and a less charged partially unfolded dimeric β-lactoglobulin at pH 7 treated with high hydrostatic pressure at 600 MPa (Fig. 1). The comparison of the different β-lactoglobulin samples revealed that a partial unfolding and thus, increased hydrophobicity of the protein structure in the aqueous solution favours the further unfolding of adsorbed proteins (e.g., decrease in intramolecular β-sheets, increase in random coil structures) due to an increased affinity with the hydrophobic oil phase.
The results of this study contribute to the structural elucidation of proteins at liquid interfaces and will allow further conclusions about structure-function relationships of interfacial active proteins in the future. In particular, more complex disperse food systems can be studied, in which proteins are specifically structurally modified by other food components (e.g., carbohydrates or secondary plant metabolites such as phenolic compounds) and thus, being affected in their interfacial properties.

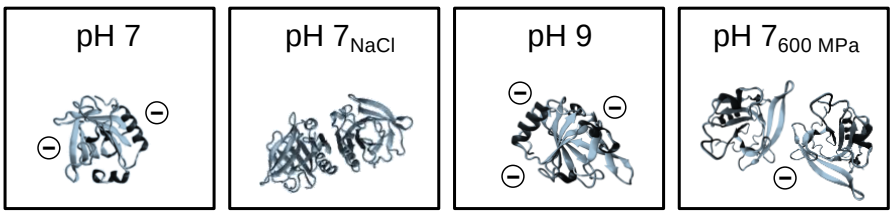

Fig.1. Molecular dynamics simulation of the four used protein structures in the aqueous bulk phase.

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Oxidative stability of beer assessed by electron paramagnetic resonance (EPR) spectroscopy

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Oxygen is both a friend and a foe to beer. In the early stages of the beer making process, oxygen is needed for the proper germination of the barley embryo in the malting process, and it is used later by yeast to manufacture and maintain its cell membrane. However, oxygen becomes a problem when the finished product is stored, contributing to the deterioration of the beer and shortening the shelf-life of the packaged product [1].

The oxidative reactions in beer are based in iron catalyzed trapping of oxygen leading to the initial formation of hydrogen peroxide (H_2O_2), which then reacts with Fe (II) through the Fenton reaction and leads to the generation of hydroxyl radicals (•OH). These radicals can then oxidize beer components (especially ethanol, the second most abundant constituent of beer after water) forming α-hydroxyethyl radicals [1, 2]. Moreover, the hydroxyl radicals can also lead to the formation of other aldehydes and ketones or initiate a series of secondary radical reactions that could further oxidize compounds in beer [1, 2].

Beers stored at high temperatures, frequently employed to accelerate the oxidation process, still have antioxidants that delay radical-induced reactions. These antioxidants are normally sulfites and polyphenols that protect the beer by terminating free-radical pathways, thus delaying oxidation. This is the so-called *lag phase*, the period beer takes until all the antioxidants are consumed and radicals can be detected [3].

EPR allows the detection of free radicals in the degradation process of food and beverages. However, because of the short lifetime of radicals generated in food, direct observation is feasible only at low temperatures. One method to overcome this limitation is the use of spin-trapping techniques, which allows the indirect detection of food-derived radicals by forming spin adducts in detectable quantities, allowing for both characterization and quantification of the trapped radical [3]. The α-hydroxyethyl radical, •$CH(CH_3)OH$, has been identified by using different spin traps once is the radical that predominantly lead to formation of EPR – detectable spin adducts [4]. One of the most used spins traps for the identification of this radical is α-pyridyl-N-tert-butylnitrone (PBN). The primary α-hydroxyethyl radical is formed during aerobic forced aging and subsequently reacts with PBN to give a stable spin adduct [5].

The aim of the present study was to evaluate the oxidative stability of beers submitted to different storage conditions: fresh beers, naturally aged beers (90 and 180 days at 20 ± 2 ºC) and forced aged beers (7 and 14 days at 37± 1 ºC). The oxidative stability was studied by monitoring the formation of radicals. For this purpose, an accelerated aging (55 ºC) process was applied to beers and the radicals were determined by spin trapping with PBN and EPR detection. The *lag phase* and the T_{150} values were evaluated and compared for beers under study. Moreover, the impact of temperature, iron and antioxidants, especially sulfites, on beer oxidative stability will be discussed.

The different storage conditions applied to beers, namely time and temperature, were shown to affect the beer oxidative stability. Fresh beers showed a greater endogenous antioxidant potential (higher *lag phase* value). However, after 150 min of accelerated aging, they have shown a higher radical content. The differences observed in the endogenous antioxidant potential and the levels of radicals after 150 min of accelerated aging (55 ºC) are related to the levels of sulfites and iron found in each one of the analyzed beers. Lower levels of iron are related to the lower radical generation in beers stored at high temperature. Higher levels of sulfites are related to the higher *lag phase* values, and consequently higher oxidative stability, determined for fresh beers.

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Validation of an analytical methodology for mycotoxin determination by UHPLC-MS/MS in the maize value chain

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Mycotoxins are toxic compounds of low-molecular weight that commonly enter the food chain through contaminated food and feed crops, mainly cereals [1]. These toxins occur in every step of the agri-food chain due to its ability to bioaccumulate through different levels of the food pyramid, and its negative effects have impact in harvest, production profitability, animal health and safety of the end-products of this food chain [2]. Since maize is one of the oldest and most prominent cereal crops in the world, presenting a key role in food security and sovereignty, its quality and safety as an animal and human food product must be ensured and, therefore, it is important to have effective and reliable analytical methods to identify and determine mycotoxins at legislated levels for appropriate risk assessment and to allow the enforcement of regulatory limits [3].
Mycotoxin monitoring is the first step of an accurate risk assessment and, consequently, sampling plans and analytical procedures must be well established to guarantee reliable results. Several official validated methods have been made available by CEN and AOAC, though the need to develop and validate new methods is crucial to enforce the very low limits introduced by the European Commission and to encompass the constantly changing mycotoxic patterns [1,3]. Nowadays, optimization and validation of analytical methods towards the multidetection of mycotoxins has become an increasing challenge due their chemical diversity, and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has been characterized as the golden method for these multi-analysis purposes [1]. In the present work, blank maize samples fortified with mycotoxin standards were subjected to an optimized extraction procedure based on modified QuEChERS protocols, using a combination of C18 and PSA sorbents. The identification of a wide range of toxic compounds, from regulated (aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, zearalenone, T-2 and HT-2 toxins) to emerging mycotoxins (moniliformin, enniatins, beauvericin) in different samples from the maize value chain was performed using a liquid chromatography coupled with tandem mass spectrometry (UHPLC-QTRAP-MS/MS) methodology. Validation parameters, including linearity, limit of detection (LoD), limit of quantification (LoQ), precision and recovery were evaluated, and compared among the different published analytical methods. Performance criteria was performed according to specific requirements for confirmatory methods stated in Commission Regulation nº 401/2006 [4]; and non-regulated mycotoxins followed the guidelines established by FDA [5], ICH Q2 (RI) [6] and Relacre [7].
The present methodology successfully permitted the extraction and determination of 23 mycotoxins in seeds, flowering plants, harvested grains, and forage of the maize value chain. The mass detector on MRM mode for data acquisition provided an excellent specificity and linearity for all the compounds analysed, allowing an unequivocal detection and confirmation of all mycotoxins analysed at low levels in these matrices with commercially available reagents.

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Innovation in puffed breads production: asparaginase application to acrylamide diminishing

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In order to improve the nutritional quality of cereal products that meet consumer expectations, producers are expanding their portfolio with innovative non-wheat cereal products from rye, oats, spelt, buckwheat etc. On the other hand, in addition to their nutritional benefits, they may be a source of undesirable heat-induced contaminants such as probably carcinogenic acrylamide. The main precursors of acrylamide have been proven to be the non-essential amino acid asparagine and reducing sugars. Cereal-based thermally treated products are high in acrylamide depending on precursors presence in raw material, heat and processing conditions. Based on our observations of acrylamide in puffed breads, bio-rye and bio-spelt breads were recognized as the highest in acrylamide (850 and 770 µg/kg, respectively), significantly exceeding the benchmark level (300 µg/kg) set by Commission Regulation no. 2017/2158. One of the effective ways to reduce acrylamide in foods is the application of asparaginase. It is strongly recommended due to the imperceptible effect on the sensory quality of the final products. In our case study, the enzyme asparaginase was first successfully used in the technology of making rye puffed bread with prior flour extrusion. The level of asparagine in rye flour used in this treatment was more than 700 mg/kg. Asparaginase was applied during the moistening of the rye flour before extrusion, which resulted in a substantially reduced level of asparagine (below 100 mg/kg) of the rye pellets formed. This was reflected in a markedly low acrylamide puffed bread (approximately 340 µg/kg) with adequate sensory properties. The whole process of application of asparaginase in this technology was registered at the Industrial Property Office of the Slovak Republic as utility model number 9269 and subsequently applied at the European Patent Office as European patent application no. EP 3 847 903 A1 (currently under evaluation).

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Organic chicken meat in a compostable biopackaging solution: a comparative shelf-life study

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Nowadays the widespread use of plastic packaging constitutes a crucial ecological problem. This concern is leading to an important shift to biodegradable and compostable materials by packaging producers [1]. Among different meat production systems, organic one has notably increased in importance over recent years, due to its high attention to animal feed and welfare [2]. According to FAO, world poultry meat production soared from 9 to 122 million tonnes in last 50 years [3]. This work aims to assess the ability of a new biopackaging, based on biodegradable and compostable material, to preserve the quality of organic chicken meat for 14 days. Biopackaging consists on a compostable multilayer structure obtained using bio polyesters from polycondensations of diacids and diol. A comparative study was performed between the biopackaging and a traditional polyethylene terephthalate (PET) packaging. Chemical (biogenic amines, volatile organic compounds) and microbiological (meat microbiota) markers as well as sensorial parameters of packaged organic chicken breast meat were monitored during a 14-days shelflife. Results showed that the concentration of biogenic amines and of the 18 monitored volatile organic compounds (VOCs) showed a similar trend in both packaged chicken meats. For example, the total biogenic amines concentration in meat increased from 390 to 961 mg kg^{-1} in biopackaging and from 393 to 800 mg kg^{-1} in PET. Also microbiological counts, meat pH monitoring and sensorial parameters did not present important differences between biopackaging and PET preservations. In conclusion, the new proposed biopackaging showed similar properties as PET material to preserve the shelf life of organic chicken meat but with the great advantage to be completely biodegradable, compostable, and sustainable for the environment.

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Lipid oxidation in sorted herring (*Clupea harengus*) filleting co-products and its relationship to composition

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In industrial fish filleting, around 30-70 % of the total weight of the fish end up as side streams (often called by-products), such as the head, backbone, caudal fin, skin, and intestines. Currently, these fractions are dedicated to low value uses as fodder meals or mink feed, even if they contain significant amounts of protein, long chain (LC) n-3 polyunsaturated fatty acids (PUFA), and other nutritional components such as vitamins and minerals. Established and more recent techniques enabling value addition to fish filleting side streams are for example mechanical meat-bone separation using belt and drum, the pH-shift process, enzymatic hydrolysis and, for oil alone, supercritical fluid extraction. However, most factories that fillet fish mix their side streams, not least when it comes to small pelagic species like herring. This practice limits use of the co-products for food production since the raw material gets very complex, and since blood, enzymes and lipids from e.g., the viscera and head parts easily contaminate the cleaner parts like the backbones and tails, accelerating e.g., their oxidative or enzymatic degradation. Also, different filleting co-products contain different amounts of nutrients and could therefore be suitable for different end uses and value addition processes. Therefore, sorting fish filleting side streams into separate raw material fractions, and choosing the best method to add value to them individually depending on their nutritional components will be important to take co-product valorization to the next level. In present study, lipid oxidation in ice-stored sorted and minced herring fractions (head, backbone, viscera+belly flap, tail, fillet) from spring and fall, and its association with endogenous pro-oxidants, antioxidants and lipid substrates were investigated. Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) had increased significantly in all fractions after 1 day, but for both seasons, the most rapid PV and TBARS development occurred in head, which also had highest hemoglobin (Hb) levels and lipoxygenases (LOX) activity. Viscera+belly flap was overall the most stable part, and also had the highest α-tocopherol content. Pearson correlation analyses across all five fractions confirmed a significant impact of Hb, LOX and α-tocopherol on the lipid oxidation susceptibility, while content of total iron, copper, lipids or PUFA provided no significant correlation. Overall, the study showed which pro-oxidants that should be inhibited or removed to succeed with value adding of herring filleting side streams along with the fillet itself.

Fig 1. The sorted herring filleting side stream fractions and fillet

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Effect of feeding olive pomace and soybean acid oils on European seabass fillet quality

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The importance of aquaculture production to cover food fish demand has significantly raised in last decades [1]. Fish oil is an essential component in fish diets, so its demand has raised with the increase of aquaculture production, while fish oil production has remained static [1-2]. As this fact could jeopardize aquaculture sustainability, it has been mandatory to find alternative fat sources to partially replace fish oil in fish diets. Despite the options which have drown more attention are crude vegetable oils [3], the use of some fat byproducts coming from food industry, might be interesting. Acids oils (AO) are fat by-products from chemical refining of edible oils, characterized by their high proportion of free fatty acids [4]. For years, there has been an interest in using them in animal feeding because of their high energetic value and because, by being upcycled, they will increase the sustainability of the food chain. In this study, specimens of European seabass (*Dicentrarchus labrax*) were fed 5 dietary treatments with 16 % of added fat that was degummed fish oil (F), or a blend of F and another fat source (at 25:75, p/p): crude soybean oil (S), soybean-sunflower acid oil (SA), crude olive pomace oil (OP) or olive pomace acid oil (OPA). After 3 months, fillets were sampled (5 replicates per treatment). Fillets were stored under commercial refrigeration conditions ($CO_2/N_2/O_2$; 40/30/30; 2°C) for 0 or 6 days. The fatty acid (FA) composition [5]; tocopherol (T) and tocotrienol (T3) content [5]; lipid peroxide formation by the induced FOX assay [6] (incubation for 96h); TBA value [7]; color; and the sensory acceptance, with nine-scale hedonic tests, of the fillets were determined on ground samples.
The F fillets were the most unsaturated (much higher content in EPA and DHA). All treatments had α-; β-; γ- and δT, and β-T3, but their total level (T+T3) and profile differed: F showed the lowest level of total T+T3 and of α-T (the predominant tocol); S had the highest T+T3 and γ-T levels, followed by SA. The refrigeration decreased the total T+T3 in all treatments. FOX results revealed that F had the lowest oxidative stability, while OP and OPA were the most stable. S and SA had an intermediate stability because, although they were more unsaturated than OP and OPA, they had higher T+T3 content, which could have reduced the development of oxidation reactions. No influence of the refrigeration was observed on oxidative stability results. TBA values showed higher levels for F and for all refrigerated samples. Color results of fish flesh depended on the diet used and on the refrigeration time, while the sensory acceptance was not affected by these two factors.
In conclusion, the replacement of F by vegetable oils used (S, SA, OP and OPA) in European seabass diets decreased EPA and DHA content of fish fillets and increased the total tocol levels. These composition changes were accompanied by an increment of the oxidative stability and a reduction of the TBA values of fish fillets. However, the inclusion of the vegetable oils at the levels used in this study did not affect the sensory acceptance of fish fillets. The use of AO instead their corresponded crude oil did not have any impact on oxidative stability, TBA values or sensory acceptance. In addition, despite the refrigerated storage clearly increased secondary oxidation products, it did not affect the sensory acceptance of fish fillets.

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Biorefining platform for the recovery of health beneficial fractions from fruit processing by-products

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Globally around 14% of the world's food is lost from production before reaching the retail level, while the loss of fruits and vegetables comprise approx. 23% [1]. Many fruit species are known for their excellent flavour and healthy phytochemicals. However, many fresh fruit species rapidly deteriorate after harvesting and therefore the major parts of their yields are processed into various longer shelf-life products sometimes generating large amounts of by-products at various production steps. For instance, pressing juice generates large quantities of pomace (press-cake) containing on average 30% of the whole dry matter, which includes valuable nutrients [2]. Currently huge fractions of such by-products are used rather inefficiently, e.g. for composting, animal feeding, or even discarded as a waste. Therefore, comprehensive and systematic studies focused on valorisation of fruit processing by-products are of high interest both for growers and industry [3]. Such studies should encompass scientific, technological and economic issues.

Our investigations in this field during the last 6 years have proposed an integrated biorefining schemes for processing small fruit (berry-size) pomace into high value ingredients by using supercritical CO_2 (Sc-CO_2), pressurized liquid (PLE) and enzyme assisted (EAE) extractions. The results obtained demonstrated that healthy functional ingredients may be obtained from the pomace by a combination of the above-mentioned processes. Sc-CO_2 effectively recovers lipophilic fractions consisting mainly of oil, which is rich in polyunsaturated fatty acids, tocopherols, carotenoids and phytosterols: at the optimized parameters the yields of lipophilic extracts were from 3% (chokeberry) to 20% in (raspberry). The Sc-CO_2 extraction residue is further processed by using increasing polarity subcritical solvents at 10 MPa and different temperatures and extraction cycles. This process, depending on the fruit species and extraction parameters, produces 20-60% of soluble fractions, which are strong antioxidants and contain various health-beneficial bioactive phytochemicals. Finally, enzyme assisted extraction enables to recover remaining water-soluble substances such as oligosaccharides, bound polyphenolics and others. Further selection of fractionation methods and parameters enables obtaining the products with a high concentration of target phytochemicals.

Phytochemical were identified and quantified by ultra-high performance liquid chromatography (UPLC) with different detection/quantification methods, while antioxidant properties were evaluated by various *in vitro* assays. In addition, cytotoxicity assays were performed in various cell-lines. The presence of bioactive phytochemicals in pomace fractions at high concentrations makes tem suitable for various applications, e.g. in functional foods, nutraceuticals and cosmeceuticals. Some fractions have already been tested in various foods; for instance, strong antioxidants recovered from various pomaces improved oxidative and microbiological stability of meat products, increased antioxidant potential of bakery goods. Moreover, it may be hypothesized that berry pomace phytochemicals might mitigate adverse effects (carcinogenicity) of processed meat products to human health. The results of some recently performed preliminary assays with cancer cell lines are in favour of such hypothesis. Currently some products with berry pomace fractions are patented in Lithuania and EPO.

Keywords: supercritical fluid extraction, pressurized liquid extraction, enzyme assisted extraction, nutrients, antioxidant capacity.

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Betacyanins from *Gomphrena globosa* L. as natural food colorants: application in different foodstuff

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Nature can be an inexhaustible source of natural molecules, such as colorants, which can be extracted and applied in different industries. Looking at the high diversity of colours present in nature, this work intends to target colouring compounds, in the range of pink colours (betacyanins) for the application in different foodstuff. Betacyanins, have been described as unstable to some specific conditions, thus, different stabilization techniques have emerged to enhance their applicability. Bearing this in mind, herein, *Gomphrena globose* L. was explored as a natural alternative source of betacyanins. For this purpose, different extraction procedures were applied, namely dynamic maceration (DM), microwave (MAE) and ultrasound assisted extraction (UAE), supported by the application of mathematical models in order to optimize the extraction procedure and achieve a betacyanin enriched extract. Afterwards, stabilization techniques (freeze drying and spray drying) were applied, when needed. As an extra benefit, the final extracts were also evaluated for biological activities, namely antioxidant, antimicrobial and cytotoxic. The obtained extract was incorporated in ice creams and cookies to analyse their stability and viability after incorporation processes and to assure their resistance to the storage conditions of each food matrices. The final products were evaluated for their nutritional value, physicochemical characteristics and microbial load, to guarantee the organoleptic integrity, and food safety. Regarding the extraction procedure, UAE was chosen due to its higher yield and purity, allowing to obtain 45.5 g betacyanins/kg plant material [1]. The results obtained for the ice cream and cookies incorporated with the enriched extract of *G. globosa* flowers, were compared with different formulations, (*e.g.,* formulations without added food colorants and formulations with added artificial and natural food colorants) in different storage times (ST), and the interactions among both factors were also assessed. Regarding the nutritional composition, the interaction between ice cream formulation (ICF) and ST influenced all the studied parameters. Concerning the colour, the ST did not significantly affect this parameter, enhancing the good stability of these compounds [2]. The chemical composition of the cookies was not significantly altered by the incorporation process, as well as the physical studied parameters. The colour of the cookies incorporated with the *G. globosa* extract presented a deep pink colouration, even after the cooking process, since the colour intensity did not undergo drastic changes along the ST, demonstrating once more a good stability of these compounds, even when subjected to high temperatures [3]. Therefore, this work supports the exploration of these betacyanins as natural colourants, and provides the industry a natural alternative to artificial colorants, being able to offer consumers healthier alternative products.

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Characterization and quantification of apple pomace's phenolic compounds extracted using conventional and pressurized liquid solvent extraction techniques

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Food waste is considered as one of the major current environmental issues, and about 20% of this waste is generated by the food processing industry [1]. Drink and juice manufacturing are among the food industry that generate a considerable amount of waste, and apple juice is among the top five juice flavours. Apple pomace is 25%-30% of the total processed fruit obtained after the juice extraction [2]. Apple pomace is considered as a poor animal feed due to its low content of protein and high amount of sugar, however it is rich in natural antioxidant such as phenolic compounds. Phenolic compounds are powerful antioxidants with potential applications in food and pharmaceutical industries [3]. The objectives of this work were to characterize and quantify the phenolic compounds extracted using conventional solvent extraction technique from apple pomace of six different varieties, and compare this method of extraction to pressurized liquid extraction (PLE) which uses minimum amount of organic solvent at high pressure. The apple pomace studied were from Golden, Fuji, Inord Story, Granny, Pink Lady, and Royal Gala varieties. For conventional extraction of the phenolics, homogenizer-assisted methanol extraction technique was applied [4]. In order to optimize the extraction, PLE was done using methanol as a solvent under various pressures, extraction times, and cycles. PLE extractions were performed on Dionex ASE 200 system. For both extraction techniques, well homogenised fresh apple pomaces were used. After evaporation of the solvent, phenolic compounds were collected in water and analysed using a Nexera-i-LC2040 UHPLCPDA coupled with a LCMS-8040 triple quadrupole mass spectroscopy, and the column used was a Raptor ARC-18 2.7 µm. The quantities of the phenolics were reported based on Epicatechin equivalent (Epi-Eq), and the total phenolics and their profiles were compared within the varieties and the different extraction techniques. When conventional extraction was applied, the total phenolic contents were between 11.2 to 13.6 mg(Epi-Eq)/g DM, and they did not vary significantly among the varieties. The major phenolics tentatively identified were Caffeoylquinic acid, Quercetin-glucoside, Caffeoylarbutin, Quercetin-rhaminoside, B type-procyanidin, Epicatechin, and Caffeoylshikmic acid based on their abundance within all the varieties. The profiles of the phenolics, however varied significantly among the varieties. When PLE was applied, the yield of the extractions almost doubled. Principal Component Analysis (PCA) was used to analyse the phenolic profiles extracted under various conditions. PCA results revealed a clear separation between the PLE and conventional extraction techniques where PLE was more effective in the extraction of peak 1, 2, 3, 6, and 10 (Figure 1). In conclusion, the major phenolic compounds were identified in different apple pomaces. PLE had a significant effect on the extraction yield and showed to be more effective on the extraction of certain phenolics when compared to the conventional extraction.

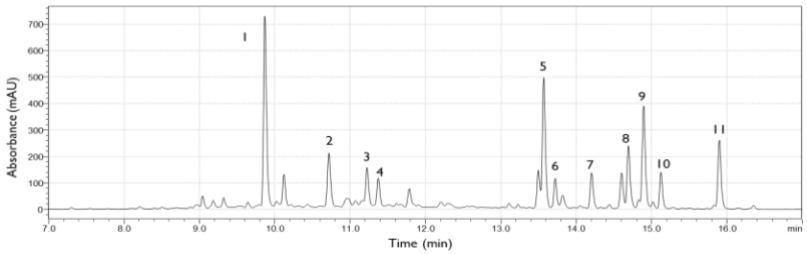

Fig.1. Chromatogram of conventionally extracted Golden apple pomace recorded at 280-370nm. 1, Caffeoylquinic acid; 2, B type-procyanidin; 3, Epicatechin; 4, Caffeoylshikmic acid; 5 and 6, Quercetin-glucoside; 7 and 8, Caffeoylarbutin; 9, Quercetin-rhaminoside; 10 and 11, unknown.

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FLASH COMMUNICATIONS

Synthetic Musks in shrimp and seawater samples from the NW Portuguese coast

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The rapid commercial and industrial development over the years contributed to the increase of coastal environmental pollution. Synthetic Musks (SMs) are chemicals used as fragrance additives in diverse personal care and household products, including perfumes, shampoos, deodorants, and detergents. The European Union forbids the use of musk ambrette, moskene and tibetene in cosmetic products and limits musk xylene and ketone to less than 0.003–1% and 0.042–1.4%, respectively. These emergent pollutants are endocrine disruptors and lipophilic with the capacity to accumulate in biota and one of exposure routes for these chemicals is diet.
Shrimp is one of the most popular seafood consumed worldwide and can be a healthy addition to our diet. Shrimp is low in fat and calories, rich in omega-3 fatty acids and a good source of key nutrients, such as iodine, phosphorus, choline, copper, zinc, B-complex vitamins, vitamin A and E and antioxidants, especially astaxanthin.
The aim of this study was to measure the presence of SMs in two shrimp species, *Palaemon serratus* and *Palaemon varians*, and in the seawater from their natural habitat on the northwest (NW) Portuguese coast. *P. serratus* was collected along the NW Portuguese coast (namely in Vila do Conde, Matosinhos, Aveiro, Ria de Aveiro and Figueira da Foz) by local fishermen and *P. varians* (wild and aquaculture origin) was collected in the Sado estuary. The sampling was performed in autumn and spring between 2017 and 2019 in all the locations. Surface water was collected in the same locations of the shrimp samples and during the same period. The extraction of the SMs from surface water samples was performed by solid phase extraction method using Strata C18-E cartridges in a vacuum system manifold. For shrimp samples the extraction was performed by Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodology. The extent of the contamination in shrimps and water from their habitat was reached through the quantification of 6 SMs (celestolide, ambrette, tonalide, galaxolide, xylene and ketone), using gas chromatography mass spectrometry.
The results for the evaluation of the SMs in water from shrimp's habitat, showed the presence of SMs, galaxolide, tonalide and ketone, although all below the method detection limit (MDL). Regarding shrimp samples the same three SMs were detected, in this case above the MDL. When comparing the average concentration values between spring and autumn for the three detected SMs, higher values were observed in spring. The average concentration value for galaxolide was 4.89 ng/g w/w in spring and 4.15 ng/g w/w in autumn; and ketone presented values of 5.40 ng/g w/w for spring and 4.55 ng/g w/w for autumn. Tonalide only presented values above the MDL in shrimp samples from spring, with an average of 2.86 ng/g w/w.
Regarding the two shrimp species, the average for *P. serratus* was 4.64, 1.86 and 4.41 ng/g w/w and for *P. varians* it was 4.40, 1.83 and 6.33 ng/g w/w for galaxolide, tonalide and ketone, respectively. Aquaculture shrimps have slightly lower values of galaxolide and moderately higher values of tonalide and ketone [1]. These results highlight the need to establish specific legislation for aquatic environments and fishery products, highly valuable in the human diet.

Acknowledgments: This work was supported by UIDB/50006/2020 by the Fundação para a Ciência e a Tecnologia (FCT)/Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds. The authors also thank FCT and the European Union's H2020 Research and Innovation Program 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 (Fundação para a Ciência e a Tecnologia) and ESF (European Social Fund) through POCH (Programa Operacional Capital Humano) for the PhD grant SFRH/BD/128817/2017.

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Extension of shelf-life of lager beer can be a solution to prevent beer wastage resulting from its reduced consumption during the SARS-Cov-2 pandemic?

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Beer is the most consumed alcoholic beverage in Europe, and its aroma characteristics are essentially associated with its volatile components, which have different origins, namely they can result from the metabolism of yeast, from raw materials, or they can be (bio)transformed during beer production and storage. In the current situation of the SARS-CoV-2 pandemic, several sectors have been affected, being one of them the beer market. During the lockdown period, brewers had to destroy beer, since it was not possible to dispose of all production within the shelf-life defined for these products (commonly defined as an expiration date of 12 months). Thus, a forced ageing experiment was performed on lager beers (pilsner style) for an estimated time of 16 months, i.e. greater than the storage time indicated for these beers. To achieve this objective, the beer volatile composition was monitored using a highly sensitive methodology that consisted of the combination of solid phase microextraction (HS-SPME) with comprehensive two-dimensional gas chromatography with mass spectrometry detection and time-of-flight analyser (GC×GC-ToFMS). In addition, the total polyphenol content and colour properties was also evaluated.

For the dataset of 21 compounds under analysis, reported as ageing, thermal abuse and/or oxidation markers [1], only 12 analytes showed increments over the time, namely methional, diethyl succinate, phenylacetaldehyde and 5-ethyldihydro-2(3H)-furanone that increased from the estimated 12 to 16 months. Methional and phenylacetaldehyde, which derived from amino acids degradation (methionine and phenylalanine, respectively), may contribute with negative notes to the beer aroma characteristics: methional presents a cooked potato-like, bread, and aged beer-like notes, and phenylacetaldehyde exhibits sweet-honey notes. As these compounds increased over the time of the experiment, specially from 12 to 16 estimated months, it represents some concerns to expand the shelf-life of beer.

In summary, chromatographic analysis unveiled that occurred changes in the beer volatile profile over the time of forced aging, and the fact that there was an increase in compounds that can negatively contribute to the aroma, raises some concerns regarding the possibility of extending the lifetime of these lager beers beyond 12 months. Nevertheless, these results must be supported by sensory analysis to confirm the impact of storage periods, beyond their expiration date, on their sensory characteristics.

Acknowledgments: Authors acknowledged FCT/MEC for the financial support to the LAQV-REQUIMTE (UIDB/50006/2020), through national funds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement.

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Development of a prototype GC-(ion trap)MS/MS-IMS-system

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In recent years, gas chromatograph-ion mobility spectrometry (GC-IMS) has become a more and more popular method in different fields of analytics, especially in the analysis of complex mixtures e.g. of foods and flavors, as well as of trace gases and in environmental applications. Generally, GC-IMS allows for a rapid analysis of volatile organic compounds (VOC). These VOCs allow to monitor food and fermentation processes or they can serve as an indicator for shelf life of different food products. For an increase in selectivity, our group has previously described the use of GC-IMS-MS systems [1]. This prototype proved to be valuable for the simultaneous generation of electron ionization mass spectrometry (EI-MS) spectra and IMS spectra in particular for differentiation of isomers in complex matrices.
While the combination of single quadrupole MS and IMS increases availability of sample data in a general fashion, the often highly similar EI spectra and limited selectivity of fullscan/selected ion monitoring diminish the additional benefit. Therefore, the aim of our recent development is the implementation of a headspace (HS)-GC-MS/MS-IMS system based on an ion trap MS detector, which can be used in EI and chemical ionization (CI) mode. MS/MS in general provides an increase in selectivity and sensitivity of different analytes and shows promising advantages over single quadrupole MS detection. The setup is based on a prototype HS-GC-MS-IMS system with a Polaris Q ion trap mass spectrometer (Thermo Quest CE Instruments, Austin, USA) and an OEM ion mobility spectrometer module (G.A.S. Gesellschaft für Analytische Sensorsysteme mbH, Dortmund, Germany). Both are coupled to a HS-GC system (Trace – GC 2000, Thermo Quest CE Instruments, Austin, USA) via a three-way capillary flow technology splitter (Agilent Technologies, Santa Clara, USA). To optimize the gas flow for both detectors, an active mass flow controller (EL Flow® Prestige 201CV, Bronckhorst High-Tech B.V., Ruurlo, Netherlands) is used. While EI was used for these first trials, CI ionization is more optimal to be used in conjunction with MS/MS. The soft ionization of CI results in less fragmentation of the precursor ion and consequently allows for a better identification of substances by subsequent MS/MS. The system is intended to be used in the field of flavors authentication analysis.
As examplary food products, citrus juices such as orange, blood orange and grapefruit were selected, as they are one of the most consumed beverages and frequently adulterated products. The quality of citrus juices decreases with storage duration and also often due to adulteration with water, sugar or other juices of inferior quality. While it could be demonstrated that the parallel detection of IMS and MS bring a clear benefit for the classification of citrus juices, most of the volatile compounds were only detected in the IMS, not in the EI-MS system [2]. This study describes the use of soft chemical ionization to improve the MS detection with regard to formatio of intact precursor ions in order to improve classification by multimodal data. The strategy will furthermore be transfered other complex products such as cocoa or vanilla.

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Food fingerprinting techniques for the authentication of oregano

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Food fraud is one of the most important issues in the food industry today and is attracting much attention from authorities around the world. In the study of Black et al. [1], it was shown that about 25% of the analyzed commercial oregano samples were adulterated; up to 70% of bulk agents can be detected in samples. Targeted approaches do not offer a complete solution when it comes to authenticity analysis; non-targeted approaches are more interesting to solve food fraud-issues[2]. Several analytical techniques, i.e. spectroscopic techniques as near infrared (NIR) and mid-infrared (MIR), hyperspectral imaging, gas chromatography coupled to mass spectrometry (GC-MS) and proton-transfer reaction time-of-flight mass spectrometry, combined with chemometrics, were examined to evaluate their potential to solve different food fraud and quality control issues: geographical origin and variety assessment, processing control and adulteration with look-alike agents, e.g. sumac, myrtle, olive leaves and cistus leaves. In total, 102 oregano samples were analyzed for origin-assessment, 159 adulterated oregano samples for adulteration-assessment, 72 oregano samples for batch-to-batch control, and 25 samples for quality control. The Gaussian Process Latent Variable Model (GP-LVM) [3] was selected as technique and applied to obtain a reduced two-dimensional space. In figure 1, the GP-LVM plot for the geographical origin assessment of oregano with NIR is displayed. Differentiation between the different origins (Italy, Turkey, Israel and South-America) was successfully done, with validation showing over 90% correct classification. Batch-to-batch control could be easily performed with infrared spectroscopy, clustering oregano from a single supplier together. Adulteration was detected successfully at a level of 10%. The potential of data fusion of the different data was examined and showed some improvement over the use of single techniques.

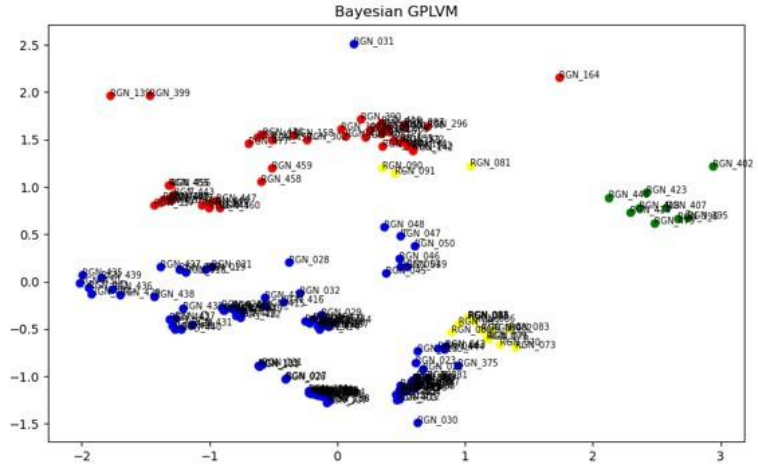

Fig.1. GPLVM-plot for geographical origin assessment on NIR-data: oregano samples originating from Italy (blue), Israel (green), South-America (yellow) and Turkey (red).

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Non-targeted VOC profiling by GC-IMS and machine learning - principles and applications

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Powerful analytical strategies for the authentication and quality analysis of foodstuffs, such as citrus juices, essential oils or spices are more than ever an important pillar in the battle against food fraud. While high-end techniques, such as high-resolution mass spectrometry or NMR are ideal tools for structure identification-driven metabolomic approaches, they are usually not suitable for routine applications far away from the specialized laboratories they require for operation. Apart from the fact that these systems have a high cost of ownership and need expert personnel to operate, the point of care is typically in producing countries, at distributor sites or at the boarders, where suitable equipment is needed - benchtop, "low-tech" and robust in operation.

In this context, GC-IMS is one of the most promising techniques of the last years, as it can be hyphenated to standard GC equipment and features orthogonal, complementary 3D data (retention time x drift time x intensity) with extraordinary sensitivity at levels comparable with GC-MS systems, but without the need for sophisticated vacuum techniques.

VOC profiling based on headspace GC-IMS is a very powerful analytical strategy when it is paired with modern machine learning methods, starting from simple exploratory data analysis, such as PCA over to complex supervised techniques, such as PLS-DA, PLSR or non-linear SVM [1,2]. Machine learning allows for the extraction of hidden information from the analytical data most efficiently and can help in identifying correlations or anomalies in complex samples, that require full-spectrum based fingerprinting approaches.

Furthermore, data fusion of analytical data coming from multimodal data sources, such as IR or MS will be discussed as an option to increase discriminative power in difficult tasks, such as geographic differentiation of products.

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DNA-based methods as a powerful tool for the entomological authentication of honey

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Honey is a food widely consumed worldwide and much appreciated for its nutritional, organoleptic and health properties. However, it is also considered one of the food products most prone to be adulterated in the EU. Up until now, honey authenticity addressed mainly the issue of sugars addition and botanical origin. Still, increased attention has recently been paid to honey entomological origin as it also relates to its geographical origin since honeybees carrying mitochondrial DNA (mtDNA) of distinct ancestries can be found across Europe. While in Portugal mtDNA of the autochthonous subspecies *Apis mellifera iberiensis* belongs to the African (A) lineage, in the northeastern part of Iberia African mitotypes are replaced by mitotypes of western European (M-lineage) ancestry. The native distribution of the M-lineage *A. m. mellifera* expands from the Pyrenees to Scandinavia and from the British Isles to the Ural Mountains while the C-lineage *A. m. ligustica* and *A. m. carnica* subspecies are naturally found in the Apennine and Balkan peninsulas, respectively [1]. Also, certain honeys holding the protected designation of origin (PDO) label should be produced by autochthonous *A. mellifera* subspecies, as mentioned in their EU geographical indications register. Because honey's entomological origin also relates with geographical origin, the development of entomological authentication tools can contribute to detect geographical origin mislabelling, which is a fraud difficult to identify. Within the project Autent+, new tools are being developed to discriminate the honey produced by the native A-lineage *A. m. iberiensis* from others of different lineages. To that end, two methodologies were developed using different technologies, namely: (i) a real-time polymerase chain reaction (RT-PCR) coupled to high-resolution melting (HRM) analysis and (ii) a DNA-metabarcoding approach using next generation sequencing (NGS). The mitogenomes of 121 individuals, representing *A. m. iberiensis* (lineages A and M), *A. m. mellifera* (lineage M), *A. m. carnica* (lineage C) and *A. m. ligustica* (lineage C), were used to select the most promising regions for primers design. A total of 35 honeys, including samples of known entomological origin provided by beekeepers from Portugal, Spain and Italy, and honeys purchased in supermarkets, were submitted to DNA extraction using an in-house optimized pre-treatment step to eliminate interferents and the NucleoSpin Plant II kit (Macherey-Nagel, Germany). The extracts were analysed using both HRM and NGS methods. For RT-PCR, the optimized conditions allowed establishing an absolute limit of detection (LOD) of 0.1 pg of honeybee DNA, a reaction efficiency of 93.4% and a R^2 of 0.998. For NGS, DNA extracts were first amplified using the newly designed primers attached to suitable adapters. Then, the products were amplified in a second PCR with a set of appropriate indexes and sequenced on the Illumina MiSeq platform. The obtained sequences were analyzed using a bioinformatics pipeline tailored for assigning sequencing reads to the different mitochondrial lineages and corresponding *Apis mellifera* subspecies.

The developed HRM analysis allowed the successful differentiation of honeybees from lineages A, M and C in three different clusters with high percentage of confidence (>99%) and when applied to honey analysis, the authenticated samples provided by beekeepers were correctly assigned proving the efficacy of the proposed method. However, some commercial samples were not clustered, suggesting the presence of a mixture of honeys produced by honeybees of different ancestries. NGS confirmed the HRM results, allowing to further identify the honeybee subspecies and estimate their percentage for the samples having a mixture of honeys. In particular, some honeys from Spain showed the presence of DNA mixtures from lineages A and M, which is consistent with the distribution of *A. m. iberiensis* in the Iberian Peninsula. For a sample produced in Faial, Azores, a mixture of DNA from lineages A and C was identified by NGS, which is also consistent with the subspecies used in beekeeping in that island. Overall, RT-PCR amplification with the fluorescent dye EvaGreen followed by HRM analysis proved to be a simple, fast and cost-effective approach, although it does not allow for the identification of honeybee lineages in case of honey mixtures. In contrast, this can be achieved by NGS that also allows for high-throughput analysis despite being a more laborious approach, requiring the availability of expensive equipment.

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Monitoring of fermentation processes by gas chromatography-ion mobility spectrometry (GC-IMS) and machine learning

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GC-IMS is a powerful technique for the fingerprint analysis of complex samples in conjunction with multivariate analysis. Due to the high sensitivity of IMS, headspace sampling is commonly used. The analysis of the exhaust air of fermentations allows for the generation of profiles of volatile, extracellular metabolites without disturbing the process or the risk of contaminations. The obtained GC-IMS data can be correlated to variables that cannot be measured directly in real-time, e.g. the formation of a product or the presence of contaminations.
This talk presents an offline proof-of-concept-study which demonstrates that microorganisms can be categorized simply by headspace analysis as a first step towards detecting contaminations. For this experiment *E. coli*, *S. cerevisiae*, *L. brevis* and *P. fluorescens* were cultivated on an incubation shaker with an hourly sampling by headspace GC-IMS. Classification was carried out by a newly developed, Python-based toolbox for chemometric analysis of multimodal data. It includes methods for I/O, preprocessing and convenient workflows for various common statistical techniques that will be demonstrated with code snippets.
Furthermore, the progress and in particular, challenges in development of a custom GC-IMS setup to record and analyze data online will be explained.

Production of *Opuntia ficus-indica fermented* beverage: The effects of fermentation time and pasteurization methods on the physicochemical proprieties

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Prickly pear fruits (*Opuntia ficus-indica*) possess high levels of micronutrients and phytochemical compounds, such as betalains and phenolic compounds, therefore, presenting a high nutritional value and health benefits, namely in the prevention of several diseases [1, 2]. The pulp is characterized by a high water content (84% to 90%) and reducing sugars - glucose and fructose (10% to 15%), 0.3% ash, and less than 1% protein [3]. The high soluble solids content and pH (approximately 6) makes prickly pear pulps a very attractive medium for the growth of microorganisms [4], requiring treatment in order to control the growth of pathogenic microorganisms. Thus, this work resorted to alcoholic fermentation and posterior pasteurization to ensure its microbial safety. Centrifuged prickly pear pulp juice was used as raw material, to produce a value-added fermented beverage.
The juices were fermented for 18h and 42h, and two pasteurization processes, namely temperature-TP (71.1 ºC for 30s) and high pressure-HP (500 MPa for 10 minutes), were applied to the fermented beverages. To evaluate their characteristics, several physicochemical parameters were analyzed, including the alcohol content, reducing sugars, pH, titratable acidity (TA), total soluble solids (ºBrix), browning degree, and turbidity. Globally, the fermentation time of 42h was shown to be preferential, resulting in a beverage with lower pH and high TA -characteristics that prevent microbial growth; and a lower browning and turbidity - that are considered consumer acceptance factors. Furthermore, enzymes such as polyphenol oxidase (PPO), pectin methylesterase (PME), and peroxidase (POD), which are usually present in fruits, were measured after HP and TP processing techniques. The enzyme PPO showed a reduction in its activity of approximately 48% and 65% when compared to the non-pasteurized juice and the processed samples, respectively. Complete inactivation of the PME enzyme was achieved in the samples subjected to thermal and high-pressure pasteurization.
In general, a 42h fermentation and high-pressure processing proved to be effective in maintaining the stability and overall quality of the desired end product, thus suggesting a possible market acceptance of a new fermented beverage with similar characteristics to a traditional cider.

Table 1. The effects of fermentation time and pasteurization methods on the physicochemical parameters of prickly pear fermented beverages.

	Sample	pH	Tritatable acidity	Total soluble solids (°Brix)	Browning	Turbidity	[Reducing sugars]	Alchool content
Fermentation time	Juice	5.84 ± 0.14^{a}	0.049 ± 0.002^{a}	13.10 ± 0.21^{a}	1.139 ± 0.082^{a}	0.432 ± 0.004^{a}	158.87 ± 18.07^{a}	*
18h	NP	4.15 ± 0.04^{b}	0.393 ± 0.010^{b}	8.60 ± 0.00^{b}	0.816 ± 0.025^{b}	1.724 ± 0.034^{b}	62.34 ± 5.13^{b}	2.494 ± 0.000^{a}
	HP	4.11 ± 0.03^{b}	0.178 ± 0.004^{c}	8.60 ± 0.00^{b}	0.837 ± 0.011^{b}	0.970 ± 0.001^{c}	49.56 ± 2.55^{b}	2.494 ± 0.000^{a}
	TP	4.31 ± 0.02^{c}	0.222 ± 0.008^{d}	7.67 ± 0.12^{c}	0.768 ± 0.009^{b}	1.969 ± 0.069^{d}	39.88 ± 1.62^{b}	2.975 ± 0.076^{b}
42h	NP	3.91 ± 0.03^{d}	0.494 ± 0.005^{e}	3.93 ± 0.12^{d}	0.936 ± 0.004^{c}	0.518 ± 0.025^{ea}	2.123 ± 0.536^{c}	4.900 ± 0.076^{c}
	HP	3.84 ± 0.02^{d}	0.283 ± 0.007^{f}	4.13 ± 0.06^{d}	0.794 ± 0.005^{db}	0.076 ± 0.006^{f}	2.139 ± 0.676^{c}	4.856 ± 0.000^{c}
	TP	4.10 ± 0.03^{b}	0.328 ± 0.020^{g}	4.53 ± 0.12^{e}	0.704 ± 0.001^{eb}	0.160 ± 0.011^{f}	3.120 ± 1.265^{c}	4.637 ± 0.076^{d}
			$g_{citric\ acid}$ /L				mg_{sugar}/mL	% (v/v)

*The values represent the mean (N=3) ± SD

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Determination of benzyl isothiocyanate-protein conjugates in a vegetable-enriched bread with different cress genera

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The daily intake of vegetables is still not as high as suggested by the WHO even though the intake can have health-promoting effects based on the content of secondary plant metabolites (SPM). To increase the supply studies suggest to enrich traditional foods, such as bread, by adding raw material rich in SPM [1].
Glucosinolates are a group of SPM in Brassica vegetables, which are believed to have health-promoting potential. Possible benefits like inflammatory and antimicrobial effects seem to be associated with the degradation products, especially the isothiocyanates (ITC), which are formed enzymatically, or heat induced. Next to the health-promoting properties, ITC are electrophilic and can react with other nucleophilic food ingredients such as proteins and form irreversible reaction products [2]. Such ITC-amino acid conjugates can have an influence on the protein's biological value and functionality.
Consequently, the aim of the present study was to analyze the influence of baking on glucosinolates and their degradation, migration of the latter into the bread matrix, as well as the identification of potential ITC-wheat protein conjugates.
For this research, a wheat dough was enriched with fresh garden cress (Lepidium sativum L.) microgreens or freeze-dried material of nasturtium (Tropaeolum majus L.) containing mainly benzyl glucosinolate. During the baking process benzyl glucosinolate is degraded to benzyl isothiocyanate (BITC) and benzyl cyanide (BC).
The analysis of possible amino acid conjugates was based on the LC-ESI-MS/MS-method developed by Kühn et al. (2018) and the glucosinolate degradation products were analysed according to Wermter et al. (2020) [3, 4]. The results underline the assumption that the breakdown products of glucosinolates migrate into the bread crumb and ITC undergo reactions with other ingredients, analysed as protein conjugates [5].

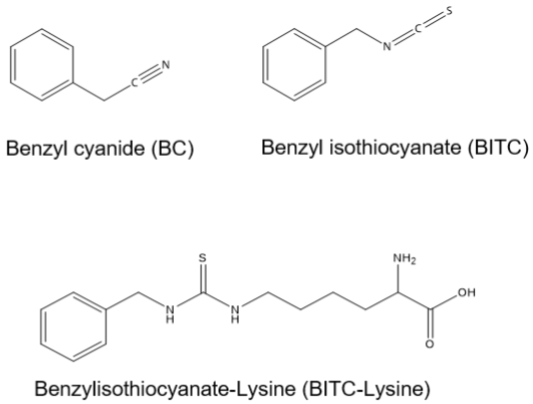

Fig. 1.: Identified benzyl glucosinolate degradation products and ITC-amio acid conjugates.

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Recovery of polyphenols and polysaccharide-polyphenols conjugates from grape pomace. Application for type II diabetes mellitus prevention

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Diabetes Mellitus is a relevant public health problem that is defined by high blood sugar levels and it can be accompanied by metabolic abnormalities of proteins, lipids, electrolytes or minerals salts [1-3]. The most frequent type is type 2 diabetes [4] The available therapeutic options include, in addition to insulin, several oral antidiabetic agents. However, many of them have seriously adverse effects [5]. Thus, new antidiabetic agents with therapeutic efficacy are needed [6].
Recently, the association between bioactive compounds in food stuff and health benefits has gained a attention [7]. In our daily life, foods rich in bioactive compounds, which includes, for example, phenols, flavonoids, polysaccharides, xanthones and alkaloids can be consumed [8]. The interest in the health effects of phenolic compounds has grown exponentially in the past few years and one of its biological effects that is studied most intensively is their antidiabetic effect [9-10].
Beside extractable phenolic compounds, a considerable quantity of non-extractable phenolic compounds that still remains bounded to cell-wall matrix, namely to polysachharides, can be present in the residues that are disregarded in the aqueous organic extractions [11]. This way extracts rich in polyphenols and polyphenols-polysaccharides conjugates may be an alternative therapeutic strategy to the ones typically applied.
In this word, it is intended to extract free polyphenols and polyphenols-polysaccharides conjugates from agro-food by-products and to study the impact of these extracts on digestive enzymes, such as pancreatic α-amylase and α-glucosidase, on the formation of antiglycation end products and on modulation of glucose transepithelial transport using *in vitro* intestinal cell models (Caco-2).

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Volatile composition and bioactive properties of lemon verbena (*Aloysia citrodora Palau*) essential oil: comparison of two extraction methods

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Several aromatic plants and spices commonly used for food purposes due to their aromatic and flavoring characteristics have also played an important role in traditional medicine for their beneficial properties. Those are frequently associated to the essential oils (EOs) present in these plants, whose antimicrobial and antioxidant activities have been described in numerous studies [1]. These properties have gained prominence in recent decades since an increasing number of consumers are seeking for food products that include natural preservatives in substitution of synthetic additives. Additionally, EOs have been described as promising ingredients in active packaging towards the shelf-life extension of food products. Therefore, several studies have been conducted in the last years focusing on different aspects including on their applications (packaging materials, films, nanoemulsions, etc), on solving their applicability limitations (low solubility, organoleptic properties) but also on their characterization and novel extraction approaches.

In this work, the essential oil of Aloysia citriodora Palau (lemon verbena) well known for its antiseptic properties [2,3], was extracted by using two methods: Clevenger hydrodistillation and microwave assisted extraction (MAE). The extractions were carried out using the same sample and a ratio plant:water of 1:20. Hydrodistillation was performed in a 5L system for 3 hours according to the European Pharmacopoeia instructions. MAE extractions were performed on a NuWav-Uno microwave (NµTech, India), operating 15 min at 98 ºC and 600 W. The obtained EO was characterized for its chemical composition using gas chromatography coupled with mass spectrometry (GC-MS) and for its bioactive potential regarding antioxidant and antimicrobial activity. The analysis of volatile compounds was carried out on a GC-2010 Plus (Shimadzu) with AOC-20iPlus automatic injector (Shimadzu), and SH-RXi-5ms column (30 m x 0.25 mm x 0.25 µm; Shimadzu, USA), multi-resistant clinical bacterial strains were used to screen the antimicrobial activity of the essential oil and the antioxidant activity was evaluated by two different methods, namely DPPH (2,2-diphenyl-1-picrylhydrazyl) and Reducing Power.

A slightly higher extraction yield of EO from lemon verbena leaves was obtained using MAE when compared to hydrodistillation method. The EOs extracted by the two methods showed the same qualitative profile, with a total of 71 compounds identified. However, some differences were observed in quantitative terms (relative % of individual compounds). Oxygen-containing monoterpenes was the main group (53.1% MAE vs 49.6% hydrodistillation) with the isomers geranial and neral being the major compounds. Monoterpene hydrocarbons group (6.8% MAE vs 6.9% hydrodistillation) was the only group that did not showed statistically significant differences ($p>0.05$) in the quantitative composition of the essential oil, contrary to the oxygen-containing monoterpenes, sesquiterpene hydrocarbons and oxygen-containing sesquiterpenes groups. Regarding the results of antioxidant activity, both Eos presented interesting antioxidant properties, showing values between 9.58 µg/mL (Clevenger) and 8.63 µg/mL (MAE) for DPPH and 1.88 µg/mL (Clevenger) and 2.04 µg/mL (MAE) for Reducing Power. In general, both Eos performed well against foodborne bacteria, inhibiting all tested gram-positive bacteria and 4 out of 5 gram-negative bacteria. Yersinia enterocolitica and Bacillus cereus were the most sensitive gram-negative and gram-positive bacteria, both presenting a MIC of 0.07 %. By the contrary, no inhibition was observed for Pseudomonas aeruginosa at the maximum concentration tested (2.5%).

Overall, MAE showed to be a promising alternative to the traditional hydrodistillation method being faster and thus spending less energy, at the same time allowing obtaining an EO richer in neral and genarial, which have been associated with interesting properties such as antimicrobial and anti-inflammatory [4,5].

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Preliminary study of winery by-products from Dão Region: Phytochemical potential to fight multidrug bacteria resistance

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It is well established in the scientific community that agro-food wastes represent economic advantages and contribute to circular economy. For instance, wine industries of Dão Region, one of the Regions with a lack of information on this topic, involve the production of large quantities of by-products, such as stem, pomace, trimmed vine shoots, or wine lees, presenting a remarkable valuable composition in phytochemicals with putative health-promoting qualities. The incidence of nosocomial infections (HAIs) caused primarily by bacterial pathogens such as methicillin-resistant Staphylococcus aureus (MRSA) is high. In chronic wounds where the healing process can take up to 3 months, it is important to find alternatives to the antibiotic molecules available on the market and for which there are multiresistant bacteria (MDR).
Thus, and taking this into consideration, this work aims to identify the potential of the by-products of the winery industry of Dão Region, as sources of compounds with biological activity, through the characterization of the polyphenolic composition (qualitative and quantitative) and the determination of the biological activity of the extracts obtained in relation to experimental models in vitro, namely, antioxidant and antibacterial activities.
The by-products used were pruning wood, stems and lees of the varieties Touriga Nacional, Tinta Roriz, Alfrocheiro, Jaen, Borrado das Moscas and Encruzado, in which Jaen stands out in almost all the analyses performed, the highest values in ortho-diphenols, flavonoids in pruning wood and total phenols *ortho*-diphenols and flavonoids in the stem. In the case of lees, the red wine lees contain the highest values of total phenols, *ortho*-diphenols and flavonoids. Concerning the antioxidant activity, the pruning wood and stem samples from Jaen presented the highest values for ABTS, DPPH and FRAP methodologies. Furthermore, from a circular economy perspective, the results obtained concerning the antibacterial study indicates that pruning wood can be a matrix with antibacterial potential to be used in the pharmaceutical industry.

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The production of low-cholesterol milk products

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Cardiovascular diseases (CVD) are the leading cause of mortality in the world and the most frequently proposed mechanism for CVD consists of the increase of blood lipids content, especially total cholesterol. As milk fat is composed of a high content of saturated fatty acids and cholesterol and milk products are consumed on a large scale, the importance of the reduction of dietary cholesterol content in these products has risen in the past years [1]. The methods for the reduction of cholesterol content in dairy products can be based on the enzymatic conversion of cholesterol, steam distillation, adsorption on several sorbents, supercritical extraction, or the removal of lipids using liquid solvents [2]. However, most of these methods lack specificity and affect negatively the nutritional and textural properties of final products. The most selective method is based on the adsorption of cholesterol molecules onto a cyclodextrin (CD) cavity. The advantage of this method is also an easy sample preparation procedure, as it is composed only of the mixing of the sample with β-CD, the settlement of β-CD-cholesterol complex in the sample, and the precipitation of complex by the centrifugation. Thus, this study aims to the suitable treatment conditions and β-CD concentration on the measure of cholesterol removal in milk and cream. In our previous study [1], it was shown that mixing speed significantly influence the final measure of cholesterol removal in milk with the most suitable parameters such as mixing speed at 840 rpm, mixing time 10 min, mixing temperature of 25°C, and the β-CD concentration of 2.0%. The cholesterol content in such treated milk samples was decreased to almost 99%. The proper precipitation of the complex was achieved by settling at 4°C for 120 min and by centrifugation at 1100 rpm for 20 min. As cream consists of a higher fat content (30%), the treatment conditions were slightly different than in milk. The optimised parameters were mixing at 480 rpm for 20 min at 40°C, settling at 4°C for 30 min, and centrifugation at 2000 rpm at 20 min. The measure of cholesterol removal in cream achieved almost 95%. So, these conditions can be applied for the production of milk and cream base functional foods with the decreased cholesterol content.

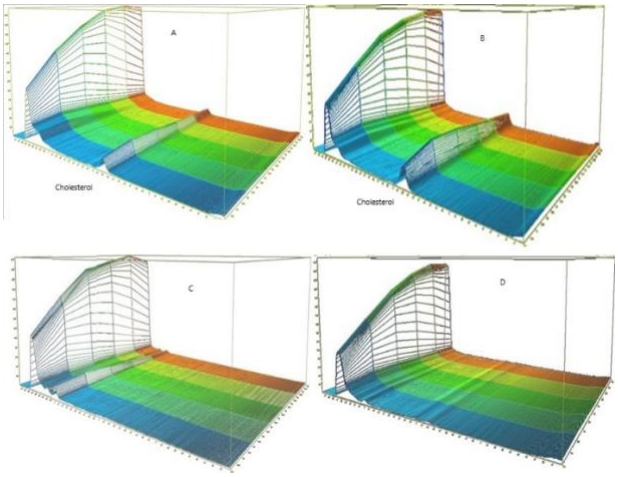

Fig.1. The 3D records of chromatograms for: A – control milk sample, B – control cream sample, C – milk treated with 1.5% of β-CD, D – cream treated with 5.0% of β-CD.

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Vitamin D from edible mushroom waste: a new sustainable approach

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The current pandemic situation linked to Covid-19 has accompanied a growth in demand for Vitamin D-based nutraceuticals, having an important role in the regulation of the immune system [1]. Mushrooms contain high levels of ergosterol, a precursor of vitamin D_2. Mushroom wastes (20% of the production) could be exploited with a view to the circular economy and environmental sustainability to produce vitamin D for nutraceutical purposes [2]. Mushroom wastes of the *Agaricus bisporus* and *Pleurotus ostreatus* varieties were studied through non-conventional extraction techniques and subsequent instrumental analysis by HPLC-MS. High levels of ergosterol (6.7 - 7.5 mg/g) were determined in the freeze-dried mushrooms through an optimized ultrasound-assisted extraction method (30 min, 40°C, 40 kHz) with ethanol as extraction solvent. Photo-irradiation parameters (sample form, irradiation time, temperature, and intensity) have been studied to improve the production of vitamin D_2 limiting the formation of inactive photo-isomers such as tachysterol and lumisterol. The optimized conditions of UVC irradiation (254 nm, 0.16 mW/cm^2, for 240 min at 20°C) allowed a high production of vitamin D (0.7 - 1 mg/g) in extracts, with a conversion rate from ergosterol between 10 and 15%. The extracts enriched in Vitamin D could be purified to obtain vitamin D ingredients intended for companies that deal with the production of food supplements and nutraceuticals, as well as the functional food market.

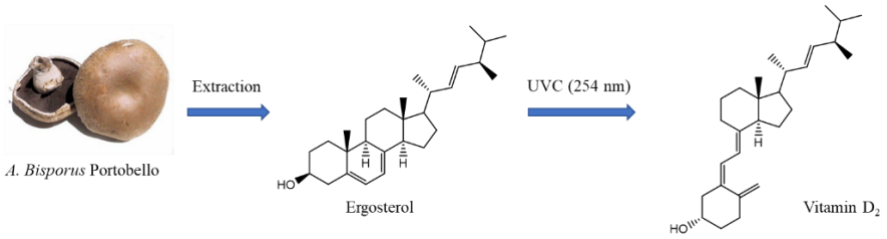

Fig.1. Graphical abstract.

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Microalgae hydrolysates as functional ingredients: antihypertensive potential

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Microalgae have been used in food and cosmetic industries due to their richness in compounds with high biological value, such as proteins, essential amino acids, vitamins and minerals [1]. Several microalgae contain high protein content, similar to other common protein sources such as meat and soybean [1], making them a promising source of bioactive peptides. Bioactive peptides are inert inside proteins but can show several interesting properties when isolated [2]. Antioxidant, antihypertensive, antidiabetic, anticancer, anti-inflammatory and anti-aging are some properties that can be found described in bioactive peptides. Bioactive peptides may be more easily absorbed by the gastrointestinal tract than the intact protein, which allied with their potential bioactivities make them interesting for the development of functional foods, with health benefits for the consumer. Hypertension is one of the main causes of cardiovascular diseases, which can lead to heart attack or stroke. Angiotensin-converting enzyme (ACE) is involved in blood pressure regulation, thus inhibiting it can help to control high blood pressure. Thus, this research aimed to produce water soluble hydrolysates rich in proteins and bioactive peptides, with antioxidant and anti-hypertensive potential, from the five microalgae species *Chlorella vulgaris*, *Nannochloropsis oceanica*, *Tetraselmis* sp., *Scenedesmus obliquus* and *Phaeodactylum tricornutum*. The five microalgae species were submitted to an enzymatic hydrolysis (one of the most described methods for producing bioactive peptides) with a cellulase and a subtilisin protease, using previously optimized methods. Prior to the enzymatic hydrolysis, *C. vulgaris* was submitted to an acid hydrolysis, using a weak and food-grade acid. The anti-hypertensive potential was evaluated by the hydrolysate's ability of inhibiting ACE. Previous studies [3] showed that *C. vulgaris* and *S. obliquus* hydrolysates stood out with the higher antioxidant potential. All the hydrolysates demonstrated anti-hypertensive potential by showing an IC_{50} lower than 500 µg protein/mL for ACE inhibition (Table 1). Thus, production of peptide hydrolysates from microalgae may represent an interesting approach for the development of sustainable, natural functional ingredients to be used to prevent hypertension on the consumers by incorporating it in food matrices.
In conclusion, the enzymatic hydrolysis of microalgae allowed to produce hydrolysates with antioxidant and anti-hypertensive potential. Further studies should be done to confirm the anti-hypertensive ability after the gastrointestinal digestion of the hydrolysates. If the bioactivity is maintained, these hydrolysates may be incorporated in food matrices as functional ingredients, contributing to the development of functional foods with antioxidant and anti-hypertensive benefits for the consumers.

Table 1. Antioxidant and antihypertensive potential of microalgae hydrolysates.

Property	*Chlorella vulgaris*	*Nannochloropsis oceanica*	*Tetraselmis sp.*	*Scenedesmus obliquus*	*Phaeodactylum tricornutum*
Antioxidant (µmol TE/g extract) [1]	462 ± 39.9	361 ± 49.2	156 ± 9.5	572 ± 24.3	360 ± 54.6
Antihypertensive (IC_{50} µg protein/mL) [1]	286 ± 55.0	239 ± 17.4	249 ± 23.1	253 ± 52.2	297 ± 7.9

[1] Values expressed as mean ± SD of three replicates (N=3).

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Seasonal variation of mineral content in the muscle of fish species with no or low commercial value in Portugal

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The Portuguese population is the largest per capita consumer of seafood in Europe, consuming 57 kg live weight per year [1]. However, the most consumed fish species in Portugal (codfish and salmon), do not correspond to the most caught fish on the Portuguese coast (sardines, mackerel, tuna and horse mackerel, in decreasing order) [1]. From a perspective of ocean sustainability, and consequently economics, it is important to study unexploited or poorly exploited fish species that have the potential to be included into the food market, not only for their quantity caught in fishing gear but also for their nutritional value. Whit this premise in mind we highlight the mineral elements content in the edible part of fish, which are important micronutrients from the nutritional point of view. However, some trace elements can be harmful for health, even in low concentrations. Thus, it is important to evaluate not only the average annual concentration but also the seasonal variation of mineral elements such as Ca, P, Mg, Na, K, Fe, Cu, Mn, Zn, I, As, Ni, Cd, and Pb.

This work is part of a project that studied 5 fish species with potential to be brought to the market (figure 1): three species with low commercial value (*Trachurus picturatus*, *Spondyliosoma cantharus* and *Trigla lyra*) and two species without commercial value (*Serranus cabrilla* and *Capros aper*), caught throughout the year in the coast of Portugal. Mineral elements were quantified by inductively coupled plasma - optical emission spectrometry (ICP-OES) with exception of iodine that was quantified by inductively coupled plasma - mass spectrometry (ICP-MS). Furthermore, with the available data it was possible to determine the quotient hazard (HQ) for the ingestion of elements by the consumption of these fish spices.

The results showed that the fish species studied present interesting elements concentrations, with some seasonal variations that may be related to fish physiological events. These concentrations were similar or even better than those found in the most consumed and/or most caught fish species in Portugal. Toxic elements such as Cd, Pb, Ni and As were not detected. Regarding the HQ, although values higher than 0.13 were obtained for Fe in *Trachurus picturatus* and I in *Spondyliosoma cantharus*, these are these essential elements and, therefore, these fish may be important sources of these nutrients.

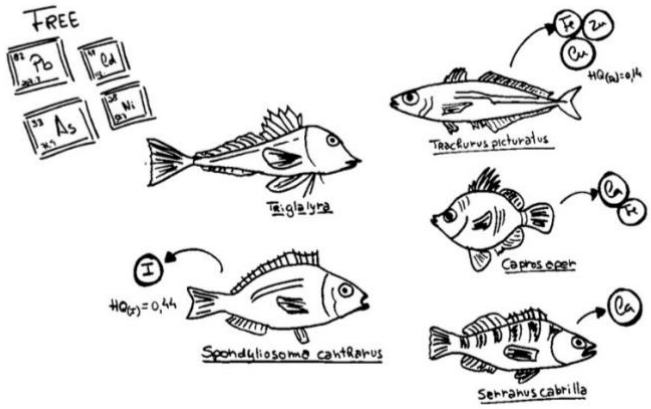

Fig.1. Representation of the species under study and highlighted results.

Acknowledgments: Authors thank FCT (Fundação para a Ciência e a Tecnologia) for the financial support attributed to MARE – Marine and Environmental Sciences Centre, Polytechnic of Leiria (UID/MAR/04292/2020). This work was also funded by project VALOREJET (16-01-03-FMP-0003), Operational Program Mar 2020, Portugal.

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Influence of a magnesium sulfate application on the content of sulfolipids in green multi-leaf lettuce

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Sulfur is a macronutrient and can influence growth, development and quality parameters of food plants. It is an essential component of various compounds such as amino acids, glutathione, selected vitamins, secondary plant metabolites, as well as sulfolipids. For example, green multi-leaf lettuce showed an increase in production performance and changed quality characteristics resulting from a fertilization with magnesium sulfate [1].
In the present study, the effects of a 1 mM or 1.5 mM $MgSO_4$ application on sulfolipids were analyzed by characterizing the different lettuce samples. Sulfolipids (sulfoquinovosyldiacylglycerol derivatives, SQDGs) belong to the most abundant sulfur compounds in the biosphere, comparable to cysteine und methionine [2]. They are located in the thylakoid membrane of chloroplasts, and can be characterized by a glucose derivative, a sulfonate group, and a glycosidically-linked glycerol molecule with one or two fatty acids esterified (at positions sn 1 and sn 2). Due to the differentiation through the linkage of various fatty acids, a large number of different SQDGs were already described [3,4].
Preparation of the green multi-leaf (cv. Hawking) lettuce (*Lactuca sativa* L.) was carried out according to the extraction method developed recently by Fischer et al. [3]. Various SQDGs were detected with a targeted-HPLC-ESI-QqQ-MS/MS multi-method. A correlation between fertilization level and total sulfolipid content as well as significant changes in sulfolipid composition were found in the different lettuce samples. The SQDG with an *m/z* of 815 (Figure 1) was the most common sulfolipid. Its content increased with the fertilization levels. Total SQDG content was significant increased by a factor of five. An enhanced formation of SQDGs linked with unsaturated fatty acids were observed, which might serve as an indicator of a modification of the stress potential of S treated lettuce.

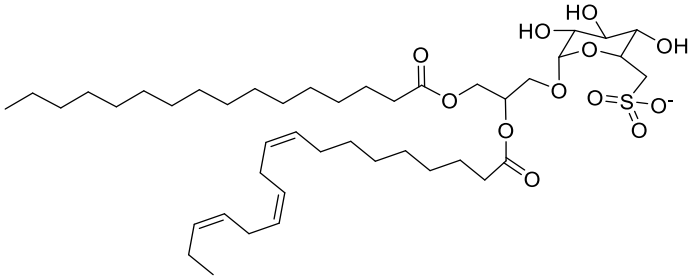

Fig.1. SQDG *m/z* 815.

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Melanoidin formation based on aldol reactions of norfuraneol and short-chain MAILLARD intermediates

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The chemical composition of food is strongly affected by non-enzymatic browning reactions during food processing and storage. Subsequently, different pathways of parallel and consecutive reactions lead to a wide range of products, for example, aroma compounds [1], antioxidant species [2], and colorants [3]. Antioxidant properties as well as food browning can be attributed to the high molecular weight end products of the MAILLARD reaction, known as melanoidins [4]. The chemical structures of these reaction products are still mostly unknown. Recent studies proved the effectiveness of model experiments with selected MAILLARD precursors to elucidate relevant reaction mechanisms such as aldol reactions and MICHAEL additions leading to the formation of low and high molecular colorants [5,6].
To gain a deeper understanding of the structural properties of melanoidins, norfuraneol was selected as a heterocyclic model compound with an active methylene group. It was incubated at elevated temperatures (130 °C, pH 5) with typical 1,2-dicarbonyl intermediates of the MAILLARD reaction, in detail methylglyoxal or diacetyl. The color formation of the reaction mixtures were measured as absorption at 420 nm. The concentration of the reactants during the reaction was analyzed by RP-HPLC-DAD. Size-exclusion chromatography was used to determine the molecular weight of colored reaction products. Structural characterization of the reaction products was carried out by means of high-resolution mass spectrometry (HRMS) whereas the structure of a novel condensation product of norfuraneol and diacetyl was elucidated by NMR experiments.
Heat-treatment of the binary model systems led to synergistic color formation. Overall, the absorbance at 420 nm was higher in reaction mixtures containing methylglyoxal and in incubations with low (60 wt%) water content. In addition, the reaction products formed in aqueous solutions (99.6 wt%) were well soluble in water whereas apolar colorants were predominantly formed in model systems with reduced water content. HRMS experiments showed that mostly aldol addition products were present in aqueous solutions. In contrast, primarily condensated reaction products with varying degrees of oxidation were formed in model systems at reduced water content.
In conclusion, the herein described reaction products consisted of up to five units of each, norfuraneol and methylglyoxal or diacetyl, and can be categorized as chromophore sub-structures of complex melanoidins. Such units may be present in melanoidins of foods rich in pentoses or pectins. The present study gives insight into the water-dependency of aldol reactions of MAILLARD intermediates and shows the importance of redox activity at reduced water content which are typical for roasting or frying. Further investigations, combining the findings to date, are needed to unravel the complex structures of heat-induced colorants in food.

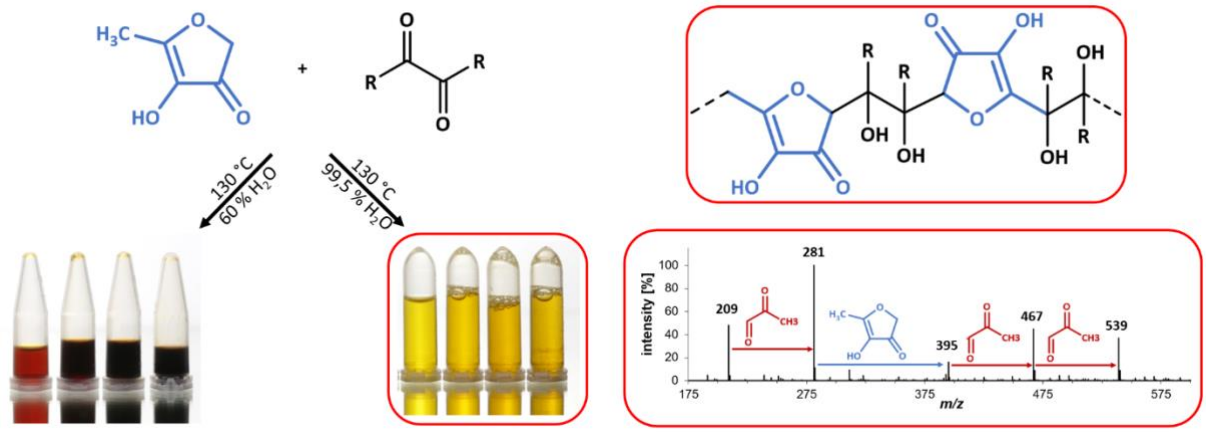

Fig.1.: Formation of (pre-)melanoidins by heat treatment of norfuraneol with α-dicarbonyl compounds.

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POSTERS

Nutritional and antioxidant characterisation of the peel of 10 species of coloured potatoes

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The use of by-products from the food agro-industry reduces production costs, increases the total use of food and reduces the impact that these by-products may cause when disposed of in the environment. In this way, some potato by-products are used and transformed into food ingredients, such as peelings. Thus, innovative measures are needed to stimulate the full reuse of these foodstuffs, since, whenever possible, the final waste should become raw material for a new process, constituting a second transformation, leading to the reuse of all the raw materials of the potato industry. In this sense, the aim of this work was to carry out a study aimed at peeling ten potato species to which the nutritional parameters were carried out. The samples were from different countries, grown in Greece and obtained under optimal consumption conditions. A total nutritional characterization of the twenty-nine potato peels was performed, as well as of the individual organic acids and soluble sugars. The antioxidant activity was carried out using the thiobarbituric acid reactive substances (TBARS) assay. According to the results obtained, the sample Peru Blue (1.57 ± 0.05 g/100g) obtained the highest value for crude fat, followed by Blaue Hindelbank (1.12 ± 0.13 g/100g); while the sample Blaue Hermans Blaue presented the lowest value (0.33 ± 0.01 g/100g). For protein, Fleuer Bleue (9.5 ± 0.2 g/100g), Blaue Hindelbank (8.9 ± 0.1 g/100g), Pink of Bolivia (9.3 ± 0.5 g/100g), Purple from Congo or Congo (8.9 ± 0.6 g/100g) and Blue from Peru (9.1 ± 0.3 g/100g) were not significantly different from each other and showed the lowest value, while Highland Burgundy Red (12.6 ± 0.1 g/100g) showed the highest value. The ash content showed statistical differences among the results, with the highest content found in Pink of Bolivia (8.0 ± 0.2 g/100g) and the lowest for Fleuer Bleue (1.3 ± 0.1 g/100g). In terms of energy there was also a difference between the results with lower values for Pink of Bolivia (373 ± 1 Kcal) and Purple from Congo (375.8 ± 0.4 Kcal) and higher for Fleuer Bleue (398 ± 1 Kcal). Two soluble sugars were identified and quantified, namely fructose and glucose. For fructose the values were highest for Fleuer Bleue (3.29 ± 0.11 g/100g) and lowest for Highland Burgundy Red (0.04 ± 0.02 g/100g), also, glucose showed lower values compared to fructose, the lowest content being quantified for Purple Fiesta (0.516 ± 0.052 g/100g) and the highest for Blue from Peru (1.07 ± 0.07 g/100g). Three organic acids were quantified, namely oxalic, malic and citric acids. Oxalic acid was highest for Hermans Blaue (2.394 ± 0.015 g/100g) and lowest for Purple Fiesta (0.91 ± 0.02 g/100g). Malic content was found highest for Purple Fiesta (3.12 ± 0.017 g/100g) and lowest for Purple from Congo or Congo (0.213 ± 0.003 g/100g DW). For citric acid, two potato samples showed higher values, namely Purple Fiesta (2.8 ± 0.2 g/100g) and Blaue Hindelbank (2.9 ± 0.1 g/100g) and two other potato samples showed lower values, namely Highland Red Fiesta (1.53 ± 0.03 g/100g) and Blaue Hermans (1.57 ± 0.01 g/100g). The antioxidant activity carried out using TBARS showed similar values for five of the 10 potatoes analysed, Highland Burgundy Red (343.4 ± 8.7 µg/mL), Violet Queen (328.4 ± 11.9 µg/mL), Purple Fiesta (305.7 ± 8.0 µg/mL), Blaue Hindelbank (366. 7 ± 12. 7 µg/mL), Pink of Bolivia (369.5 ± 12.7 µg/mL) and Blue from Peru (323.7 ± 3.5 µg/mL) showed the lowest EC50 concentrations while Blue Star (740.9 ± 33.9 µg/mL) and Fleuer Bleue (692.1 ± 13.3 µg/mL) showed the highest concentrations. This work showed that potato peel has an interesting nutritional profile and can be used to enrich other foods. Furthermore, future work will be carried out on antimicrobial activities, and cytotoxicity.

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Olive leaves as a source of biophenols: extraction, quantification, and antioxidant activity evaluation in Portuguese olive trees

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Portugal is one of the world´s largest olive oil producers [1]. Due to the extensive development in the sector, it is expected that by 2030 Portugal will be the third world´s largest producer. Consequently, an increased generation of olive waste (leaves, wood) and by-products (olive pomace, mill wastewater and olive stones) is foreseen [2].
Olive leaves represent 10% of the waste produced during the harvesting or pruning of the olive fruit. These leaves are rich in bioactive compounds (biophenols) with claimed therapeutic benefits including anti-inflammatory, antioxidant, hypoglycemic, hypocholesterolemic, antiproliferative and neuroprotective effects [3]. Therefore, and considering the food industry goal of waste valorisation and reuse, aligned with the circular economy action plan, the application of olive waste and by-products to generate added-value food products has been pursued [4].
In this work, olive leaves from different locations in Portugal (Fig. 1) were analysed regarding their content in biophenols. Different extraction solvents were tested to set the most suitable strategy for compounds extraction. The quantitative profile of the leaves was determined resorting to an HPLC-UV-Vis method (Fig. 1). Then, the content in biophenols was correlated with the antioxidant capacity determined by several *in vitro* methodologies (Folin-Ciocalteu reducing assay, copper(II) reducing assay, and scavenging capacity against synthetic radicals and against the peroxyl radical), towards establishing a correlation between the quantity of biophenols and Trolox equivalent antioxidant capacity values. The occurrence of synergistic effects was also evaluated. The best extract was selected for further bioaccessibility studies, envisioning future application in food fortification.

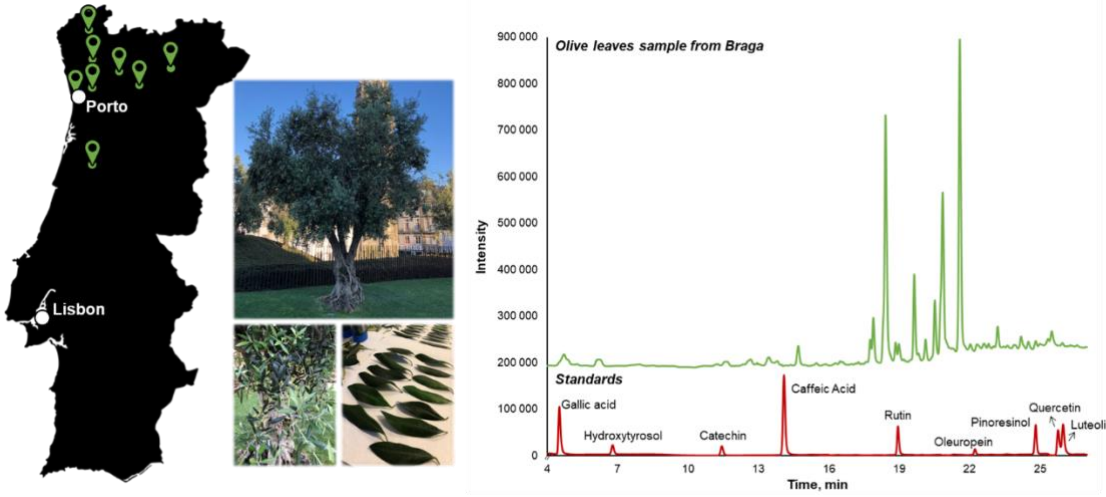

Fig.1. Schematic representation of the sampling spots in Portugal *(left)*, olive tree and leaves *(center)*, and chromatogram for quantification of phenolic compounds in olive leaves extract by HPLC-UV-Vis *(right)*.

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Analytical tools to support the development of new protein ingredients – chemical analyses and nutritional quality

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Sustainable food systems require diverse sources of protein for our diet. There is a growing interest among the food sector and researchers in utilizing new, nonconventional protein sources. Over the past few years, the food industry has actively developed and commercialized plant protein products, and the protein markets are forecast to grow [1,2]. Demand for plant protein ingredients has been significant. However, there is also a need to utilize animal-based ingredients as effectively as possible, preferably for human nutrition. In the development of new food ingredients several types of quality parameters need to be taken into account. The holistic data provided by this research will facilitate and guide the development process of ingredients for new consumer products.

The aim of the study was to investigate the macronutrient composition and amino acid profile as well as technological and sensory properties of selected protein ingredients under development. The combination of three posters [3,4] presents a set of analytical methods to provide comparative information on the chemical, technological and sensorial properties of protein ingredients in support of the development process. Together they provide an understanding of the inter-relationships between the ingredient composition, functionality, and sensory properties. This presentation focuses on the chemical and nutritional properties of the protein ingredients under development.

The chemical properties of the fractions were determined by analyzing their protein, fat, moisture and ash content. The carbohydrate and energy contents were calculated from the above-mentioned determinations. In addition to these, pH measurements were performed at various points in the development process. Determining pH is important because it is one of the most important environmental factors influencing protein functionality, especially solubility [5]. It should be noted that the solubility of proteins affects several technical and sensory properties of the fractions [5]. The results of the chemical analysis are used when estimating the nutritional composition of the possible consumer products. For the assessment of the nutritional quality, the amino acid (AA) profile of the fractions was estimated based on literature values.

Protein content of the fractions was 70-90%, covering most of their energy content. The fractions were low in fat (max. 5g/100g). The ash content indicating the mineral content of the fractions varied from 2 to 7%. The pH of the fractions was close to neutral or slightly acidic. Protein solubility is minimal at the isoelectric point, which is unique to each fraction. Plant protein fractions tend to lack certain essential amino acids (EAAs) needed by our bodies like lysine or sulfur-containing methional. The AA profile provided information about the limitation of the EAAs and how a balanced amino acid composition could be achieved by combining different fractions.

The development of new protein ingredients is a complex process that requires knowledge of many areas. It is important that a set of analytical tools is involved from the beginning and that the interaction between the different properties is evaluated throughout the development process. In this study, the results of the chemical and nutritional analysis will be evaluated together with technological [3] and sensory properties [4]. In terms of nutritional quality, the digestibility and solubility of the proteins should also be considered. A wide range of analytical tools will help to develop new protein ingredients with a wider understanding of the inter-relationships between the different properties.

Acknowledgments: This work is funded by the European Regional Development Fund (ERDF).

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Analytical tools to support the development of new protein ingredients – sensory analyses

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Sustainable food systems require diverse sources of protein in our diet. There is a growing interest among the food sector and researchers in utilizing new, non-conventional protein sources. Over the past few years, the food industry has actively developed and commercialized plant protein products, and the protein markets are forecast to grow [1,2]. Demand for plant protein ingredients has been significant. However, there is also a need to utilize animal-based proteins as effectively as possible, preferably for human nutrition. In the development of new food ingredients several types of quality parameters need to be considered. The holistic data provided by this research on the ingredients will facilitate and guide the development process of ingredients for new consumer products.

The aim of the study was to investigate the sensorial and technological properties as well as the macronutrient composition and amino acid profiles of the selected protein ingredients under development. The combination of the three posters [3,4] presents a set of analytical methods to provide comparative information on the sensorial, chemical, and technological properties of protein ingredients in support of the development process. Together they provide an understanding of the inter-relationships between the ingredient sensory properties, composition, and functionality. This presentation focuses on the sensorial properties of the protein ingredients under development.

Lehikoinen & Salonen (2019) [5] stated that according to Finns food enjoyability and taste are important when making choices. In addition, health awareness and information about the origin of the food were more important than environmental awareness. The researchers suggest that while developing new, sustainable products attention should be paid to consumer liking in addition to healthiness and an emphasis on local food. As taste plays such an important part in consumer choice it is important to study the sensorial properties of new protein fractions and to link the results to consumer studies.

By means of a sensory profile, information was obtained about the appearance, odor, texture, taste, and flavor properties, together with their intensities, on two protein fractions in development and two commercial protein fractions. Twenty sensory attributes were recognized. The sensory profile between the two reference protein fractions resembled each other especially as regards odor, taste, and flavor properties. The sensory profiles of the protein fractions in development differed from each other as well as from the reference protein fractions.

Properties that could affect the end product such as texture or produce off-odors or -flavors can be recognized with the sensory profile. By combining sensory data with chemical [3] and technological [4] data, it is possible to focus further product development on the correct aspects and, as a result, new protein rich products can be produced more accurately from protein rich raw materials. Since production side streams would be diverted for human consumption, food waste could be reduced, and thus, the impact of the food industry on climate change would decrease.

Acknowledgments: This work is funded by the European Regional Development Fund (ERDF).

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Analytical tools to support the development of new protein ingredients – technological properties

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Sustainable food systems require diverse sources of protein in our diet. There is a growing interest within the food sector and among researchers in utilizing new, non-conventional protein sources. Over the past few years, the food industry has actively developed and commercialized plant protein products, and the protein markets are forecasted to grow [1,2]. Demand for plant protein ingredients has been significant. However, there is a need to utilize animal-based proteins as effectively as possible, preferably for human nutrition. In the development of new food ingredients, several types of quality parameters need to be taken into account. The holistic data provided on the ingredients will facilitate and guide the development process of ingredients for new consumer products.

The aim of the study was to investigate the technological and sensory properties as well as the macronutrient composition and amino acid profile of the selected protein ingredients under development. The combination of three posters [3,4] presents a set of analytical methods to provide comparative information on the chemical, technological and sensorial properties of protein ingredients. Together they provide understanding about the inter-relationships between the ingredient composition, functionality, and sensory properties. This presentation focuses on the technological aspect of the protein ingredients under development.

The studied technological properties were water holding capacity (WHC), oil holding capacity (OHC), solubility, viscosity, and emulsification. WHC and OHC were determined by using centrifugation to be able to measure the ability of protein fraction solutions to bind and hold added water or oil. The amount of proteins dissolved into WHC supernatant was studied by dry solid matter measurement. Viscosities were measured in different concentrations and velocities to study the resistances of fluid to flow at a given rate. The formation and stability of the protein fraction emulsions were measured by hydration and homogenization with canola oil and by filming the stability of the emulsion. These properties have a distinct effect on the product quality, such as texture, flavor, juiciness, foaming, gelling, thickness, and coherence [5].

Two protein fractions were compared with a commercial reference fraction. The WHC measurements were made with ultra-filtered water and 0.6 M NaCl-solutions, and the OHC measurements with canola oil. The water-based results varied significantly from 2.08 to 7.33 g/g, and NaCl-based measurement from 2.84 to 4.65 g/g. In comparison, canola oil results varied only slightly from 1.58 to 1.85 g/g. The protein fractions with the highest WHC values might be the most suitable for products with juicy and moist structure. Fractions with lower WHC could be used in products with dry structure, or as protein enrichment in nutritional products. The results also indicate that the fractions with the lowest WHC and OHC might need more process development to achieve the wanted results in production.

The results concerning the technological properties should be evaluated together with the nutritional [3] and sensory [4] properties of the protein ingredients under development. A wide range of analytical tools will help to develop new protein ingredients with a wider understanding about the interrelationships between the ingredient composition, functionality, and sensory properties.

Acknowledgments: This work is funded by the European Regional Development Fund (ERDF).

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Chemical characterization and bioactive properties of different winemaking residues towards their valorization

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In the last decades, there has been an increasing concern in the search for strategies towards the valorization of agricultural residues. Annually, wine production is responsible for the generation of large quantities of phytotoxic waste, whose disposal is challenging as these residues can be hazardous to the environment when they are overused as fertilizers or simply discarded [1]. However, some of these residues can be a source of interesting compounds such as proteins, fibers, and phenolic compounds. In particular, the bioactive phenolic molecules have attracted considerable attention from the pharmaceutical, cosmetic, and food industries. Up until now, different studies have been conducted on the characterization of grape pomace and their components such as seeds, skins, and stems, particularly focusing on residues from red grape varieties [2]. However, less attention has been paid to other by-products generated during winemaking such as residues from white wine production, wine lees, and diatomaceous earth, which are used in the filtration of wine and represents approximately 250 tons/year of residues from the wine sector just in Portugal. In this context, as part of the project BacchusTech that seeks to develop a new innovative process, including the extraction, purification, and concentration of bioactive compounds present in winemaking residues, this work aimed in characterizing the pomace obtained from red and white wines production, the residues obtained after white pomace distillation, wine lees and diatomaceous earth in terms of phenolic compounds composition and extracts bioactivity. The 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-MS/MS). Additionally, the biological activity was assessed through TBARS, DPPH, and reductive power assays to determine the antioxidant activity, and the antimicrobial activity was evaluated by broth microdilution against eight bacteria and two fungi.

The non-anthocyanin and anthocyanin phenolic composition were in accordance with the previously reported by Sun et al. and He et al. [3,4], respectively, in red wines. 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-glycosilated flavanols (isorhamnetin and quercetin derivatives), three flavanol aglycones (quercetin, kaempherol, 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 amounts 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, especially the red and white grape pomace before distillation and diatomaceous earth in the bacteriostatic activity and the wine lees in the 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 in red pomace before distillation (EC_{50} = 0.08±0.04 mg/mL).

Overall, the obtained results demonstrated that the evaluated wastes are good sources of bioactive compounds, namely anthocyanins and other phenolic compounds, that can be used as raw materials for subsequent steps of concentration, purification and/or isolation of added-value compounds.

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Multi-response optimization of enzyme-assisted extraction of bilberry (*Vaccinium myrtillus* L.) pomace

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Bilberries contain many bioactive compounds and nutrients such as anthocyanins, flavonols, flavon-3-ols, stilbenes, procyanidins, tannins, vitamins, and phenolic acids [1]. After juice production, seeds and skins rich in berry polyphenols are typically discarded, thus generating significant amounts of waste [2]. However, the remaining pomace can serve as a basis for food additives, nutraceuticals, functional foods, or cosmetics [3]. Although several techniques have been proposed to recover bioactive fractions from berry pomaces, data on enzyme-assisted extraction (EAE) are somewhat limited.

This study aimed to optimize critical EAE parameters using Viscozyme L ® to obtain a high-yield extract with enhanced *in vitro* antioxidant capacity. Towards this, a central composite design and response surface methodology evaluating the effect of pH, temperature, extraction time, and enzyme concentration on three responses, chosen as independent variables, were utilized to define optimal EAE conditions. The total yield and *in vitro* antioxidant capacity as measured by two assays, namely the total phenolic content (TPC) and the $ABTS^{\bullet+}$ radical scavenging assay, were chosen as responses.

Multi-response optimization indicated a pH: 4.5, temperature 46 °C, one h of extraction, and two active units (AU) of Viscozyme L/g of pomace) as optimal conditions. Under these conditions, EAE yielded 56.15 g/100 g DW of the water-soluble fraction. In comparison with conventional maceration, EAE, besides the yield, has shown significant increases in the antioxidant capacity as measured by the TPC, $ABTS^{\bullet+}$, CUPRAC, and ORAC assays. Furthermore, an increase was observed for the mono- and disaccharide content measured by HPLC-RI and anthocyanin content measured by HPLC-UV.

Overall, these data indicate the improved efficiency of EAE over conventional extraction to isolate fractions with a higher yield and enhanced functional properties in a fast and sustainable manner.

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Effect of supplemental red grape pomace on proportion of valuable meat parts of Ross 308 broiler chickens

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The wine industry generates a huge amount of waste, which consists of stems, grape pomace (GP), sewage waters and yeast sludge. The treatment or disposal of these can have a toxic impact on the environment [1]. According to [2] for every 6 l of wine, about 1 kg of GP is produced. Traditionally, GP is incorporated into the soil or as feed for livestock. Thanks to the various organic acids (tartaric, malic and citric acid), oils, alcohol, fiber, proteins and especially various polyphenols, including flavonoids, anthocyanins, proanthocyanidins, and phenolic acids, GP can also be used to produce value-added products and grape seed oil with a positive fatty acid composition [3,4]. The meat performance of broiler chickens is evaluated by several indicators, especially the carcass weight or yield [5]. In any case, the proportions of valuable meat parts and muscle are also important for the economy of production, because of their high prices on the shelves. Therefore, the aim of this work was to determine the effect of red GP, variety Alibernet (1, 2 and 3% – experimental groups E1, E2 and E3, respectively) on the proportion of valuable meat parts and muscle after their application to the feed mixture (FM) of broiler chickens Ross 308. Experiment was designed according to [6]. At the end of fattening, 5 females and 5 males were selected from each group based on average weight, weighed and killed by neck cut bleeding incision. By weighing and calculation, the proportion of the breast part (PBP) and muscle (PBM), the proportion of the thigh part (PTP) and muscle (PTM) and their total proportions (TPP and TPM) as the % of the carcass weight were determined. Generally lowest observed proportions of valuable meat parts and muscle were observed in the control group in both females and males (significantly, P≤0.05, in PBP + PBM in ♀ and TPP + TPM in ♂). In females, significantly highest proportions were namely in PBP and PBM in E2 and E3 groups, while in males namely in TPP and TPM in group E3. Exact proportions are presented in Table 1. Although these results were very promising, further investigations should be made do confirm them.

Table 1. Proportion of valuable meat parts of broiler chickens Ross 308.

Group		PBP (%)	PBM (%)	PTP (%)	PTM (%)	TPP (%)	TPM (%)
C	♀	30.894±4,971^{b}	25.880±3,100^{b}	30.689±0,225	21.436±0,311	61.583±4,897	47.315±3,330
	♂	30.870±2,645	26.385±1,966	29.812±1,315	21.038±1,117	60.682±3,609^{b}	47.423±2,908^{b}
E1	♀	34.009±2,887^{ab}	28.099±1,874^{ab}	31.149±0,958	22.296±1,057	65.157±3,595	50.395±2,824
	♂	34.088±4,243	27.930±3,928	30.951±0,767	21.477±0,577	65.039±3,479^{ab}	49.407±3,543^{ab}
E2	♀	36.053±1,363^{a}	29.395±1,091^{a}	30.250±3,106	21.015±2,677	66.303±3,863	50.409±2,812
	♂	34.701±2,514	28.214±2,121	29.633±1,107	20.323±0,524	64.335±1,896^{ab}	48.537±1,731^{ab}
E3	♀	36.280±2,238^{a}	29.785±1,758^{a}	29.798±1,495	21.017±1,358	66.078±2,971	50.801±2,658
	♂	35.857±3,014	29.814±1,686	31.439±1,019	22.266±0,733	67.297±3,358^{a}	52.080±1,990^{a}
p-value	♀	0.042	0.041	0.669	0.564	0.208	0.223
	♂	0.085	0.189	0.135	0.059	0.034	0.073

Notes: C – control group; E1, E2, E3 – experimental groups; PBP – proportion of breast part; PBM – proportion of breast muscle; PTP – proportion of thigh part; PTM – proportion of thigh muscle; TPP – total proportion of parts; TPM – total proportion of muscle.

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Microwave-assisted extraction of phenolic compounds from pine nut skin

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Nuts skins have been demonstrated as valuable sources of phenolic compounds. These compounds are related to multiple bioactivities, including antioxidant, anti-inflammatory, antibacterial, anti-carcinogenic, and anti-mutagenic effects [1]. Moreover, skins are richer in these compounds than the kernels themselves [1, 2, 3], as the skin is the ultimate protective layer of seeds against bacterial, fungal, and other environmental stress.
Pine nut skin (PNS) is a by-product with an annual volume of approximately 550 metric tons worldwide [4]. PNS is easily recovered at the nut processing mill, has low moisture content and low density, reducing the costs associated with drying, transportation, and storage. PNS is currently used for heat production, however, the extraction of value-added compounds may allow its use as a functional food ingredient, enabling its valorization.
To be utilized in the industry, low-cost and time-saving extraction methods must be employed. Microwave-assisted extraction (MAE) allows to attain high temperatures and is considered a green extraction methodology, due to the shortened extraction time, the possibility of multiple extractions, and reduction of solvent and energy consumption [5]. Within this work, the MAE procedure was optimized to extract phenolic compounds from PNS. A full factorial design was used to estimate optimum extraction conditions of microwave, namely temperature (120, 150, 180 °C), time (1, 5.5, 10 min), and the ratio of sample mass to volume (w/v) (1, 2, 3 g to 60 mL) on the yield (% w/w), total phenolic content (TPC), and ABTS (free radical scavenging capacity).
The three evaluated responses were significantly affected by the temperature, with a higher yield, TPC, and ABTS being obtained at 180 °C. Besides, the yield was affected negatively by w/v, and by the interaction between time and temperature, with the effect of temperature more noticeable when extraction time was lower. The interaction between time and w/v was significant on ABTS, which increased with time when the w/v was low and decreased with time when the w/v was high. Thus, the condition giving the best results for the three responses, simultaneously, was 180 °C, 1 min, and 1 g skins, which resulted in 18.8 % (yield), 229.1 mg gallic acid equivalent/g skins (TPC), and 310.5 mg ascorbic acid equivalent/g skins (ABTS).
This work demonstrated PNS potential as a natural source of phenolic compounds, that could be incorporated into food formulations.

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Ultrasound-assisted extraction of bioactive compounds of olive seeds from three cultivars with valuable antineurodegenerative properties

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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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Determination of enzymes activity in mango (*Mangifera indica* L.) peel extracts

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Mango (*Mangifera indica* L.) is considered as one of the most important and widely traded tropical edible fruits [1]. By-products such as seeds and peels are produced during mango processing, for example, in the production of juices and jams. Mango peels represent about 15-20% of the total fruit weight and are discarded as waste material after the processing, thus representing a source of environmental pollution, as they are not used for any commercial purposes [2]. At the same time, peels have been found to contain important bioactive substances with therapeutic values [3,4]. They represent a rich source of various polyphenolic compounds, enzymes, carotenoids, vitamins and other bioactive substances that show good positive health benefits, including antioxidant activity [1,5]. Therefore, they could be effectively used as a functional ingredient in food products. Various studies have shown the antitumor effects of mango on various cancer cell lines, also antibacterial, antifungal, anti-inflammatory, hepatoprotective, and immunomodulatory properties [1,6,7]. The peels have been shown to contain various types of enzymes, such as protease, peroxidase, and amylase. Mango peels contain even more specific bioactive compounds than mango flesh; therefore, mango peels represent an essential opportunity for the production of value-added products. In addition to the positive effects on health, this would also reduce and eliminate environmental pollution [2].

Our study aimed to determine the total protein content and activities of certain enzymes in mango peels such as α-amylase, glucoamylase, cellulase, lipase, laccase, catalase, protease, peroxidase, transglutaminase, and superoxide dismutase. Samples were prepared from fresh and dry mango peels. Ethanol extracts were obtained using the Soxhlet extraction process, while aqueous extracts were obtained using the homogenizer-assisted extraction process. The total protein content was determined by the Bradford method. With specific enzymatic assays for each individual enzyme, enzyme activities were examined.

The obtained results showed that certain enzymes are present in the extracts of mango peels. As a result, mango by-products, such as peels, which are mainly discarded as waste, can potentially be used in various industries, such as food, cosmetic, and pharmaceutical industries, and biotechnological applications, as they represent an excellent natural source of numerous important bioactive substances, including proteins and enzymes. The enzymes present in mango peels can have a positive effect on human health; therefore, peels could potentially be used, for example, in dietary supplements.

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Extraction of essential oils from the residues of two shrub species aiming for their revalorization: chemical characterization and antioxidant, antimicrobial and cytotoxic activities

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In recent years, essential oils have been subject of research for their bioactive properties, such as antimicrobial, fungitoxic, anti-viral, anti-inflammatory and antioxidant activities. Owing to these properties they are potentially interesting for diverse industries including the food industry since one of its main problems concerns microbes and associated toxins that are responsible for food spoilage. Although the application of essential oils in the food industry may have some limitations, such as impact on the organoleptic properties and low solubility, different delivery strategies such as nanoencapsulation, active packaging and coatings are promising technologies that may overcome these issues without compromising nutritional properties in food systems [1]. In this view, increased knowledge on the composition and activity of different essential oils is needed, particularly regarding novel potential sources of essential oils such as agricultural wastes or species grown in marginal lands, on a perspective of circular economy. Therefore, in the scope of the BeonNAT project, biomass from different tree and shrub species are being screened as possible sources of essential oils and respective bioactivity evaluated.

In this work, the essential oil extracted by steam distillation from the branches (>20mm) of two shrub species grown in Spain, *Juniperus communis* L. and *Cistus ladanifer* L., was characterized for their chemical composition by gas chromatography coupled with mass spectrometry (GC-MS) as well as for their antioxidant, antimicrobial, anti-inflammatory and cytotoxic activities. GC-MS analysis allowed the identification of 98.1% of compounds in *J. communis* EO, corresponding to a total of 63 identified compounds, with α-pinene being the major compound (35.1%), followed by limonene (15.0%), sabinene (6.7%), cis-tujopsene (8.0%), β-myrcene (3.2%) and β-caryophyllene (3.5%). In general, the chemical composition is in agreement with that of juniper berry EO, defined in the European Pharmacopoeia and ISO 8897 standard, except for limonene (15.0%) which was slightly higher than the defined range (Eur. Ph of 2-12% and ISO standard of 2-8%). For *C. ladanifer* EO, 61 compounds were identified corresponding to 92.8% of the total compounds, with viridiflorol being the main compound (24.0%), followed by α-pinene (19.3%), ledol (6.9%), camphene (6.7%) and bornyl acetate (5.0%), which is in good agreement with previous data [2]. Both oils showed potential against the panel of bacteria selected according to their importance in public health and foodborne diseases, highlighting the rock-rose EO that showed interesting activity against *Escherichia coli*, *Morganella morganii*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Listeria monocytogenes* and methicillin resistant *Staphylococcus aureus* in a concentration range of 0.039-2.5%(v/v). Regarding the antioxidant activity, both oils showed promising results, with EC_{50} values of 1.35 ± 0.19 mg/mL and 1.30 ± 0.07 mg/mL in the reducing power assay and 68% and 83% inhibition of oxidation according to the cellular antioxidant activity assay, for *J. communis* and *C. ladanifer*, respectively. The essential oils showed anti-inflammatory (IC_{50} of 24±1 µg/mL and 21±2 µg/mL for juniper and rock-rose, respectively) and cytotoxic activity, with the best results obtained with the rock-rose EO in the inhibition of stomach-AGS, colon-CaCo, breast-MCF-7 and lung-NCI-H460 cancer cell lines (GI_{50} between 47±5 µg/mL and 58±1 µg/mL). Juniper EO did not evidenced cytotoxicity in non-tumoral Vero cells at the highest tested concentration (400 µg/mL) which can be an indicator of its safety. Overall, the results demonstrated that shrubs biomass can be a source of EO with similar composition to that reported for respective berries and leaves. The EOs showed interesting antibacterial and antioxidant activity thus being potential candidates for further studies on their safety and potential application in food systems.

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Extraction of phenolic compounds with antioxidant activity from cherry seeds: preliminary study

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The food processing industry is responsible for the generation of a significant amount of wastes and by-products, resulting from the transformation processes applied to food. These residues are highly rich in bioactive compounds with antioxidant activity, like for example the phenolic compounds [1]. To recover these compounds from the residues, extraction operations usually apply, but their optimization is important to maximize yield, minimize costs and reduce the environmental impacts. One of the main issues is related with the use of organic solvents derived from petroleum [2].
The objective of this study was to test different extraction conditions to obtain phenolic compounds from cherry seeds. For this the seeds used were pre-dried in the sun and grounded, and the powder was then dried in a stove for 24 hours at a temperature of 40 ºC. The extraction was performed using different solutions: water, methanol, ethanol:water (in variable percentages from 50% to 100% water) and ultrasounds. Also, the temperature was varied from 35 to 80 ºC. The total phenolic compounds (TPC), anthocyanins, flavonoids and antioxidant activity (DPPH and ABTS methods) were measured using spectroscopic methods.
The results showed that the extraction of total phenolic compounds was higher for extraction with water or mixtures of water and ethanol, rather than with methanol or water plus ultrasounds (Fig.1). Additionally, testing different temperatures and percentages of water, it was verified that maximum extraction of phenolic compounds was obtained for 70 ºC, and using an extraction solution of ethanol:water (40:60, % v:v), which was 2.65 mg/g (galic acid equivalent). Under these conditions, the anthocyanins were 0.65 mg/g, and flavonoids were 1.05 mg/g (malvidin-3-glucoside equivalent). The antioxidant activity was 2.26 mg/g measured by ABTS method and 0.65 mg/g measured by DPPH method, in both cases expressed as Trolox equivalents.
In this way it was possible to obtain extracts rich in phenolic compounds with antioxidant activity, using an aqueous solution with 40% ethanol, and by performing the extraction at 70 ºC and using magnetic stirrer.

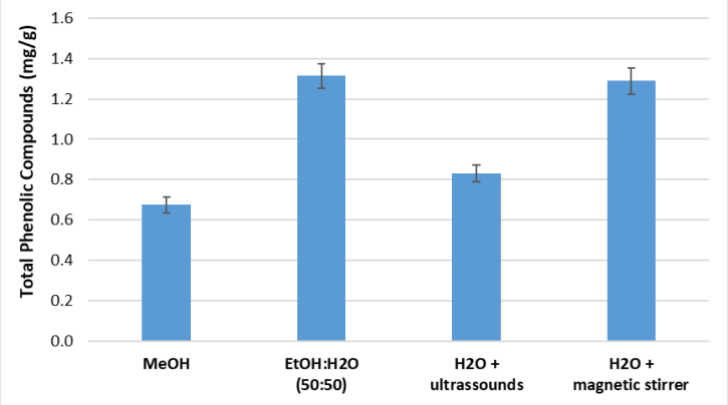

Fig.1. Extraction of Phenolic compounds with different conditions.

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High hydrostatic pressure, a green processing for apple by-product valorisation

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The European Commission has recently developed a Europe's new agenda for sustainable growth, known as, the European Green Deal. Hence, an innovative Circular Economy Action Plan has been set as one of the fundamental issues. The new Action Plan discloses different actions along the entire life cycle of products, in order to promote the sustainable use of EU resources, protect and restore natural ecosystems, and improve human health.

It seems of relevance that approximately 20% of the produced food in Europe is lost along the food supply chain, being 30% from primary production and food processing. In this regard, food by-product valorisation could provide an effective solution for this issue. The fundamental idea is to turn the food losses into added value products. Thus, a transformation process appears to be necessary for the mentioned purpose. Accordingly, the application of green technologies has proved to be a suitable alternative. In this context of emerging technologies, high hydrostatic pressure (HHP), has demonstrated to be an appealing option for the recovery of the natural components from food by-products. HHP treatment is a cold pasteurization technique in which products are subjected to a high level of isostatic pressure transmitted by water. In addition, the combination of HHP with food grade enzymes, has been proposed for intensifying the extraction of bioactive compounds from plant-based materials [1].

Apple by-product is the remaining solid derived from the manufacturing of apple-based products such as, cyder or apple juices. It is estimated that 3 to 4.2 million tonnes of apple by-product are annually discarded as waste. However, apple by-product has proved to be natural source of bioactive compounds including soluble dietary fiber and polyphenols. Soluble dietary fiber has demonstrated a great positive effect over human health. Specifically, it exhibits a potential prebiotic effect, by means of its composition, with an interesting fermentability profile. Besides, the phenolic compounds have been suggested to be responsible for the health benefits in many foods including, fruits and vegetables. Polyphenols have attracted much interest because of their potential prevention of cardiovascular diseases and its antioxidative properties[1]. However, the beneficial effects of both, soluble dietary fiber and polyphenols, depend on its availability for absorption.

The main objective of this work consists in the reintroduction of the apple by-product, after a transformation process, back into the food chain, with the aim of developing new natural resource products with an added value while reducing food loss. As it was mentioned, HHP technology is an interesting approach. Indeed, the application of HHP treatments over apple by-product, namely, 200 MPa- 600 MPa for 15 -30 minutes, induced to a considerable cell wall disruption revealed by scanning electron microscopy [2]. The increase of the cell wall accessibility led to changes in the dietary fiber distribution and, increased in more than 1.2-fold the soluble dietary fiber content. Additionally, HHP modified the techno-functional properties, enhancing the swelling and oil-holding capacity, acquiring therefore the apple by-product, new interesting characteristics [2,3].

Furthermore, the combined treatment of HHP and food-grade enzymes over apple by-product resulted effective for the release of soluble polysaccharides and oligosaccharides, achieving after the mildest treatment applied, an increase of 1.8-fold and 3.8-fold respectively. In addition, the potential prebiotic effect of the apple by-product improved [3,4]. The total phenolic content (Folin-Ciocalteu and Fast Blue) exhibited an enhance, probably due to the increase of different phenolics such as, catechin, quercetin or chlorogenic acid, that could increase the antioxidant capacity measured as ORAC and DPPH.

Concluding, HHP, and HHP assisted by food grade enzymes procedures are of interest on the one hand, for recovering the valuable compounds of the apple by-product and developing high-fiber and antioxidant functional ingredients. On the other hand, apple waste generation is decreased, and the environment is preserved.

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How the extraction method affects the bioactive and antimicrobial properties of pomegranate peel and seed extracts

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According to data from the European Union, food waste generated during food processing represents up to 39% of the total food waste, causing a negative environmental impact and significant expenses [1]. However, many of these discarded biomaterials are rich in value-added compounds with bioactive activities.
During fruit processing, bagasse, peel, trimmings, stems, husks, bran and seeds may represent more than 50% of fresh fruits, which in some cases have a higher nutritional or functional content than the final product [2]. Regarding to pomegranate fruit, the ratio of peel:arils:seeds is, respectively, 50:40:10 [3,4], which means that during juice processing about 50-60% of the fruit is discarded [5]. Nevertheless, it is widely recognized that pomegranate by-products contain considerable amounts of phenolic compounds (e.g., flavonoids and tannins), sugars, organic acids and minerals, that possess antioxidant, antifungal and antibacterial activities [3–7]. The recovery and valorisation of these bioactive compounds depends not only on the type of by-product, but also on the applied extraction methodologies, which also play a key role in the quality and bioactive composition of the extracts produced.
There are several extraction methods for recovering these compounds, such as supercritical fluid extraction, microwave-assisted extraction, pressurized liquid extraction and pressurized hot water extraction [8], but the simplest and most applied is the conventional solvent extraction. However, conventional methods often have technological, economic, and environmental limitations that are difficult to overcome and fail to achieve sustainability. The sonication-assisted extraction, which is a type of ultrasound extraction, has been recognized as a potential method to extract oils, proteins and bioactive compounds from plants or animals [9]. Its main advantages include high reproducibility, significant reduction in extraction time [10,11], non-destruction of bioactive compounds from plant matrices and intensification of their extraction [8].
This work intended to compare and study the influence of two extraction methods, namely, conventional solvent extraction (4 h, 50 ºC, 200 rpm) and sonication-assisted extraction (20 min, 20 kHz), using 50%EtOH as solvent and a S/L ratio of 20 mg/L, on the quality of peel and seed extracts from three pomegranate cultivars (Acco, Big Full and Wonderful). The lyophilized extracts were first submitted to a qualitative phytochemical screening to determine which families of compounds were present in the matrices. Subsequently, the extracts were evaluated for bioactive potential, such as total phenolics compounds (mg GAE/mg extract), total flavonoids (mg CATE/mg), tannins (% w/w, db), anthocyanins (µg cyanidin-3-glucoside/mg, db), and antioxidant activity by the DPPH method (expressed as IC_{50}, mg/mL). The antimicrobial potential was evaluated through the determination of minimum inhibitory and bactericidal concentrations, inhibition halos and inhibition curves against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus* and *Yarrowia lipolytica*. Using garden cress seeds as a case model, the phytotoxicity of the extracts was also studied.
Regarding the qualitative screening (**Table 1**), all extracts showed the presence of total phenolics, flavonoids and tannins. All the peel extracts and the Acco seeds extract showed the presence of saponins. Free and combined anthraquinones were only observed in Big Full seeds. The sonication-assisted method was shown to positively contribute to the presence of triterpenoids in the extracts. Alkaloids and polysteroids were not detected and only few extracts tested positive for cardiac glycosides.
The best results regarding the bioactive composition were obtained for total phenolic compounds (0.16-0.73 mg GAE/mg extract) using the conventional extraction. Extracts obtained by the sonication-assisted extraction were richer in total flavonoids (0.019-0.068 mg CATE/mg extract), anthocyanins (0.06-0.60 µg cyanidin-3-glucoside/mg, db) and showed greater antioxidant activity (IC_{50} 0.01-0.20 mg/mL). The tannin content was similar for both methods, with 1.8-25.3% (w/w, db) for the conventional and 1.3-26.7% (w/w, db) for the sonication-assisted method.
Regarding the antimicrobial potential, all extracts (at a concentration of 0.30 mg/mL in 10% DMSO) were more effective against the tested Gram-positive bacteria (**Fig.1**) and yeasts than Gram-negative bacteria. *E. coli* was not inhibited by any extract. The average inhibition halos (for all by-products) for conventional and sonication-assisted extraction were, respectively, 7.65 and 8.65 mm for *P. aeruginosa*, 10.39 and 11.43 mm for *S. aureus*, 8.85 and 8.90 mm for *B. cereus* and 10.66 and 11.34 mm for *Y. lipolytica*, indicating greater antimicrobial activity for extracts produced by sonication-assisted method. All extracts presented phytotoxicity against garden cress seeds (**Fig.2**) in the tested concentrations (0.003, 0.010, 0.030 and 0.300 mg/mL). All seeds germination and root growth were observed only in the case of 0.003 mg/mL. Although the observed toxicity, the sonication-assisted extracts showed the highest Munoo-Liisa vitality index (51.30%) compared with conventional extracts (38.41%).
Overall, sonication-assisted extraction produced pomegranate peels and seeds extracts with greater bioactive and antimicrobial potential and less phytotoxicity.

Table 1. Phytochemical screening of pomegranate peels and seeds from Acco, Big Full and Wonderful cultivars, according to the extraction method.

Extraction	Matrix	Cultivar	TP	Flav	Tan	Sap	FA	CA	Terp	CG
Conventional	Peels	Acco	+ + +	+ + +	+ + +	+ +	-	-	-	-
		Big Full	+ + +	+ + +	+ + +	+ +	-	-	+	+
		Wonderful	+ + +	+ + +	+ + +	+ +	-	-	+	+
	Seeds	Acco	+ + +	+ +	+ + +	+	-	+	-	-
		Big Full	+ + +	+ +	+ + +	-	+ + +	+ + +	+	+ +
		Wonderful	+ + +	+ + +	+ + +	-	-	-	+	+
Sonication-assisted	Peels	Acco	+ + +	+ + +	+ + +	+	-	-	+ + +	-
		Big Full	+ + +	+ + +	+ + +	+	-	-	+ + +	+ +
		Wonderful	+ + +	+ + +	+ + +	+	-	-	+ + +	+
	Seeds	Acco	+ + +	+ +	+ + +	+	-	-	+ + +	-
		Big Full	+ + +	+ +	+ +	-	+ + +	+ + +	+ + +	-
		Wonderful	+ + +	+ +	+ + +	+	-	-	+ + +	-

+++ large response; ++ moderate response; + minor response; - no response in the assay.
TP – total phenolics, Flav – flavonoids, Tan – tannins, Sap – saponins, FA – free anthraquinones, CA – combined anthraquinones, Terp – triterpenoids, CG – cardiac glycosides.

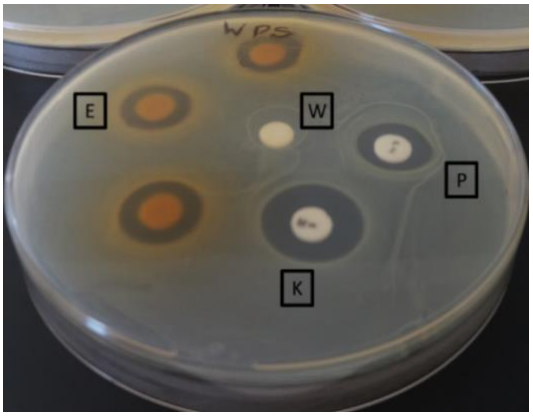

Fig.1. Example of inhibition hallos against *S. aureus* exhibited by Wonderful peel extracts obtained by sonication-assisted extraction. (W) control with water (negative control), (P) control with penicillin (positive control), (K) control with of kanamycin (positive control) and (E) extracts. (n=9).

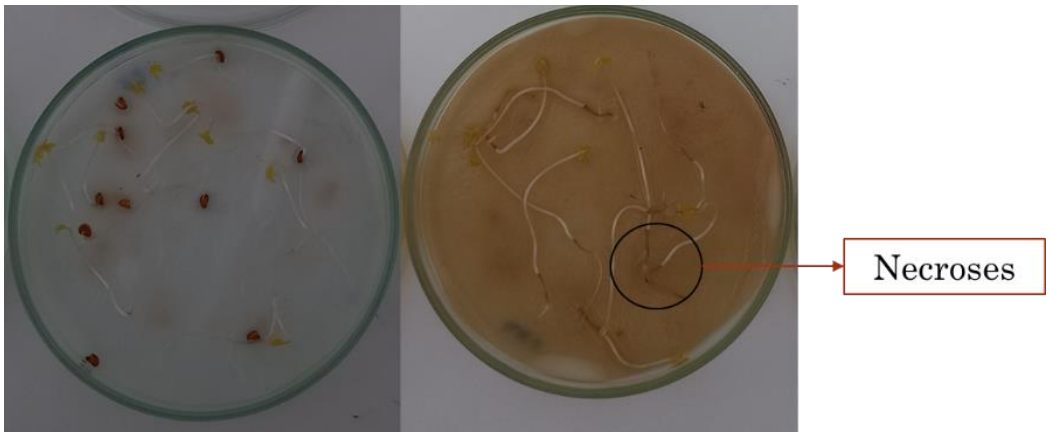

Fig.2. Example of phytotoxicity essays. Control with water (left) and pomegranate extracts at 0.003 g/mL (right).

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Physicochemical Characterization and Bioactive Potential of Coffee Silverskin

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The recovery and valorisation of waste from food industry is becoming a priority with the aim of achieving a more sustainable industry, reducing environmental impact, and generating applications for waste materials that increase their potential market value [1,2].

Coffee is one of the most consumed beverages worldwide. During coffee roasting process, a by-product is produced, called coffee silverskin, which is a thin tegument that comes off the coffee bean during processing. It is currently used as fuel and soil fertilizer since effective methods for its valorisation have not yet been developed. Some studies report that coffee silverskin is a good source of bioactive compounds [3] that can be extracted and applied in various fields, meeting the growing market demand for natural and ecological products.

Research regarding the biological profile of coffee silverskin is growing, as silverskin has been reported to have not only antioxidant, but also anti-inflammatory and antimicrobial activities [3-5]. The bioactive compounds present in this matrix are mainly phenolic acids (gallic acids, catechin chlorogenic acid and caffeic acid), diterpenes and vitamin precursors [6] that can be extracted by conventional or unconventional methods that vary according to the targeted bioactive compound or the technology available. During extraction, many factors can affect the process, such as time, temperature, pressure, and solvent [7]. Conventional methods often have practical, economic, and social limitation that are difficult to overcome and most often give rise to environmental problems. Thus, greener technological processes, like sonication-assisted extraction, have emerged, which seem to overcome some of these problems [8,9].

This work aimed to compare the bioactive composition of coffee silverskin extracts produced by two methods: conventional solid/liquid (S/L) extraction (50 ˚C, 4 h, 200 rpm) and sonication-assisted extraction (20 min, 20 kHz), both applied to whole and ground matrices of coffee silverskin (Fig.1).

Three different ethanol:water mixtures were used as solvents (25%, 50% and 75% ethanol) with the S/L ratio of 2 g/100 mL. The extracts obtained were then submitted to quantitative analysis to determine the total phenolic compounds (TPC, expressed as mg of gallic acid equivalent per mg of silverskin, db), total flavonoids (TF, expressed as mg of catechin equivalent per mg of silverskin, db), tannins (TAN, expressed as % g tannins/100 g silverskin, db), anthocyanins (ANT, expressed as µg cyanidin-3-glucoside/mg silverskin, db) and antioxidant activity (AA, determined through the DPPH method and expressed as IC_{50} mg/mL).

The extraction yield regarding both extractions was also evaluated and the sonication-assisted method using EtOH 50% and the whole matrix led to the best extraction yield, 19.7±1.9%. Also, the sonication-assisted method using ETOH 50% and the whole matrix was the most effective method for the extraction of TPC (0.18±0.01 mg GAE/mg silverskin, db), TF (0.028±0.001 mg CatE/mg silverskin, db) and TAN (3.97±0.25% w/w, db) (**Fig.2**). For anthocyanins, the conventional extraction with EtOH 75% and the ground matrix led to the best results (0.18±0.01 µg cyanidin-3-glucoside/mg silverskin, db). With respect to AA, even though the overall best results were achieved by the sonication-assisted extraction, with EtOH 75% and the ground matrix, the best result of 0.20±0.00 mg/mL was with EtOH 50%.

Even though it would be expected that the use of smaller particles sizes (ground material) would lead to the extraction of higher contents of compounds of interest (due to the increase contact area between solids and solvent), this was not verified in this study since the whole silverskin led to the best overall results. In this case, the material grinding operation can be avoided by simplifying the extraction methodology.
Comparing the methods used, it is possible to conclude that sonication-assisted method presented best results for all the groups of compounds analysed, except for anthocyanins.
It was confirmed in this study, regarding the composition of the extracts, that the coffee silverskin is a rich source of bioactive compounds, namely antioxidants and anthocyanins. Its composition makes coffee silverskin a promising raw material when applied in the food, pharmaceutical or cosmetic industry.

Fig.1. Visual aspect of the coffee material (coffee berries, raw and roasted coffee beans) and coffee by-product (whole and ground silverskin).

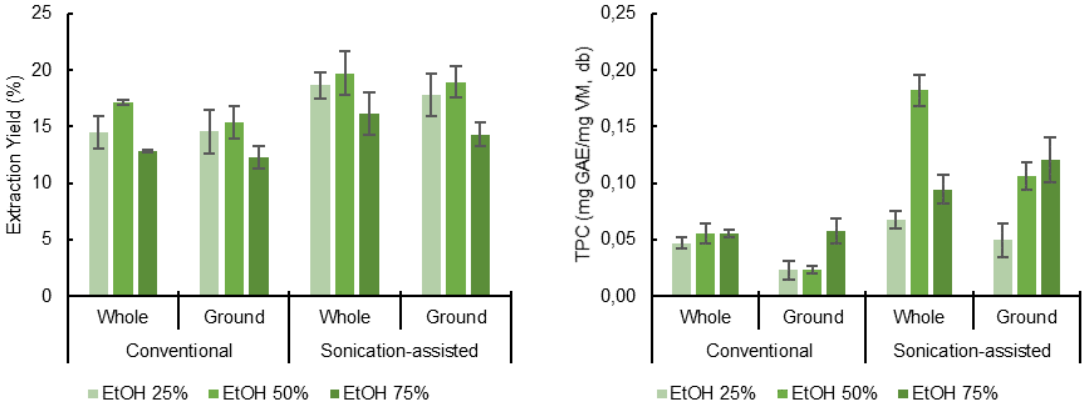

Fig.2. Extraction Yield (EY) and total phenolic compounds (TPC) of coffee silverskin extracts according to the type of material (whole, grounded); the extraction method (conventional solid/liquid (S/L) extraction (50 ˚C, 4 h, 200 rpm) and sonication-assisted extraction (20 min, 20 kHz); and the extraction solvent (25%, 50% and 75% ethanol).

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NMR a tool for unicity evaluation of Feteasca Neagra traditional Romanian wine

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Romania is well known for having a plethora of grape varieties grown across its 33 DOC regions. Many of the Romanian wines are exported, but what is particularly impressive is that 40% of wine production comes from its indigenous grape varieties. Featеasca Neagra is one of the traditional Romanian vine varieties which produces high quality red wines.
This work evaluates the compositional characteristics of Feteasca Neagra wines which makes it unique and defines it among other high quality red wines. Using NMR spectral information in chemometric approach a good discrimination of this specific variety was obtained against other high-quality wines from international well-known varieties (for instance Cabernet Sauvignon, Merlot etc). A total of 25 wines from different DOCs of Romania were investigated, alongside of other international recognized varieties of red dry wines produced in Romanian vineyards. NMR wine metabolomics proved an important tool for wine classification in term of variety, geographical origin or even harvest year. Considering that no sample preparation is needed prior to analysis of the wine, NMR proves to be a fast, reproductible and reliable tool for unicity evaluation of Feteasca Neagra wines.

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Optimization and validation of HS-SPME GC-MS method for the analysis of volatile organic compounds (VOCs) in dry-cured ham

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The numerous volatile organic compounds (VOCs) in dry-cured ham formed during complex biochemical and enzymatic processes are responsible for the final product's unique aroma and quality [1]. In this work, VOCs in Protected Designation of Origin (PDO) protected and non-specific dry-cured hams were identified using a rapid, sensitive and reliable method based on headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME GC-MS) [2]. A 50/30 µm divynilbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre was used to extract volatile compounds from the sample. The factors affecting the SPME process, such as equilibration (20-60 min), extraction (20-60 min), desorption time and temperature (40-70 °C) and salt addition, were optimised. The optimal SPME conditions were 60 min of equilibration followed by an extraction time of 40 min at 70 °C, and 4 min of desorption. In contrast, the results of response surface methodology (RSM) by sophisticated statistical Design-Expert® software have shown a slightly higher optimal extraction time (60 min). The method was validated by determining the linearity, limits of detection (LOD) and limits of quantitation (LOQ), working ranges, and method sensitivity [3]. The method was then used to analyse four different samples from the Slovenian market. These included Slovenian Karst prosciutto (Kraški pršut), dry-cured ham from the Krškopolje pig (only preserved Slovenian autochthonous pig breed), prosciutto made from pork of the Mangalica variety, and Spanish Iberico ham. Individual VOCs' were identified from their mass spectra by comparison to mass spectral libraries (NIST 14.0) and by comparing their retention indices (RI) and retention times (RT) with those of authentic standards. In total, 64 VOCs were successfully identified, among which aldehydes were the predominant group, followed by the fatty acids and alcohols. The developed method will help to determine quality among different hams from Slovenian and foreign markets.

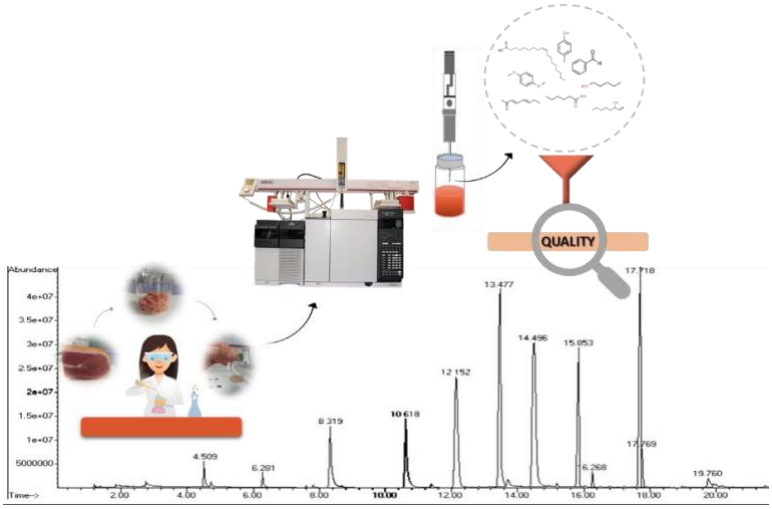

Fig.1. Graphical abstract.

Keywords: dry-cured ham, HS-SPME/GC-MS, VOCs, response surface methodology.

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Authenticity of Coffee: Arabica or Robusta?

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Coffee is one of the most valuable and most consumed commodities in the world. The most traded coffees are Arabica *(Coffea arabica)* and Robusta *(Coffea canephora*) [1,2]. Compared to Robusta, Arabica is more demanding on growing conditions and more susceptible to disease [1]. Due to this and also concerning sensory properties, Arabica is more appreciated and is thus approximately twice as expensive as Robusta [2,3]. Roasted Arabica beans are prone to adulteration, they are either substituted or diluted by cheaper Robusta without declaration on the label. Besides others, Arabica and Robusta differ in the content of 16-O-methylcafestol (16-OMC), which is present mainly in Robusta. Roasting has a minimal effect on the content of 16-OMC, therefore, it is one of the most widely used markers for distinguishing Arabica and Robusta [2,4-6]. The German standardized method DIN 10779, which uses a combination of liquid chromatography and UV detection, is focused on its analysis. Because 16-OMC occurs in coffee mainly in esterified forms, the sample preparation for this analysis is time and money consuming [1,2,5,7]. Another complication is the large variability of the content of the 16-OMC marker in different Robusta samples, which makes difficult the verification of the declared composition of Arabica and Robusta in mixtures [8]. Within this study, a simple extraction procedure for sample preparation was optimized. The methanolic extracts were analyzed by ultra-high performance liquid chromatography (UHPLC) on a reversed phase followed by detection by high-resolution tandem mass spectrometry (HRMS/MS). A total of 100 different coffee samples (76 roasted and 24 green beans) were analyzed. 48 samples were provided to the study by 3 different Czech roasters, which buy coffee directly from farmers. Other coffee samples were purchased in the Czech market. Based on the measured metabolomics fingerprints with following statistical analysis, it was possible to distinguish Arabica and Robusta samples, both within green and roasted been sets. In the next step, a set of mixtures of roasted Arabica and Robusta with defined ratios prepared in the laboratory was analyzed. The minimum weight addition of roasted Robusta to Arabica, which could be distinguished by principal component analysis (PCA), was 15 % (w/w). Thanks to statistical data analysis, PCA and partial least squares-discriminant analysis (PLS-DA), potential characteristic markers (19 of Arabica and 12 of Robusta) were selected. These markers were most responsible for the separation of roasted Arabica and Robusta. The marker of Robusta with the greatest influence on the distribution of the samples was identified as 16-O-methylcafestol palmitate. The variability of this marker in all 21 analyzed samples of roasted Robusta was determined to be 15%. The variability of the 16-OMC was determined to be 60% under the same conditions. On this account, monitoring of more than one marker to verify the declared composition is advisable. The suitability of this concept (monitoring of multiple markers) was documented on prepared mixtures with a defined ratio of Arabica and Robusta. It was found that it is possible to estimate the ratio of Arabica and Robusta in mixtures with the accuracy of +/- 10%. Thanks to the selected markers and based on statistical analysis, two samples from the Czech market were identified as suspected of counterfeiting. The investigation within this study continues, the conditions for extraction and analysis of selected markers are currently being optimized to determine lower undeclared additions of Robusta to Arabica and to more accurately estimate the composition of their mixtures.

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Fiber and low molecular weight carbohydrate composition in new industrial milling fractions of rye varieties

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In our study, the composition and variability of non-starch carbohydrates (NSPs) in rye varieties were examined. Rye has favourable high fiber content, the arabinoxylans (AXs) are the most important components. Nutritionally, fiber components are attributed to varying degrees of beneficial effects. In contrast, certain low molecular weight sugars and oligomers, such as the FODMAP components (fermentable oligo-, di-, monosaccharides and polyols) cause adverse health effects in patients with irritable bowel syndrome (IBS). However, there is not enough knowledge about the variability of NSPs in varieties and different fractions. Our aim was to investigate the variety and harvest year effect. The novelty of our study is the investigation of the variability of FODMAP and AX content together. The other novelty is that the NSPs were investigated in new milling fraction of several rye cultivars developed by our research group in collaboration with industrial partners.
In 2019 three varieties of rye and one general grade rye mixture used in industry were examined. In addition to the grain and the usual three milling products (flour, bran, wholemeal), six newly developed industrial milling subfractions were investigated. The effect of the harvest year (as complex environmental factor) was investigated on two varieties from 2020 The AX content of rye samples were measured by GC method [1]. Measurement of low molecular weight carbohydrates (include FODMAP components) was performed by an adapted complex method [2]. A ligand exchange column was used for the separation of monomers, while a hydrophilic interaction liquid chromatography method was use for the separation of oligomers. Both were detected by an evaporation light scatting detector (ELSD). The fructan content was measured by Megazyme Fructan HK Assay.
In the first year, the variability was the greatest for bran total arabinoxylan (TOTAX) values. About water-soluble arabinoxylans, there is not much difference between the fractions and the varieties. The fructan content is higher than water-extractable arabinoxylan (WEAX) in all cases, there is no outstanding value in terms of variability. The effects of harvest year are most spectacular for bran TOTAX results. In the second year, both the values of the two varieties and the difference between them also decreased. For WEAX and fructan content, the values remained nearly the same. The composition variability of fibers and FODMAPs in the six experimental industrial mill fractions described. One of the fractions has high water-soluble fiber and fructan content. Another has a lower fructan content than flour, while TOTAX has a higher content. The effect of three factors (harvest year x variety x fraction) on each carbohydrate component and the correlation were investigated using statistical tools.
The measurement of the variability of fiber and FODMAP composition together means a new approach for the evaluation of real nutritive values and health promoting effects of cereal products. Additionally, these results can contribute the understanding the relationships among the technological properties and (macro)molecular composition of cereal based milling fractions.

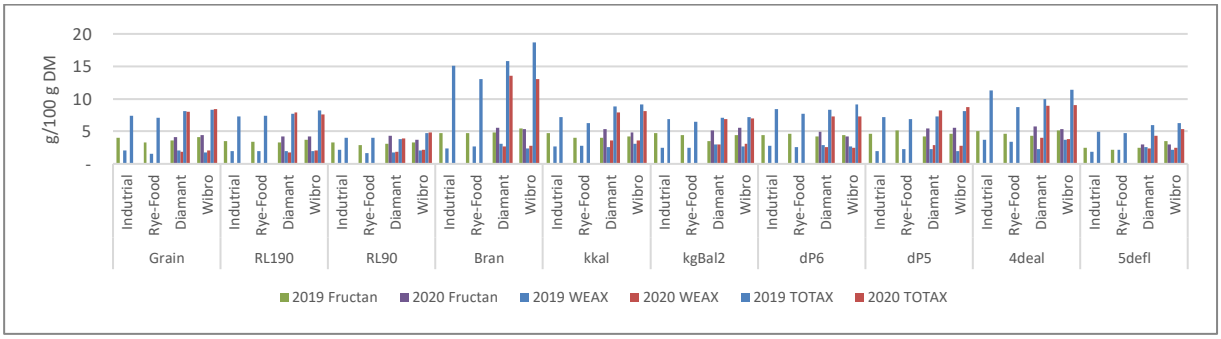

Fig.1. Results of four general and six experimental fractions of the four varieties of rye in two years.

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An innovative control strategy based on machine learning and miniature near infrared spectroscopy to assure the geo-traceability of cephalopods

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Octopus represents an important fishery resource with a high economic, social, cultural, and nutritional value for Mediterranean countries, although local catches are facing progressive decline due to overfishing practices, new fishery policies and climate change. The consequent increasing importation of octopus from other areas calls therefore into question the truthfulness of the nature, the genuineness, and the quality attributes of these products. Modern analytical techniques using miniaturized and portable near infrared (NIR) spectroscopy instruments are particularly suited for quality, safety, authenticity, and traceability testing of fishery products, offering not only high throughput results in a very short time, but also fulfilling the goals of 'White Analytical Chemistry' in terms of analytical, practical, economic, and ecological efficiency [1].
Hence the objective of the present study is to propose a rapid, cheap, eco-friendly and automatable analytical methodology based on NIR spectroscopy and machine learning, to identify the country of origin of two different octopus species and guarantee their traceability along the supply chain.
A total of 118 musky and 29 common octopus specimens (*Eledone spp*. and *Octopus vulgaris*, respectively) were collected from Western Mediterranean Sea (FAO fishing area 37.1.1, Balearic waters) and North-East Atlantic Ocean (FAO fishing area 27.9.9, Portuguese waters). The 908–1676 nm NIR spectra recorded in quadruplicate for each sample (i.e., 472 and 116 musky and common octopus) via the ultracompact, portable and wireless NIR device MicroNIR OnSite-W (Viavi Solutions). Data elaboration was performed on the first derivatized NIR spectra by comparing the performances of the following machine learning classification algorithms: logistic regression (LR), random forest (RF), support vector machine (SVM), and multilayer perceptron-artificial neural network (MLP-ANN).
The results were found to be optimal for all the trained models according to the average classification accuracy (94%), specificity (96%), and sensitivity (87%) values achieved when 10-fold cross validation performed on 75% of data. When the prediction ability of the models was compared by evaluating the percentages of the validation samples correctly identified by the trained models (i.e., by using 25% of the remaining spectral data) it was found that, for musky octopus, SVM performed better compared to the other models since only one Mediterranean sample was wrongly recognized as Atlantic (Fig. 1). No misclassifications were instead observed for Mediterranean and Atlantic common octopus, following the geographical origin recognition by the SVM, as well as MLP-ANN models (Fig.1). In general, it was also observed that both musky and common Atlantic octopuses were better predicted than the Mediterranean counterparts. This evidence can be the consequence of the specific characteristics of the FAO fishing area 27.9.a, whose body of water is smaller and much closer to the coast, thus potentially reflecting a more uniform environment responsible for the composition of octopus originating from this area to be more stable and preserved.
In conclusion, the achieved results achieved suggest the combination of portable NIR spectroscopy and machine learning as a promising plan of action to be adopted for the creation of an integrated analytical platform with capabilities for automated data recording, processing, and reporting in on-site or in-line monitoring of fishery products.

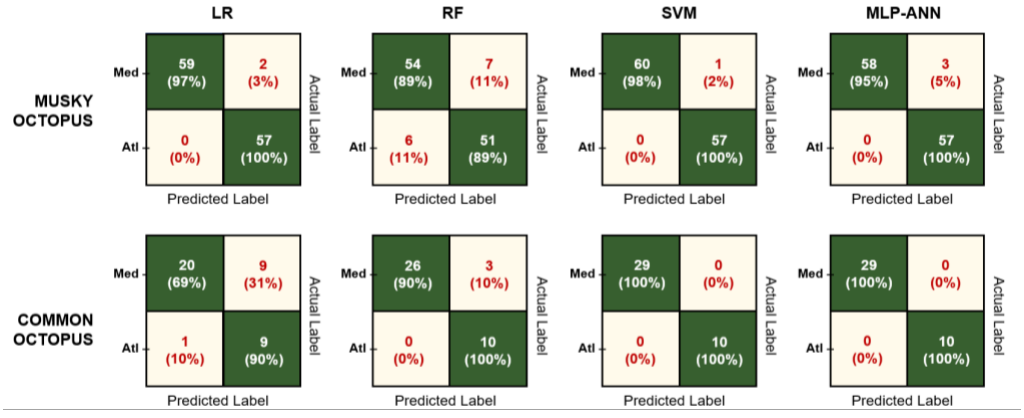

Fig.1. Confusion matrices showing the outcome of different classification algorithms in predicting new samples of musky and common octopus by geographical origin.

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Mineral profile of sea cucumber caught off Northeast Atlantic (Portugal)

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Sea cucumbers from Portugal have a good lipids profile allied to n-3 PUFA and can be a possible source of essential mineral elements that play the most varied and important roles in biological systems, as zinc (Zn), iron (Fe), copper (Cu), or calcium (Ca). However, to date, there are only few studies on the mineral profile in sea cucumbers of the Northeast Atlantic coast. In this sense, the present study aimed to determine the principal mineral elements in *Holothuria arguinensis*, *Holothuria forskali* and *Holothuria mammata* captured in the Northeast Atlantic (Portugal), three of the five species from Portugal. A total of 370 sea cucumber were studied, *H. arguinensis* (*n*= 123), *H. forskali* (*n*= 124) and *H. mammata* (*n*= 123). Mineral analyses were performed by Energy Dispersive X-ray Fluorescence (EDXRF). Results showed that all three species have a high content of minerals, being Cl>Ca>S>K the macro elements and Sr>Fe>Cu>Zn>Rb the micro elements. There was not significant difference ($p>0.05$) in mineral content between *H. arguinensis* and *H. mammata*, however, *H. forskali* was the one with the highest values of Cl, K and S. The mineral profile showed by the three studied species indicates that these species can be a good source of essential elements. These results can be suitable for nutritionists, health professionals and seafood industry.

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Physicochemical properties of inulin isolated from dandelion (*Taraxacum officinale*) roots by "green" extraction

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The aim of study was the isolation and physicochemical characterization of inulin from defatted dandelion roots by "green" extraction techniques - microwave extraction (MAE). The results were compared with the conventional extraction procedure and chicory inulin as a reference. The isolated inulin presented a white, tasteless powder with a degree of polymerization (DP) 17-20. Swelling properties, water and oil-holding capacity, wettability, angle of repose, flowability and cohesiveness and other phytochemical properties of dandelion inulin were evaluated (Table 1). FT-IR spectra contained typical bands for 2-ketose and α-D-Glcp residue. The linear inulin-type structure was also confirmed by ^{1}H and ^{13}C NMR spectroscopy (Fig 1). Polysaccharide was composed mainly of fructosyl units β-(2→1) linked to a terminated α-D-glucose unit that confirmed the structure of glucofructan GFn (2.7-3.2 kDa). MAE can be considered as an appropriate approache for the simultaneous extraction of dandelion inulin with a high average DP in large scale with high punrity (82-89%). The physicochemical properties of dandelion inulin showed its potential as an additive in food and pharmaceutical application.

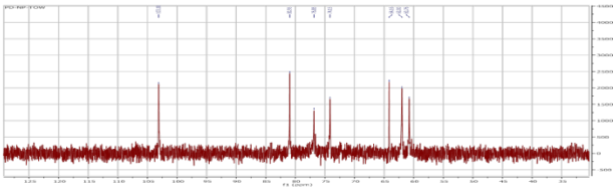

Fig.1. ^{13}C NMR (126 MHz, D_2O) spectra of MAE of inulin from dandelion roots.

Table 1. Physicochemical properties of dandelion inulin and chicory inulin.

Characteristics	Dandelion MAE	Dandelion Classic	Chicory DP=25
Yield, %	11	20	-
Purity, %	82	89	85
Fructose content, %	62	72	75
Moisture, %	11,26	10,79	11,25
Ash, %	1.65	3.31	0.13
Melting point, ºC	177-179	177-179	175-179
Angle of rotation, º	-25	-24	-25
Wettabity, %	28.28	7.77	0.70
Hygroscopicity, %	5,3	7,0	3,7
Swelling index, g/cm^3	3,98	1,46	1.05
Water holding capacity, g water/g	2.01	1.52	1.81
Oil holding capacity, g oil/g	3.50	4.31	3.32
Angle of repose,°	28,37	27,47	42,61
True density, g/cm^3	1,14	1,44	1,25
Bulk density g/cm^3	0,47	0,38	0,50
Tapped density, g/cm^3	0,61	0,51	0,61
Carr`s index (CI) %	24	25	18
Hausner index	1,37	1,33	1,21
Flowability	Fair	Fair	Good
Cohesiveness	Intermediate	Intermediate	Intermediate
Molecular weight, Mw Da	3284	2885	4020
Number molecular weight Mn, Da	3135	2762	3890
Polydispersity index	1.05	1.04	1.03
Degree of polymerization (DP)	20	17	25

Analytical tools to identify authenticity markers of PDO “Pera Rocha do Oeste” and PGI Alcobaça Apple var. Golden Delicious

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The PDO “Pera Rocha do Oeste” pear and the PGI "Maçã de Alcobaça” apple, due to its sui generis characteristics, are important Portuguese products due to its economic value to Portugal. The designation of a food product as Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) is a guaranty of authenticity and traceability.

Apple and pear fruits have their consumption in fresh, as well as industrially processed, as constituents of cake fillings. Although fresh fruits are easy to identify, their 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. To identify authenticity markers based on volatile compounds, it is important to select only the contribution of compounds that do not vary according to the storage conditions of the fruits. Aromatic compounds with description of pear and fruity odor are influenced by the environmental conditions of the orchards and fruit storage.

In this study, two analytical tools were used to identify authenticity markers of PDO “Pera Rocha do Oeste”, PGI Alcobaça Apple var. Golden Delicious. On the one hand, the mineral profiles of PDO pear and PGI apple (fresh fruit and fruit fillings) were analyzed by ICP-MS and ICP-OES and identified to evaluate the applicability of multielement data on the determination of geographical origin and authenticity. 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 characteristic. On the other hand, a method based on headspace solid-phase microextraction (HS-SPME) coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-ToFMS) was used to analyze volatile compounds of pears PDO “Pera Rocha do Oeste” from different orchards stored in different atmospheres and of pears from Alentejo area stored in normal atmosphere. From a total of 130 volatile compounds identified in PDO pears, 14 compounds (1-butanol, 1-hexanol, ethyl acetate, butyl acetate, pentyl acetate, butyl butanoate, ethyl hexanoate, hexyl acetate, heptyl acetate, ethyl octanoate, hexyl hexanoate, ethyl 2,4-decadienoate and α-farnesene) allowed to separate PDO pears and pears from Alentejo area. The 14 selected compounds can be proposed as markers to track the authenticity of the PDO “Pera Rocha do Oeste”.

The present study shows that multielement analysis by ICP-MS and ICP-OES enable to the identification and authenticity of the geographical origin of pear and apple fillings, even if industrially processed.

The HS-SPME / GC × GC-ToFMS methodology permit to distinguish the characteristic compounds of the fruits and to authenticate the origin of Rocha pears.

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Interactions of apocarotenoids with β-lactoglobulin

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In foods, carotenoids play important roles as antioxidants or colorants and have potential health benefits. Their chemical or enzymatic conversion leads to apocarotenoid formation *in vivo* and *in vitro* [1, 2]. Particularly the reactivity of short chain carotenoid metabolites (Table 1) formed during asymmetrical enzymatic cleavage or under pro-oxidative conditions, remains elusive. It has been demonstrated that oxidation of secondary plant metabolites such as phenolic compounds, produces reactive metabolites which can interact with proteins. For instance, nucleophilic additions of quinones to thiols- and thioether groups, causing altered physicochemical properties, have been observed [3].
Our project aims to provide first insights into how C_9-C_{13} apocarotenoids modify proteins. Preliminary studies revealed interactions with cysteine. The bovine whey protein β-lactoglobulin (β-Lg) was chosen, to investigate a potential cysteine specific reaction within proteins. β-Lg contains a free thiol group (Cys 121) as well as two intramolecular disulfide bonds (Cys66-Cys160, Cys106-Cys119) in its native conformation and has been proposed as a vector for (covalent bound) bioactive compounds [4].
First, adduct formation of intact β-Lg with apocarotenoids was examined *via* matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) analysis. For α-ionone, β-ionone and damascenone a mass shift corresponding to the $[M+H]^+$ of one analyte molecule bound to β-Lg was observed. Adducts were not detected in experiments with native β-casein, which does not possess a free thiol group. Next, targeted LC-MS/MS analysis of tryptic β-Lg digests was performed to determine the binding sites (Table 1). The free cysteine residue (121) turned out to be the major binding site for α-ionone and β-ionone. In contrast, damascenone adducts bound to β-Lg were detected exclusively for Cys 66 and Cys 160.
As apocarotenoids may interact with proteins, further studies should address these reactions in more detail to enhance the understanding of structures that can be formed during processing and storage as well as resulting changes in functional properties.

Table 1. Overview of apocarotenoids investigated for adduct formation with β-lactoglobulin (β-Lg).

Compound	Adduct with β-Lg
α-Ionone	**X (Cys 121)**
β-Ionone	**X (Cys 121)**
Damascenone	**X (Cys 66, Cys 160)**
β-Cyclocitral	**-**
Dihydro-β-ionone	**-**

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Amino acid composition of Rugova Cheese

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Rugova cheese (RC) is a traditional hard cheese produced in the Rugova Mountains of the Dukagjini region in western Kosovo. This cheese is made from cow's milk, and ripened for fifteen days. The objective of this study was to determine the quality of Rugova cheese based on its amino acid composition. Amino acid composition was determined by Automatic Amino Acid Analyzer.AAA400 (Ingos Ltd., Prague, Czech Republic) equipped with an Ionex Ostion LCP5020 cation-exchange column (22×0.37 cm). Of the 17 amino acids detected in Rugova cheese, glutamic acid (23.98%), proline (16.85%), leucine (9.76%), lysine (7.01%) and aspartic acid (6.63%) were the main amino acids, while cysteine (0.16%), glycine (1.89%) and histidine (1.95%) were the minor amino acids (Fig. 1). Essential amino acids accounted for 39.51% of the total amino acid content. Leucine and lysine were the most abundant essential amino acids. Amino acid score is a method of determining whether a protein is complete based on the limiting amino acid. The limiting amino acid calculated by amino acid score (AAS) was isoleucine (AAS:0.87) (Table 1). The high essential amino acid content, as well as the high amino acid score of the limiting amino acid make Rugova cheese a highly nutritious source of protein.

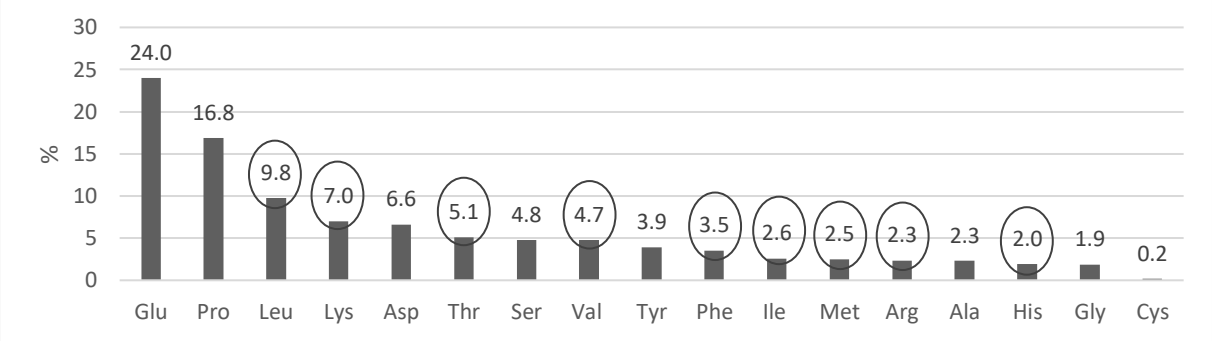

Fig.1. Amino acid composition of Rugova Cheese (essential amino acids are marked in the picture).

Table 1. Amino acid score of Rugova cheese.

	SAA	BCAA			AAA	Thr	Lys	His
		Val	Ile	Leu				
3–14 years	1.14	1.19	0.87	1.59	1.81	2.05	1.46	1.22
15–18 years	1.14	1.19	0.87	1.62	1.85	2.13	1.49	1.22
> 18 years	1.19	1.22	0.87	1.65	1.95	2.23	1.55	1.30

*SAA: sulphur-containing amino acids (methionine and cysteine); BCAA: branched chain amino acids (valine, leucine and isoleucine); AAA: aromatic amino acids (phenylalanine and tyrosine); *Amino acid score is calculated as described by the Food and Agriculture Organization, (2018).

Key words: amino acid, cheese, nutrition value, protein.

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Microwave-assisted extraction: an eco-friendly alternative for extraction of antioxidant compounds from blueberry

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Introduction

Anthocyanins are widely found in red fruits, responsible for the color and partially for the antioxidant activity. The conventional extraction method, maceration, requires a longer extraction time, which implies high energy consumption. Thus, the purpose of this study was to compare the extraction of blueberry anthocyanins using a traditional maceration extraction (TME) and microwave-assisted extraction (MAE).

Material and Methods

A solution of ethanol/water (80:20 v/v) was used as a solvent in a solid/liquid ratio of 33.3 g/L. TME was carried out at 40 °C, 120 rpm, 1 h, while MAE was performed at 100 °C, 1 min. The solutions were immediately filtered, evaporated, freeze-dried, and resuspended in water (20 mg/mL) before analysis. Anthocyanins were identified through HPLC-DAD-ESI/MSn, and the antioxidant activity was determined through the TBARS and DPPH assays [2,3]. Student's *t*-test through Excel (Microsoft Office 365®) was carried out to determine differences between samples.

Results and Discussion

Anthocyanins such as delphinidin-3-*O*-(6"-*p*-coumaroyl)hexoside, delphinidin-3-*O*-glucuronide, cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, pelargonidin-3-*O*-(6"-rhamnosyl)glucoside, cyanidin-*O*-(malonyl)hexoside, and pelargonidin-3-*O*-acetylglucoside were identified in the blueberry extracts.

Blueberry extracts obtained through MAE had better antioxidant activity through DPPH assay than those obtained by TME (Table 1).

Table 1. Antioxidant activity of blueberry extracts.

	TME	MAE	*p*-value
TBARS (IC_{50}; mg/mL)	0.07 ± 0.01	0.08 ± 0.01	≥0.05
DPPH (IC_{50}; mg/mL)	0.32 ± 0.03	0.13 ± 0.02	<0.05

Note: IC_{50}: The half-maximal response.

Although the higher temperature in microwave-assisted extraction, the time of exposure to the matrices with these conditions are lower, contributing to higher recovery of the antioxidant compounds.

Conclusion

Microwave extraction of antioxidant compounds can be an eco-friendly alternative to the traditional maceration method.

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UHPLC-PDA-MS analysis of vitamin B_{12} and its pseudo-form in nutritional supplements based on microalgae

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Cobalamin plays an important role as enzymatic co-factor within the human metabolism [1]. The natural occurring native forms (so-called vitamers) are 5'-deoxyadenosylcobalamin (AdCbl), methylcobalamin (MeCbl) and hydroxocobalamin (OHCbl). Due to chemical reaction of these B_{12}-vitamers with cyanide, the artificial but more stable cyanocobalamin (CNCbl) originates [2]. Only MeCbl and AdCbl show physiological activity being vital for the enzymes *methionine synthase* and *L-methyl-malonyl-CoA mutase*, respectively [3]. OHCbl and CNCbl can be metabolized in order to become useful for human beings. Moreover, MeCbl and AdCbl are interconverted into OHCbl when exposed to UV light [4]. The vitamin B_{12}-molecule is a six-coordination complex with cobalt as central atom that is bond to four pyrrole nitrogens and a 5,6-dimethylbenzimidazole (DMB) moiety. The structure is based on a corrin ring system. The upper ligand can be substituted due to weaker bonding forces resulting in different structural vitamers of B_{12}. Furthermore, the lower ligand (DMB) can be substituted by other bases such as adenine resulting in a non-active form of B_{12}, which is called pseudo-cobalamin (PsCbl). It cannot be metabolized by the human body [5,6].
Vitamin B_{12} is synthesized *de novo* by bacteria and certain archaea, hence it is almost exclusively found in food of animal origin, e.g., liver. However, it is crucial for a balanced diet, and as a consequence, of great concern especially to vegetarians and vegans [2,7,8]. Therefore, nutritional supplements have gained the attraction of consumers' awareness. Algae are believed to be a suitable source of cobalamin, which is why algae-derived supplements in various forms have been highlighted over the past few years. This also states the importance of being able to separate and analyze cobalamin and pseudo-cobalamin in algae-derived nutritional supplements.
Cobalamin is considered a challenging compound for analysis. Moreover, false-positive results are likely caused by pseudo-forms. Therefore, a fast, simple and reliable ultra-high performance liquid chromatography method with photodiode array detection was developed to separate cyanocobalamin from pseudo-cyanocobalamin using a reversed-phase system. Mass spectrometry was used for correct identification and confirmation.
Among the nutritional supplements analyzed, many samples are labeled to contain one or different *Chlorella* and/or *Spirulina* (cyanobacteria) species. Results obtained show a big variation regarding the content of cobalamin and pseudo-cobalamin. However, nutritional supplements containing (almost) only pseudo-cobalamin could be seen as useless regarding bioavailability for human beings.

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Structure and antioxidant activity relationships of dipeptides derived from foods

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Food proteins, apart from their nutritional functions, can be a source of peptides with variety of bioactivities, including antioxidant effect. Regardless the biological activity, peptides can be analyzed by in vitro and in silico methods [1], including chemometric analysis. This study uses a statistical model based on principal component analysis (PCA) to determine the impact of the structure of dipeptides on their antioxidant activity. Dipeptide sequences were acquired from the BIOEPEP-UWM protein sequence and bioactive peptide database (http://www.uwm.ed.u.pl/biochemia) [2]. The numerical variables (22 in total) were the descriptors of physicochemical properties of each dipeptide amino acid. They were derived from the AAIndex database [ftp://ftp.genome.jp/pub/db/community/aaindex/aaindex1]. 47 dipeptides with antioxidant activity were subjected to chemometric analysis. The obtained results indicated that the first 4 components explained 79.9% of the total variance. The first component consisted of the following variables related to the physicochemical properties of the N-terminal amino acid: molecular mass (+0.98), number of carbon atoms (+0.91), polarizability (+0.98), size (+0.91), percentage of buried residues (-0.82) and amino acid composition (-0.88). The values in parentheses indicate positive or negative correlations. The second component characterized the properties of C-terminal amino acid. They were as follows: molecular mass (+0.97), number of carbon atoms (+0.93), polarizability (+0.96), size (+0.86), percentage of buried residues (-0.72), and amino acid composition (-0.85). The third component consisted of the following variables: polarity (-0.87), bulkiness (+0.78), and Kyte-Doolittle hydropathy index (+0.91). They concerned the properties of C-terminal residue of dipeptide sequence. The fourth component described N-terminal residue, and included polarity (-0.90), bulkiness (+0.77) as well as Kyte-Doolittle hydropathy index (+0.86).

Final remarks:
Based on the PCA results concerning the structure- activity relationships of analyzed peptides, it was found that the presence of N-terminal amino acids such as Trp, Phe, Leu, Ile, Ala and C-terminal Pro, Val, Leu and/or His decided about the antioxidant bioactivity of dipeptide.

Keywords: antioxidant activity, dipeptides, BIOPEP-UWM database, chemometrics, PCA

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The profile of polyphenolic compounds, total phenolics and flavonoids contents, anti-oxidant and anti-microbiological properties of bee products

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Numerous studies have pointed out the high nutritional value and health benefits of bee products [1,2,3]. To reflect and understand the health benefits of the consumption and/or use of bee products in the food industry, determined the profile and content of polyphenolic compounds and the antioxidant and antimicrobial properties of four different bee products obtained from the same batch. The correlation coefficients and the principal component analysis (PCA) were also studied to find the relationship between particular bee products' determining parameters.
The total phenolic and flavonoid contents were as follows: bee pollen > beebread > beeswax > honey. According to the UPLC−PDA−MS/MS data, 20 polyphenols were identified. Sinapic acid was dominated in bee pollen, gallic acid in the beebread and honey, while in beeswax, the main compound was pinobanksin. The data showed that bee pollen and beebread characterized the highest antioxidant values than honey and beeswax. Beebread and bee pollen's antimicrobial activity was higher towards Gram-negative strains, while honey had the higher inhibitions against Gram-positive bacteria. The antimicrobial activity of bee products may be attributable to the combined effect of the gallic, ellagic, neochlorogenic, chlorogenic and protocatechuic acids.
Our study indicates that bee products may be a valuable source of bioactive compounds with value functional properties.

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Comparison of Total antioxidant capacity and Total content of polyphenols in green *Coffea arabica* from South and Central America

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Green coffee beans are the most globally important commodity and popular beverage around the world. Green coffee beans are a significant source of antioxidants and polyphenols, but caffeine, micronutrients, and chlorogenic acids are other major compounds that have health implications [1]. Coffee contains about 1500 different chemicals. Most of them are formed during the roasting process [2]. The chemical composition of green coffee beans is characterized by polyphenols, polysaccharides, minerals, chlorogenic acids, caffeine, organic acids, and lipids [3,4]. This study was aimed to analyze the total polyphenolic content (TPC) and total antioxidant capacity (TAC) of green coffee from South and Central America and describe possible differences of coffee from well-known growing regions. TPC and TAC have been analyzed in samples of *C. arabica* samples from Central America (1A Jinotega – Las Campanillas, 3A Guatemala, 5A Mexico) and South America (2A Villa Maria Colombia, 4A Colombia, 6A Peru). Samples were in altitude range of 1100 – 2180 mamsl, and all of them were wet-processed. For TPC analysis a modified method using a Folin-Ciocalteu (Lachman 2003) method according to Lachman (2003) [5] was used, the measurement was performed on a two-beam UV/VIS spectrophotometer (T-80 UV / VIS; PG Instruments Ltd; Czech Republic) at a wavelength of 765nm. For TAC, we used DPPH spectrophotometric method by Brand-Williams (1995) [6]. Dry matter content of analyzed samples was in interval 90,02% - 92,43%.

	TAC (% inhibition DPPH)	TPC (g GAE.kg -1)
Central America	72,007 a	54,069 a
South America	74,905 a	53,635 a
Pr> F(Model)	0,319	0,958
Significant	No	No

Table 1. Statistical analysis ANOVA, Duncan test.
(Notes: a,b = groups within a column with different superscripts differ significantly at p≤0.05).

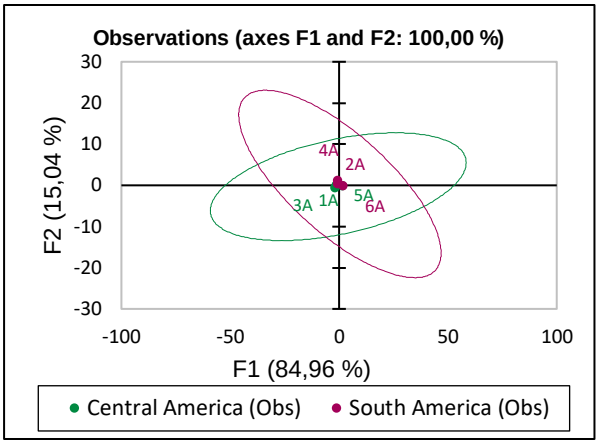

Fig.1. PCA map and variables representation.

We used PCA statistics for the visualization of differences between green samples from Central America and South America. PCA did not show any significant differences in these two geographical regions regarding the TAC and TPC parameters. Our results suggest that TAC and TPC measured in green coffee beans from South and Central America did not reach statistically significant differences. However, addition of more parameters, such as caffeine content and chlorogenic acids might provide a useful information regarding the differentiation between coffees from selected geographical origin.

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Micro- and macroalgae amino acid profile and protein content

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Algal protein has been reported to be a sustainable source of non-animal and non-allergenic proteins, for application in the food industry. There is also a recent trend in the markets towards the elimination from the food production proteins of animal sources, such as food for vegan consumers. Algae proteins have advantages such as non-allergenic proteins, complete amino acid composition, higher productivity, simple and low production cost. The algae protein content, expressed in percentage of protein in dry matter is high and is dependent on the algae species, for example, microalgae *Spirulina maxima* presented 63%, and macroalgae *Porphyra tenera* presented 44%, *Ulva lactuca* 29%, *Chondrus crispus* 20%, and *Fucus serratus* 17% [1]. However, protein amino acid composition is still underexplored. Therefore, the aim of this study was to determine the total and free amino acid profile and the protein content of micro- and macroalgae.
In this work, three samples of microalgae (*Arthrospira platensis, Chlorela vulgaris*, and *Tetraselmis chuii*) and six samples of macroalgae (*Codium tomentosum*, *Porphyra dioica*, *Gracilaria gracilis, Chondrus crispus, Fucus vesiculosus, Ulva Rigida*) were submitted to acid hydrolysis (total amino acids) and to an aqueous extraction (free amino acids). The total and free amino acid profile and the amino acid content was accessed using an HPLC-DAD. Total nitrogen was also evaluated by the Kjeldahl method.
Arthrospira platensis presented the highest total amino acid content and a complete total amino acids profile compared to other microalgae and the lowest total amino acid content was determined for the macroalgae *Fucus vesiculosus*. *Tetraselmis chuii* presented the highest levels of free amino acids content. *Chlorella vulgaris* presented the highest levels of the amino acids glycine, lysine and proline and *Codium tomentosum* the highest level of hydroxyproline. These algae have a high protein content to be explored as a green, non-animal, and non-allergenic alternative protein source for the food industry (Table 1).

Table 1. Protein and amino acid content of nine algae.

Algae Samples		NTP*	Total nitrogen % (g/ 100g)	Total amino acids % (g/ 100g)	Free amino acids % (g/ 100g)
Microalgae					
Arthrospira platensis		6.3±0.0[2]**	92.0	50.8±3.4	4.5±0.0
Chlorela vulgaris		6.4±0.1[2] *	66.6	35.6±0.8	2.7±0.1
Tetraselmis chuii		6.3	50.5	23.4±0.8	6.1±2.8
Macroalgae					
Codium tomentosum		4.2±0.5[3] **	11.3	17.9±1.5	2.1±0.3
Gracilaria gracilis		4.0±0.4[3] **	16.4	19.0±3.9	3.3±0.2
Porphyra dioica		4.2±0.5[3] **	18.1	20.9±0.3	3.1±0.0
Chondrus crispus		4.0±0.4[3] **	6.8	12.2 ± 4.2	0.1±0.0
Fucus vesiculosus		4.2±0.4[3] **	3.8	7.7 ± 0.6	0.1±0.0
Ulva Rigida		4.2±0.5[3] **	10.1	14.0 ± 4.3	0.1±0.0

***NTP:** Nitrogen-to-protein conversion factors; ** Bibliographic references

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Barley (*Hordeum vulgare L.*) grain as a source of antioxidant peptides

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Cereals are one of the main sources of energy to human. Their consumption is considered to have a positive effect on human health. The quality of food proteins is determined not only by their amino acid composition, but also by peptides that are released during the hydrolysis processes [1]. Peptides released as a result of enzymatic hydrolysis of food proteins, including those derived from barley, may show biological function, e.g. antioxidant activity.
The aim of the research was the characteristics of antioxidant peptides derived from barley grain proteins.
The amino acid sequences of all proteins were acquired from the UniProt database [2]. The research included two stages: *in silico* (bioinformatic) analysis and verification of the results using *in vitro* methodology. Bioinformatic studies included the calculation of the profile of potential biological activity of barley proteins, computation of A parameter (the frequency of bioactive fragments' occurrence in a protein sequence), simulation of proteolysis using human digestive enzymes. Above analyses were carried out by using BIOPEP-UWM database [3]. Experimental methods included the extraction of barley their proteins, enzymatic hydrolysis using the digestive protocol of Infogest [4] and assessment of antioxidant activity of extracts as well as hydrolysates.
The results showed that barley protein hydrolysates had the antioxidant activity, which may result from the presence of peptides with above-mentioned biological function. These results were consistent with those obtained using bioinformatic methods. It shows that *in silico* methods are useful tools supporting the analysis of food proteins as the source of antioxidant peptides. Our approach can also be applicable to study of proteins of different origin being the precursors of peptides with various biological functions.

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Geographical origin authentication of roasted *Coffea arabica* using volatiles profile and Linear discriminant analysis

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Coffee is one of the most traded commodities in the world market, due to that, the adulteration is relatively often. Therefore, authentication approaches are needed [1]**.** The main aim of this paper was to determine the possibility of using volatiles contained in beans of *Coffea arabica* roasted to medium roasting level to determine their geographical origin. Analyzed samples (from Africa, South America, and Central America) were purchased from Caffé ORO (Zvolen, Slovakia). Green samples were then roasted to Medium roasting level Full City++ (232 °C) using a roasting machine (Behmor 1600, model TO9500T). For volatiles determination, gas chromatography GC-MS was used according to Marek et al. (2019) [1] methodology with minor modification. Identification was performed by comparing data with reference materials and the NIST 14 library.

We separated detected volatiles into several categories based on their composition (aldehydes, alcohols, organic acids and esters, hydrocarbons (alkanes, alkenes, alkynes, and aromatic hydrocarbons), ketones, heterocyclic compounds, and amines. To obtain more detailed description, pyrazines and furan derivates were determined separately to heterocyclic compounds. Yang et al., [2] also reported that these groups are the most abundant in roasted coffee. Given the roasting process, the highest concentrations reached heterocyclic compounds, pyrazines, furan derivates.

Profile of volatiles was further used in Linear Discriminant Analysis a statistical approach that dimensionality reduction technique with class discrimination used to separate observation into groups [3]. Wilk's Lambda (Rao's approximation) showed significant results (p-value=0.002). This suggests that samples could be identified and separated into 3 groups (Central America, South America, and Africa). Most African samples are distinguished well (Fig. 1.), and centroids of South and Central American samples are closer together, which might be explained by the similarities in environmental and climate conditions of these regions. The confusion matrix for training samples and Cross-validation: Prior and posterior classification, membership probabilities to evaluate classifier performance. The first one reached an accuracy of 100%, meaning all training samples were correctly identified. The latter predicts the accuracy of 83,33% when 1 sample from Central and 1 from South America were misclassified.

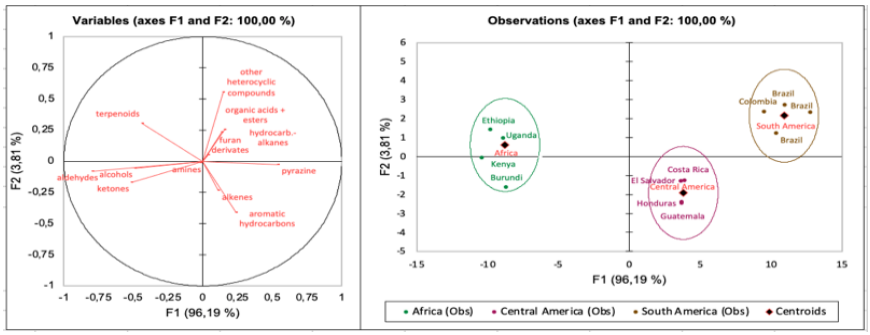

Fig.1. LDA map.

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Phenolic compounds of blackthorn (*Prunus spinosa* L.) fruits originated from Serbia

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Wild fruits are crucial for people in rural areas and unemployed youths by offering them the possibility to make new food products such as jelly, jam, preserves, marmalade, dry berries, juice, wine, syrup, sauce, and others. Blackthorn (*Prunus spunosa* L.) is one of the important wild plants with powerful health-promoting properties. It is a bush plant with pronounced thorns that belongs to the family Rosaceae. The fruit is spherical in shape with a stone inside, distinctly dark blue in color with a grayish waxy coating, 10-15 mm in diameter, with a bitter and astringent taste. Blackthorn is widespread throughout Europe. It grows alone or with other plant species like dog rose, acacia, hawthorn, dogwood, etc. [1]. The chemical composition of thorns is characterized by the presence of phenolic compounds which act as antioxidants, specifically flavonol heterosides (quercetin and kaempferol), phenolic acids (neochlorogenic and caffeic derivatives), coumarin derivatives, anthocyanins and type A proanthocyanidins [2]. Polyphenolic compounds have the greatest contribution to its antioxidant activity, whereas processes of freezing and storage of blackthorn fruits in the frozen state do not have a significant impact on the change of polyphenolic composition and antioxidant activity [3]. The blackthorn fruit contains organic acids (malic, citric and fumaric), carotenoids, pectins, tanins and vitamins (C, E) [4]. The fruits are used in food products like jellies, syrups, ice cream, vinegar or for liquor making (such as gin, kvass, patxaran, flavored beers, vodka, Porto` wine…) [5,6].

In this study polyphenolic profile of blackthorn fruits originated from a rural area of South Banat District in the Republic of Serbia was studied. Extract of ripe fruits was prepared using ultrasound assisted extraction with 75% ethanol (in 1:5 ratio) in ultrasound bath at 25 ^{o}C during 30 minutes. For determination of phenolic compounds in the fruits extract HPLC method was used [7].

The results of the analysis, revealed following phenolic acids in the hawthorn fruits ethanolic extract: caffeic, chlorogenic, *p*-coumaric, protocatechuic and syringic. The highest and the lowest concentrations were measured for chlorogenic acid (22.32 mg/kg) and *p*-coumaric acid (1.53 mg/kg). Rutin, quercetin_3-*O*-rhamnoside and quercetin_3-*O*-glucoside (glycosides of flavonol quercetin) were the most abundant phenolic compounds in the extract, with 104.12, 79.66 and 22.86 mg/kg, respectively.

Studied blackthorn fruits represent a significant source of antioxidants. This gives numerous possibilities for their application in food industry, especially for the development of new functional products or improvement of existing ones. Also, the important fact is that blackthorn fruits are available during the autumn and winter months which mean that they are sources of antioxidants in time when other sources in nature are not available.

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Classification of bee pollen samples according to their intact glucosinolate content

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In the information age in which we live, more and more research is emerging to find new compounds, which are present in foods, that can provide benefits for our health. In particular, the high number of publications concerning bee pollen analysis in the last decade demonstrates the rediscovery of this product due mainly to its associated biological activities (antimicrobial, anti-mutagenic, antioxidant…). According to the number of publications, phenolic compounds, proteins, vitamins, and carbohydrates could be considered the most representative bioactive compounds of bee pollen.

However, in the last few years, attention has also been drawn to a family of compounds, namely, glucosinolates, which are plant secondary metabolites and they are also becoming significant for human nutrition due to their preventive role in health, chiefly in terms of their breakdown products. Both the quality and the number of glucosinolates differ among plant species and subsequently in their pollen. As a result, monitoring glucosinolate content in bee pollen could be of great interest not only from a nutritional but also from a botanical point of view, as such compounds could be used as biomarkers to identify the origin of the samples.

A study is presented of the real possibilities of glucosinolate content and chemometrics (canonical discriminant analysis) to differentiate bee pollen samples from four different apiaries (Fuentelahiguera, Monte, Pistacho, Tío Natalio) located in the same geographical area (Marchamalo, Guadalajara, Spain). Fifteen glucosinolates were determined by using an ultra-high performance liquid chromatograph coupled to a mass spectrometer with a quadrupole-time of flight analyzer. The proposed sample treatment involved a solid-liquid extraction with ultrapure water at 70°C followed by a solid phase extraction with cartridges containing a weak anion exchange sorbent (NH_2) to achieve an efficient extraction of analytes. The chromatographic separation was carried out in gradient elution mode with a Luna Omega C_{18} column, and a mobile phase composed of a mixture of acetonitrile (0.1% formic acid) and water (0.1% formic acid) at a flow-rate of 0.2 mL/min. Glucosinolates were quantified by generating extracted ion chromatograms with the precursor ions under the optimized quadrupole-time of flight conditions. Low detection and quantification limits were obtained by using the selected conditions for all glucosinolates ranging from 7 to 28 µg/kg (detection limit) and 23 to 88 µg/kg (quantification limit),

Glucosinolate residues were detected in all the samples, and these differed in number and concentration, which range between LOD and 582 mg/kg. These four apiaries were differentiated by means of the first three canonical variables obtained from a canonical discriminant analysis. The data base used in the present study comprised the response of each sample to the qualitative variable (apiary of origin) and the three analyses of each individual sample for each intact-GSL (quantitative variables). Following this analysis, more than 70% of the samples could be assigned correctly to the Fuentelahiguera, Pistacho and Monte apiaries, and 100% to the Tío Natalio apiary.

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Study of the nutritional profile of *Cichorium Spinosum* L. after fertilization with different nutritional solutions

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The concentration of fertilizing via nutrient solution can affect the production and quality of the nutritional value of leafy vegetables. *Cichorium spinosum* L. is a wild edible plant used as a basic ingredient in the Mediterranean diet. Its nutritional composition has already been described and correlated with the prevention of chronic diseases and disorders, making it an important complement to human nutrition [1,2]. Considering it is usually collected in the wild, its accessibility and availability is limited; therefore, market potential cannot meet the growing consumer demands for healthier foods. Thus, the aim of the present study was to evaluate the effect of fertilization with nutrient solutions that contained different ratios of nitrogen, phosphorus, and potassium on the nutritional profile of *C. spinosum* leaves. 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) [3] were evaluated. 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 300:100:100 ppm of N:P:K (C311) stood out for its high crude protein (22.0±0.4 g/100 g dry weight dw) and fiber content (46.4±0.9 g/100 g dry weight dw), followed by the sample fertilized with 200:200:200 ppm of N:P:K that also showed promising fat values (6.8±0.1 g/100 g of dw), carbohydrates (20.2±0.1 g/100 g of dw), and energy (301±1 kcal/100g). The control sample (without fertilization) showed the lowest levels in all the studied parameters, except for the protein content in which there were no significant differences compared to the C311 sample. With the results obtained, it was possible to verify that the concentration of macronutrients in nutrient solution (N:P:K) may directly affect the nutritional value of the plant under study, with high concentrations of phosphorus and potassium having a negative impact on the protein and fiber content. It is thus possible to select the proper nutrient solution to obtain final products with a promising nutritional profile and promote their incorporation into commercial cultivation systems and the exploration of the species in sustainable cropping.

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Breads enriched with different flours: a new solution for healthier diets

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The diseases related to the lifestyle and, principally, with eating habits is constantly increasing, despite huge efforts to solve this problem. The food industry has innovated and re-invented a large part of its products, mainly by changing the traditional composition of food products, because seems to be an effective method of influence the improvement of the diet [1,2]. Baked products are one of the most widely consumed foods in the world, with an annual worldwide bread consumption of over 9 billion kg (23 billion euros). In European countries, the per capita consumption of bread is very diverse, being on average 59 kg of bread per year [3,4]. However, besides the bread having a list of simple ingredients for its preparation, there is a multitude of cereals that can be added to your recipe, improving the nutritional composition through the presence of beneficial compounds for human health [5].

In this study, a comparative analysis of nutritional and chemical parameters of four different types of bread (Portuguese rye bread, wheat bread legumes and cereals, biological seed bread and biological spelled bread), made in *Pão de Gimonde* with different raw materials, was carried out at.

The nutritional profile (protein, ash, fat, carbohydrate content and energy value) was evaluated using official analysis methodologies (AOAC) and the chemical profile was determined by chromatographic techniques, being the free sugars identified/quantified by HPLC- RI and the fatty acids by GC-FID.

In general, the water content is similar in all evaluated breads, with values around 41%, as well as, the ash content (around 1 g/100g fresh weight) and fibres (around 4% TDF fw). However, the remaining parameters showed statistically significant differences between the different produced breads. The biological spelled bread and biological seed bread showed the highest amount of proteins (7,8 ± 0,1 g/100 g fw), while the Portuguese rye bread presented the minor value (4,6 ± 0,1 g/100 g fw). The fat content was higher in wheat bread legumes and cereals (4.4 g/100g fw), that can be attributed to the seeds and legumes that take part in the breads, and lower in Portuguese rye bread and biological spelled bread with values of 0.30 g/100g fw. It was also evident, in all types of bread, the majority presence of mono (MUFA) and polyunsaturated (PUFA) fatty acids. Regarding soluble sugars, the fructose, glucose and maltose were detected in all bread samples, showing values of total sugars around 2.6 and 3 g/100g fw. As expected, the sugar present in highest amount was maltose, a common sugar found in cereal. In total carbohydrate evaluation, very close values were observed, which ranged between 41 and 47.7 g/100g fw, and the energetic value showed a variation between 220 and 244 kcal/100g for the Portuguese rye bread and biological seed bread, respectively.

Thus, considering protein intake, the highest-scoring breads were biological spelled bread and biological seed bread, while for consumers seeking high fibre breads, the recommendation would be biological seed bread, although it has a high amount of fat. The analysed bread varieties can continue to be a part of the consumers' diet. The application of these different raw material is motivated not only by consumers looking for healthier foods, but also by the industry itself, which seeks innovative measures to encourage the purchase of its products while maintaining financial balance.

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Influence of the maturation stage on the chemical composition and bioactive properties of *Cynara cardunculus* L. var. *altilis* seeds

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A good understanding of dynamics involved in food production is of great importance for improving food quality and security. *Cynara cardunculus* L. is commonly called cardoon and comprises three varieties, the wild cardoon or variety *sylvestris*, the globe artichoke or variety *scolymus*, and the domesticated cardoon or variety *altilis*. This perennial plant is well adapted to the Mediterranean environment [1]. It is used in the Mediterranean cuisine and in several different sectors, including the production of cheese, paper pulp, biodiesel, biomass, and bioenergy [2,3]. The wide industrial applications and the consequent associated commercial interest have been the main contributions to the increase in the economic value and cultivated area of this multi-purpose crop [3]. Among its edible parts, the seeds are particularly rich in high quality edible oil and health-promoting antioxidants [4], which have encouraged the nutraceutical exploitation of this species. Therefore, it is important to explore the potential of cardoon seeds at different maturity stages to better direct them to the most suitable sector and also to reduce the waste of this natural resource and thus contribute to crop valorisation.
For this study, *Cynara cardunculus* L. seeds were collected in Greece at four principal growth stages (PGS), ranging from PGS 6/7 (immature seed sample S1) to PGS 8 (mature seed sample S4). After seed grinding and extraction, their chemical constituents were analysed by different chromatographic techniques. The individual profiles of fatty acids, tocopherols, organic acids, and free sugars were characterized. The phenolic composition was assessed by HPLC-DAD-ESI/MS in hydroethanolic seed extracts. Regarding *in vitro* bioactivities, the antioxidant activity was measured through the cell-based TBARS and OxHLIA assays, which evaluate the ability to inhibit lipid peroxidation and oxidative haemolysis, respectively; the cytotoxic potential was tested by the sulforhodamine B assay against four tumour cell lines (HeLa, MCF-7, NCI-H460, and HepG2) and a primary cell culture (PLP2); the anti-inflammatory activity was determined through the extracts' capacity to inhibit the production of the pro-inflammatory mediator nitric oxide by a lipopolysaccharide-stimulated murine macrophage cell line; and the antimicrobial activity was evaluated by the microdilution method against several foodborne bacterial and fungal strains.
Six phenolic compounds were tentatively identified in the cardoon extracts and the content increased with increasing seed maturity (from 23.2 to 53 mg/g extract). Caffeoylquinic and dicaffeoylquinic acids were the major polyphenols. Our results revealed that mature seeds (sample S4) presented the highest content in lipids (23 g/100 g dw) and tocopherols (29.62 mg/100 g dw). This mature sample also showed the greatest capacity to inhibit lipid peroxidation, nitric oxide formation, and tumour cell proliferation. On the other hand, sample S3 was the one that best inhibited oxidative haemolysis and the growth of the tested bacteria. Regarding antifungal potential, sample S1 which corresponds to a less advanced state of maturation showed the best results. Overall, this work allowed to characterize the chemical composition and bioactive properties of cardoon seeds harvested at different maturity stages. According to the obtained results, the analysed quality attributes of cardoon seeds may differ depending on their maturation. However, additional studied are necessary to correlate the specific detected compounds responsible for the observed biological potential.

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Oxidative changes in potatoes caused by deep-frying process with sunflower oil and omega 3 sunflower oil: A food modelling study

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It was aimed to evaluate the oxidative changes as a result of frying potatoes in sunflower oil and omega-3 sunflower oil in this study.100 grams of potatoes were deep-fried using 1 liter of refined sunflower oil (SFO) and omega-3 sunflower oil (OSF). When the temperature of the oils reached 170-180 ℃, the potatoes were put in the frying pan and frying was done for 3 minutes. This process was repeated 10 times consecutively for both oils. Peroxide value (PV) (mEq O_2/g) of oils was determined. The malondialdehyde (MDA) level(nmol/g) was analysed. Total polar compounds(TPC) were detected with a commercial kit. The PVs (mEq O_2/kg) were found as 16.0±1.00, 21.0±1.00 and 6.60±1.38 at the initial, 5th and 10th frying applications for SFO($p<0.05$), and 3.0±1.00, 5.4±0.46 and 8.1±1.20 for OSF; respectively ($p<0.05$).While the MDA value of SFO at the initial was 0.05 ± 0.001 nmol/g, it was determined as 0.08±0.001 and 0.10±0.0005 nmol/g after the 5th and 10th frying; respectively ($p<0.05$).In OSF, MDA values were determined as 0.07±0.001, 0.10±0.001 and 0.12±0.002 after the frying applications of initial, 5th and 10th; respectively($p<0.05$). After the initial, 5th and 10th frying applications, a statistically significant difference was found between all oxidative parameters of SFO and OSF($p<0.05$). TPC values for both SFO and OSF were determined as <5%, even after 10th frying. As a result of this study, it has been determined that there are interestingly more thermo-oxidative changes in sunflower oil enriched with omega-3 compared to classic sunflower oil. However, the level of oxidative products or polar compounds with negative health effects, was not at a level that would pose a risk in frying application for both oil types.

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Kynurenic acid in honey from various botanical species

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Kynurenic acid (KYNA) is metabolite of tryptophane and possess anti-inflammatory and antioxidative properties [1]. It is also suggested, that KYNA may influence metabolism and weight gain acting as an anti-obesogenic [2]. KYNA can be found in a various food with wide range of concentration from meat having the lowest and vegetables like potato having the highest concentration [1]. The presence of KYNA was also observed in honey [3].

With gradient LC-MS method we determined content of KYNA in 136 Slovenian honey samples of different botanical sources. Study was conducted on chestnut, linden, spruce, acacia and silver fir honeys and also on mixed type honeys like flower and forest honeys. Results have shown that chestnut honey has much higher concentration of KYNA unlike other honeys.

Concentration of KYNA in chestnut honey is in range (330 – 1000) µg/g followed by linden honey in general range (25 – 200) µg/g. Next, in general order is spruce honey around 9 µg/g followed by acacia honey in general range (1 – 5) µg/g. Somewhere in between acacia honey range is silver fir honey with concentration range (1 – 2) µg/g.

Method of calibration curve was used. Whole linearty range (0.01 – 20) mg/L was covered. Concentration 0.01 mg/L was also determined to be the LOQ.

Repeatability was studied on 3 parallel samples of each representative honey type. Relative standard deviation was calculated. Repeatability was determined for K 1, S 143.1, H 118.1, L 22, A 1, G 3 and C 2 to be 2.95 %, 2.21 %, 2.79 %, 1.25 %, 2.81 %, 4.05 % and 0.96 % respectively. For linden and acacia honey recovery was also determined to be 106 % and 113 % respectively.

In some acacia honey samples we observed higher concentrations of KYNA than its general range, as the concentration in some linden honey samples are lower than its general range. Reason for this could be mixing honey with honey of different type, intentional dilution of honey or heterogeneity of honey bee apiary area. As expected, the range of mixed honey samples, such as flower honey and forest honey, varies from low to high concentrations with no observable order. Therefore, concentration of KYNA in forest honey and flower honey samples is in range (1 – 400) µg/g and (1 – 200) µg/g respectively. Besides the mentioned reasons for out of general range concentrations, explanation of this phenomenon, could be wide range of botanical sources of honey as this is not honey from one specific plant. High concentration of KYNA in some forest and flower honey may suggest a greater share of chestnut honey or even linden honey.

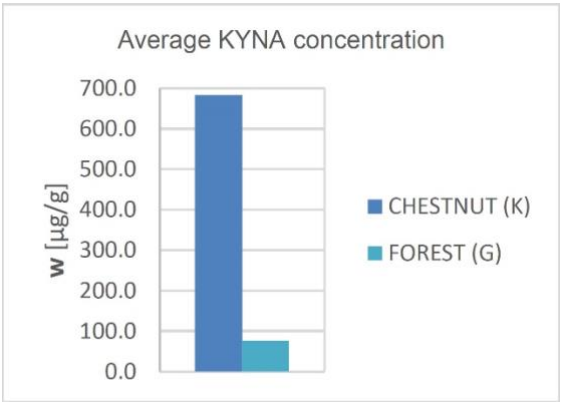

Fig.1. Average KYNA concentration in chestnut honey is approx. 10x higher than in forest honey, which has higher second highest average concentration.

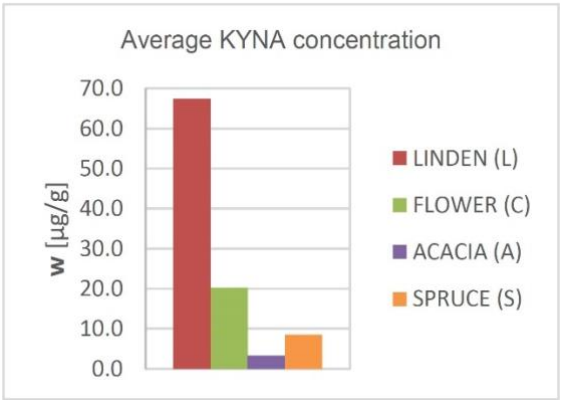

Fig.2. Lower average concentration than chestnut and forest honey has linden honey, with approx. 3x and 6-7x concentration than in flower and spruce honey respectively.

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Molecular level depiction of how stearic acid enhances β-carotene solubilization in dietary mixed micelles

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β-carotene, a precursor of vitamin A, is a natural antioxidant pigment that has many health benefits for humans [1,2]. However, β-carotene has low bioaccessibility owing to its highly lipophilic nature. In literature, the digestion products of co-consumed triglycerides have been reported to increase the bioaccessibility of β-carotene by enhancing its solubilization in dietary mixed micelles (MMs) [3,4]. The solubilization mechanism of β-carotene in MMs and their cluster-specific structural analyses have not been fully addressed so far due to the inadequacy of experimental methods to capture these properties directly. This study aims to narrow down that gap via coarse-grained molecular-dynamics simulations. We investigated the solubilization of 3 β-carotene molecules within two representative MMs formed under the duodenal fed-state conditions; i.e., one (~3.8 nm) in the absence, and another (~4.4 nm) in the presence of stearic acid (SA) to simplistically mimic lipid digestion products. The increase in size with the incorporation of β-carotenes was less marked for the MM with SA. Although the presence of SA did not affect the solubilization behaviour directly, the stability of the MM without SA was reduced after the incorporation of β-carotenes, indicated by the diminished ratio of hydrophilic to hydrophobic solvent accessible surface area. Both micelles preserved their slightly elliptic shape after the incorporation of β-carotenes. Structural analyses showed that β-carotenes occupied the micelle core and pushed the hydrophobic tails of POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and SA (if present) towards the surface. The core and surface packing of molecules were tighter for the SA micelle both before and after the incorporation of β-carotenes. The POPC tails and β-carotenes were able to adopt more extended conformations in the core of SA micelle owing to its larger size. This study is anticipated to be useful in directing future studies for the development of effective nutrient delivery systems for bioactive molecules like β-carotene.

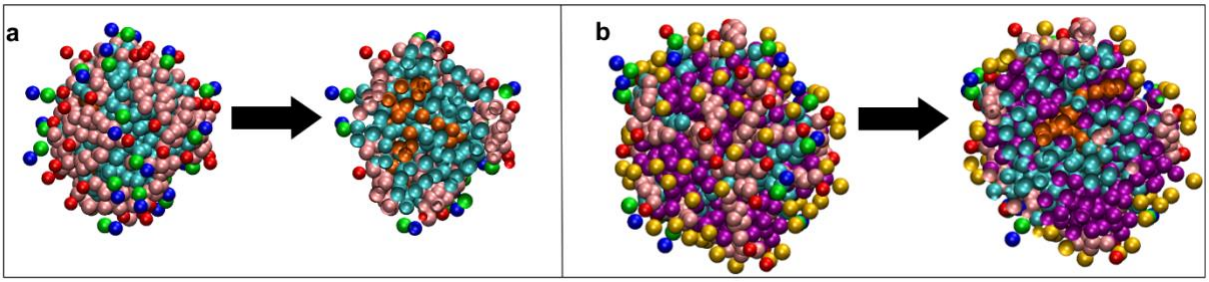

Fig.1. The snapshots and the cross-sectional views of the mixed micelles formed by **a)** cholate, POPC, β-carotene and **b)** stearic acid, cholate, POPC, β-carotene. Colouring: head groups of cholates are red, rest of cholates are pink; choline group of POPCs are blue, phosphate group of POPCs are green, tail groups of POPCs are cyan, β-carotenes are orange, head groups of stearic acids yellow, tail groups of stearic acids are purple.

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Comparison of nutritional properties and in-vitro antioxidant activity of organically grown garlic and its fermented product

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Over the past few years, fermented garlic and its extracts have been increasingly used in cooking and in the daily diet due to their specific taste, nutritional composition and health benefits. The fermentation process is performed by heat treatment of garlic with controlled humidity for a longer period of time. During fermentation, chemical reactions and transformations such as Maillard reactions and caramelization reactions cause the changes in taste, nutritional composition, content of macro- and microelements as well as the content of phenolic compounds and antioxidant activity of garlic. In this paper, samples of garlic and fermented garlic were analyzed and the content of phenolic compounds, antioxidant activity, macro- and microelements as well as nutritional composition were compared. The content of Ca, K, Mg, Na, Mn, Cu, Zn, and W increased, while the content of Fe, Al, Cr, Ni, Mo, Hg and Pb decreased during fermentation. The water content decreased during the fermentation process, while the sugar and carbohydrate content increased significantly as a result of thermal decomposition of the poly- and oligosaccharides (fructan and other complex polysaccharides). Accordingly, the energy value of fermented garlic is higher than that of fresh garlic. The content of total phenolic compounds is higher in the fermented sample compared to fresh one, indicating different chemical transformations of secondary metabolites during the fermentation process. As a measure of antioxidant activity, two assays were performed: DPPH and FIC and both showed higher activity of fermented garlic, which is positively correlated with the higher content of phenolic compounds in the sample.

Fig.1. Appearance and shape of fresh garlic and black garlic bulbs and cloves.

Table 1. Comparison of antioxidant activity of garlic and its fermented product.

Sample	TPC [mg EKG/g]	DPPH [mmol/L TE/g]	FIC [mmol/L EDTA/g]
Garlic	39 ± 5	0,014 ± 0,007	255 ± 52
Black garlic	904 ± 282	0,324 ± 0,059	365 ± 52

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Identified *Saccharomyces cerevisiae* strains from wine fermentation

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In winemaking, the alcoholic fermentation of grape must is a spontaneous process, performed mainly by *Saccharomyces cerevisiae* specie, present on the grape pruine and the cellar environment [1]. Its contribution to the final product, concretely quality and *sui generis* characteristics, is very well reported and depends on the strain that dominates during alcoholic fermentative processes [1,2].

The technological and organoleptic performance depend on agroedaphoclimatics conditions of vineyards, resulting in an adaptation of certain strains in the specific ecosystem [1]. Madeira Island, because of its volcanic origin, located in the Atlantic Ocean, subtropical climate and a pronounced orography, presents interesting agroedaphoclimatics conditions [3].

Therefore, molecular methods to typing *S. cerevisiae* strain are a powerful strategy for conservation and selection strains [1, 2]. Repetitive interdelta sequences located along to *S. cerevisia*e genome, which numbers and positions result in polymorphic patterns, represent a powerful tool to identify closely related strains [4].

During 2020 campaign, 3 grape must varieties from Madeira Island, namely, Sercial from *Seixal*, Verdelho from *Prazeres* and Tinta Negra from *São Vicente* (red grape) were supplied by certified winery. The grape musts were submitted to spontaneous microfermentation in controlled conditions, aseptically. A total of 90 isolates were, randomly, selected in the final phase of microfermentation [4]. By analysing the interdelta polymorphism, using δ2 and δ12 primers [2], Sercial showed high variability with more than 5 banding patterns.

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Assessment of γ-aminobutyric acid contents in brown rice and bran: comparison of HPLC and colorimetric methods

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The γ-aminobutyric acid (GABA) is a neurotransmitter found in the central nervous system with two important functions: tranquilizer and hypotensive. This compound has other protective actions associated with the prevention of Alzheimer's disease, the reduction of blood pressure, and the prevention of diabetes [1]. Functional foods containing GABA have been developed due to their healthy beneficial effects and the assessment of useful methodologies for GABA quantification is of great importance for their improvement in different food matrices. The GABA can be found in rice grain, essentially in brown rice and rice bran, and its content can be increased during the grain germination process [2]. The amount of GABA in white rice ranges from 0.3 to 0.7 mg/100g [3] and in rice bran ranges from 10.7 to 350.0 mg/100 g [4,5], values that varies greatly depending on rice variety and the quantification method used (colorimetric, enzymatic or chromatographic).
The main objective of this work is to determine the GABA content in brown rice and rice bran using two different methods: a colorimetric [6] and a developed HPLC method using Photodiode Detector. The rice variety selected for this experiment was Ariete (*Japonic* variety), the GABA was extracted from brown rice and bran and two different solvents were tested (distilled water and 80% ethanol).
The results obtained (Table 1) confirms the higher GABA concentrations in bran and shows higher extraction rates with distilled water than ethanol. The GABA values obtained by colorimetric method for bran are in the reported range but unexpected much higher concentrations were obtained by the validated HPLC method (r^2=0.9999). In order to explain these results, HPLC derivatization methods will be performed and also the search if other interfering components are present.

Table 1. GABA content (mg/100g) in Ariete variety (bran and brown rice).

		GABA (mg/100g)	
Sample	**Extraction solvent**	**HPLC method**	**Colorimetric method**
Rice Bran	Ethanol 80%	1962.5 ± 111.3	83.9 ± 1.4
	Distilled water	2350.3 ± 584.4	294.0 ± 20.1
Brown Rice	Ethanol 80%	255.4 ± 5.1	33.0 ± 4.8
	Distilled water	356.1 ± 144.1	95.7 ± 10.2

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In Vitro Antioxidant Activity and FTIR Characterization of Polyphenolic Extracts from Carob Kibbles Upon Roasting

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During the last years, carob has attracted interest due to its potential use as a bioactive-rich food ingredient. Carob powder that is produced after roasting and milling of carob kibbles can be utilized as a cocoa substitute [1]. The process of roasting of carob kibbles induces reactions that affect bioactive compounds and leads to the formation of Maillard reaction products (MRPs), which have high antioxidant potential. In this work, we report the ATR-FTIR characterization of extracts from raw and roasted carob kibbles to investigate the chemical changes taking place upon roasting [2]. The DPPH radical scavenging activity, total polyphenolic, proanthocyanidin and gallic acid contents were also determined for the extracts from raw and roasted carob kibbles. Antioxidant activity was enhanced by roasting at all temperatures investigated in this study. The ATR-FTIR data allowed the detection of changes in the structural features of polyphenols that were linked to the enhanced antioxidant capacity upon roasting. Moreover, the fingerprint region of the ATR-FTIR spectra contained bands that were attributed to the formation of melanoidins, providing a first indication for the structure of these diverse compounds in carob. Competitive effects arising from the thermal degradation of polyphenolic compounds at the highest roasting temperature of 175 °C used in this study was evidenced particularly for proanthocyanidins. FTIR detection of melanoidins signatures in the roasted samples, which displayed the highest antioxidant activities, laid the basis for the further investigation of these bioactive compounds in carob.

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Valorisation of Roman chamomile (*Chamaemelum nobile* L.) herb for the development of flavourings and natural antioxidants

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Natural bioactive ingredients isolated from various aromatic and medicinal plants may be valuable alternative sources for synthetic food additives. Flavonoids, phenolic acids, coumarins, procianidins, sesquiterpene lactones and essential oil (EO) isolated from Roman chamomile were reported as bioactive components possessing strong antioxidant and other activities. Roman chamomile flowers have been long used for medicinal purposes as possessing anti-inflammatory, anti-diuretic, sedative, antimicrobial, anti-fungal, soothing effects and antimutagenic properties, its essential oil, which is usually distilled from the aerial plant parts, is used as a fragrance in toothpaste, shampoos, soaps and perfumes and as flavouring in beverages and natural antioxidant in foods. The odour of Roman chamomile extracts is characterized by sweet, apple-like, floral, spicy, herbal and green tea aroma notes.
The aim of this study was to investigate the aroma profile and antioxidant properties of Roman chamomile collected in Lithuania at six different growth phases, A_3-V_6. The essential oil (EO) was obtained by hydrodistillation in a *Clevenger*-type apparatus from the aerial parts of dried herb and yielded 0.2÷0.8% (v/w). The composition of EOs was analysed by GC-MS and more than 70 volatile aroma components were identified, where the esters of angelic, methacrylic and tiglic acids being major constituents. 3-Methyl pentyl angelate (25-31%), isoamyl butyrate (6-9%), hexyl methacrylate (5-7%), 2-methyl butyl angelate (4-6%), isoamyl angelate (4-5%), isobutyl angelate (3-5%), α-pinene (2-4%), ethyl tiglate (1-2%) were dominating in EOs.
Distillation residues were separated into the liquid and solid fractions by filtration. The liquid residues were lyophilized to obtain water extract (WE), while dry solid residues were extracted with acetone to obtain deodorized acetone extract (DAE). Total phenolic content (TPC) was measured with Folin-Ciocalteu reagent, antioxidant capacity evaluated by the $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ scavenging and oxygen radical absorbance capacity (ORAC) assays. The highest TPC values, in mg gallic acid equivalents in g dry extract weight, were determined for WE-Z1 (143.2) and WE-Z2 (136.5); DAE - at Z_2 and Z_3 growth phases, respectively 66.7 and 52.7. WEs of Roman chamomile plants were remarkably stronger antioxidants than DAEs in $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ scavenging assays. WE-Z_3 and WE-Z_2 demonstrated the highest ORAC, 560 and 459 μmol trolox equivalents/g edw.

The results show that Roman chamomile are valuable source of natural phytochemicals and may be fractionated into valuable aroma and antioxidant fractions, which could find application in the development of natural bioactive ingredients for functional foods, nutraceuticals, and cosmetics.

Biobased food packaging with electrical conductivity for in-pack treatment by pulse electric field

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Biodegradable biopolymers are sustainable alternatives to conventional plastics for food packaging applications. To compete with synthetic polymers biopolymers needs to meet the requirements of cost-effective materials ensuring the mechanical and gas barrier characteristics of food packaging. Additionally, it is demanded to step towards active packaging, which means that packaging material need to interact with the food product to enhance its shelf life, contributing to reduce food waste. In this context, polysaccharides have been exploited to develop edible and biodegradable films due to their functional and sustainable characteristics and the integration of different fillers, such as clays, metal oxide particles and graphene derivatives, bring great challenges to the field of active food packaging [1].

The pulsed electric field (PEF) is a promising non-thermal food processing technique. Currently the food is processed into a treatment chamber before packaging, which represents a risk of recontamination. The use of an electrically conductive food packaging to sterilize food in-pack may overcome this drawback. In this regard, electrically conductive biocomposites are promising materials for this application due to their non-toxic nature.

The combination of the fillers, namely reduced graphene oxide (rGO), multiwalled carbon nanotubes (MWCNT), and zinc oxide, to design new formulations based on polysaccharides (starch, alginate and chitosan) allow to produce biomaterials with enhanced mechanical and barrier properties, conferring functional properties as antioxidant capacity, antimicrobial activity and/or electrical conductivity [2-5]. Electrical conductivity is a required property for the processing of food at low temperature using electric fields (Figure 1). Therefore, these bionanocomposites have a great potential as innovative and active food packaging.

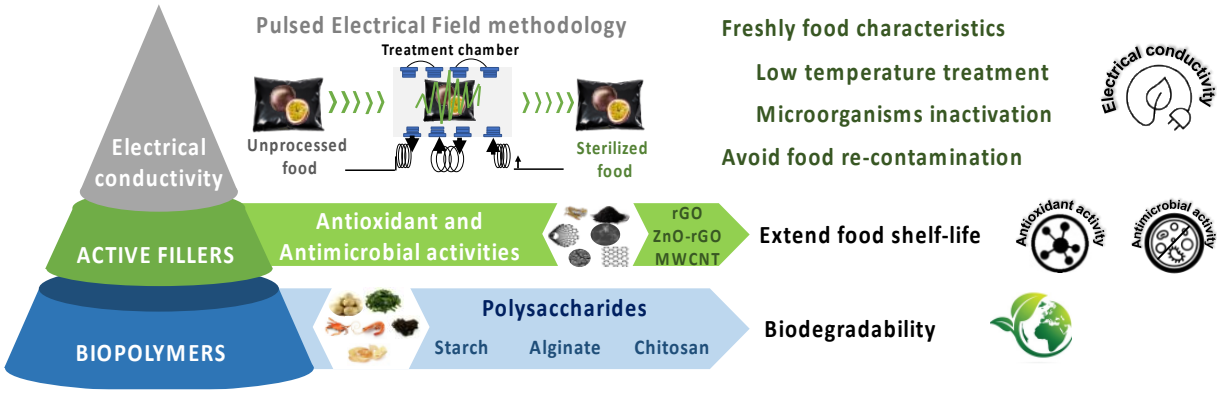

Fig.1. Electrical flexible bionanocomposite development.

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Extrusion cooking effect on arabinoxylans content in novel gluten-free flours based on rice and chickpea

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Extrusion technology has been extensively used for development of ready-to-eat snacks, most of them based on pulses flours. Moreover, the production of novel gluten-free functional food products is one of the main objectives of the food industry in order to satisfy the demands of celiac population. In this sense, there is an increasing interest in using gluten-free flours, such as rice flour or pulses flours, which stand out for their dietary fibre content and bioactive compounds [1-4]. In recent years, the arabinoxylans (AX), which are one of the most noteworthy compounds within the soluble dietary fibre constituents, have aroused great interest among the scientific community due to their potential protective role against diseases, such as diabetes, cardiovascular diseases and certain types of cancer. The chemical structure of AX consists in a backbone of a linear chain of β-D-(1→4)-xylopyranose, which is substituted on the hydroxyl groups (–OH) of the 2- and 3-positions by L-arabinofuranosyl residues linked by β-(1→4) glycosidic bonds [5]. Therefore, the aim of this study was to evaluate the changes induced by extrusion cooking on arabinoxylans content in different gluten-free formulations (rice-chickpea flours enriched with Fibersol® and passion fruit).

The arabinoxylans (total and soluble) were quantified by a spectrophotometric method according to Ciudad-Mulero et al. (2018) [2]. The absorbance was measured at 448 and 508 nm and the lecture at 508 nm was subtracted from that at 448 nm in order to remove the influence of hexoses (mainly glucose). Standard samples were prepared using different concentrations of xylose (0.005–1 mg mL−1).

In raw formulations, the content of total arabinoxylans ranged from 6.76 to 9.76 g/100 g. These values are higher than those previously reported in lentil formulations enriched with nutritional yeast (3.67 – 7.18 g/100 g) [2]. This difference could be related to the presence of rice in the analysed formulations, as arabinoxylans are the main non-cellulosic polysaccharides present in cereals [5]. In raw analysed flours, water soluble arabinoxylans were found between 0.80 – 3.00 g/100 g, being these values in accordance with those obtained in different lentil flours [2]. In general, the content of total arabinoxylans were higher after extrusion treatment achieving concentrations of 7.88 – 9.77 g/100 g. Moreover, the content of water soluble arabinoxylans was significantly ($p < 0.05$) increased as consequence of extrusion cooking, being these values between 1.78 and 4.24 g/100 g in extruded formulations. This extrusion effect has been previously reported by other authors in different food matrix, such as lentil flour [2], barley flour [6] or wheat bran [7].

The results of the present study showed that extrusion cooking is a valuable technological treatment that increase the arabinoxylans content in cereal-pulses based formulations for gluten-free snack development. Therefore, the analysed extrudates formulated with rice and chickpea flours could be healthy functional foods for celiac population.

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Water desorption kinetic curves as a tool for quality and history of products analysis

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Water activity (Aw) measurement is a routine procedure in the food industry. It is based on the measurement of partial pressure (PP) of water vapour in the system, where the sample under study is put into an enclosed volume. Aw corresponds to the value of PP in the state of thermodynamic equilibrium of the system, which means that kinetics of water desorption from the sample is not used, although it can contain additional information about properties and history of a given sample.

We put samples of flour into a chamber with very low humidity (silica gel was used as a desiccant) and monitored the process of water desorption using fast surface acoustic wave humidity sensor based on the ultrathin film of grapheme oxide. Such sensors are known to be extremely fast [1,2], thus, the kinetic curves (KC) sampling was as high as 1 Hz. The two-exponential model [3] for the time dependence of density of evaporated water molecules $n(t)$ was used in the form

$$n(t) = A_1(1 - e^{-\alpha 1 t}) + A_2(1 - e^{-\alpha 2 t}) \qquad (1)$$

where $\alpha_i = \beta \_\_ v^{iS}\ (i = 1,2)$ are time factors of correspondent processes and A_i are their amplitudes. s is the effective area of evaporation from the sample and V is the volume of gas phase inside the chamber. Note that the equilibrium density, which corresponds to Aw in this model is equal to $n(t \to \infty) = A_1 + A_2$. Parameters α_i characterize geometry of the system and water molecules adsorption rate (AR) of material. Changes in ambient conditions and industrial processes influence the AR of a product, which means that α_i can be used for monitoring them. This concept was experimentally tested by measuring KC of flour samples after their exposure to 100% RH (relative humidity) atmosphere for periods of 0, 1, 2, and 3 hours in the first stage of the experiment. In the second stage, all samples were opened and kept in ambient conditions for two hours, after which KC were measured again. All KC were used for the extraction of model parameters in (1) by the LSQ-fitting method. The results of this procedure (see fig. 1) show that processes of samples treatment affect KC and, when Aw changes by 10%, AR changes by more than 60%, giving a much clearer indication of the process.

In this work we discuss feasibility of the model used and present the algorithm of necessary experimental data treatment to guarantee applicability of the model. The method of extraction of the contribution of geometry (s and V) of the system to KC is also discussed and presented. For the experimental procedures described above we discuss the physical processes, which can lead to obtained results and general guidelines of analysis of the influence of the history of samples on model parameters of its KC.

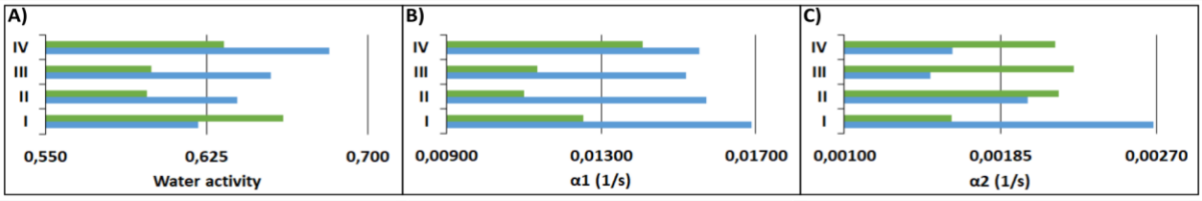

Fig.1. Results of fitting of experimental KC. A – Aw (A1+A2); B – α_1; C - α_2. Blue (green) bars correspond to the first (second) stage of the experiment. I, II, III, IV correspond to the sample which spend 0, 1, 2, 3 hours in saturated atmosphere during the stage 1.

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Extrusion process effect on resistant and total starch in corn and lentils enriched flours with grape skin (*Vitis vinifera*) by-product

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The extrusion cooking, due to its versatility, can be considered as a good technological process to obtain a gluten-free fortified food products based in pulses flours [1]. Different authors stand out that snack product formulated with lentil-based flours can stimulate the metabolic functions and prevent cardiovascular diseases, diabetes or colon cancer [2]. Moreover, grape by-products could be a great source of dietary fiber and bioactive compounds that can improve the nutritional profile of lentil-based snacks [3].
Resistant starch is related to many health benefits, similar prebiotic effects that those attributed to dietary fiber since it's defined as a portion of the starch that cannot be digested by amylases in the small intestine and passes to the colon to be fermented by microbiota [4]. In this sense, the main objective of the present study was to characterize the resistant starch (RS) and total starch (TS) content in different raw and extruded flours formulated with lentil-corn (30:70), Cabernet Sauvignon grape skin (CS-Sk; 0 – 20%) and Hylon V® (0 – 20%).
The content of RS and TS were analyzed using Megazyme kits based on the AOAC 2002.02 [5] and 996.11-76.13 [5] methods respectively.
According to the results obtained, the control raw flour (formulated with 93.75 % of lentil-corn flour), contain 3.06 % and 53.26 % of resistant and total starch, respectively. It can be observed that Hylon V (20 %) incorporation, significantly increase ($p < 0.05$) the RS content (4.45 and 5.69 % in raw flour). In general terms, the extrusion process significantly decreased ($p < 0.05$) the RS content in the analysed samples, while total starch content was not significantly affected. Other authors, reported similar TS results before and after extrusion in beans-based flours and a total decrease in RS after processing [6].
The results of the present study showed that extrusion cooking is a valuable technological treatment that maintain the resistant starch content in cereal-pulses based formulations, and therefore their potential prebiotic effect, for gluten-free snack development. Therefore, the analysed extrudates formulated with lentil-corn flours, Hylon V and grape skin flours could be considered as promising healthy functional foods for celiac population.

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Selecting ingredients and processing methods to increase carotenoid contents of carrot chips

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The benefits of consuming foods rich in pro vitamin A carotenoids have been discussed widely and are also mirrored in many countries' nutritionally guidelines. The carotenoid content of a food is affected by the abundance of these pigments in the ingredients, as well as their stability during processing.

Here, the impact of i) cultivar selection and ii) processing method on the carotenoid profiles and stability of carrots (*Daucus carota* L.) were investigated. For this, the carotenoid contents of 15 carrot cultivars of different colours were assessed with ultra-high performance liquid chromatography equipped with diode-array detector (UHPLC-DAD). Additionally, the stability of carotenoids in orange carrot slices after deep-frying, air-frying and air-drying was investigated.

The cultivar accounted for up to 12.9-fold differences in total carotenoid content between carrots of different colours and a 2.2-fold difference between orange carrots. Characteristic carotenoid profiles for each yellow, orange, red, and purple carrots were observed. Additionally, the trolox equivalent antioxidant capacity (TEAC) assay of lipophilic extracts showed a correlation between the carotenoid content and the antioxidant capacity in the fresh carrots.

When comparing carrot slices on the basis of dry weight, air-frying for 32 minutes increased extractable carotenoids 0.2-fold while deep-frying carrot slices for 10 minutes caused a 0.3-fold loss of total carotenoids in addition to *trans*-to-*cis* isomerisation of β-carotene. However, total carotenoids on a fresh weight basis increased in all treatments, with the effect of water loss outweighing that of carotenoid degradation. The highest total carotenoid content was recorded in carrot chips that were air-dried for four hours and air-fried for 32 minutes with 7.2-fold and 8.8-fold increases, respectively, indicating that these processing methods are preferable to deep-frying.

Influence of yeast strain and vessel type on aroma profile of Chardonnay white wine

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Wine aroma is characteristic for each wine variety and is one of the main quality parameters that affect consumers' acceptance of the wine. Aroma compounds in wine partially originate from the grapes, but they are mostly formed during the fermentation process and wine aging. Therefore, the final wine aroma depends on fermentation and aging conditions, like temperature, time, yeast strain, vessel type (wooden barrel, stainless steel tank). The aim of this study was to investigate the influence of two yeast strains (wine indigenous yeasts and *Saccharomyces bayanus*) and different wine aging vessels (stainless steel tank, oak barrel with medium toasting and oak barrel with heavy toasting) on the aroma profile of Chardonnay white wine. Aroma compounds were identified and quantified using a gas chromatograph equipped with mass spectrometer. The results showed that both, yeast strain and vessel type, influenced the final wine aroma. All 36 identified compounds were divided into six groups (acids, alcohols, carbonyl compounds, terpenes, esters and volatile phenols). The aroma profile of wine aged in stainless steel tank depended mostly on yeast strain, and the wine produced with selected *S. bayanus* had higher concentration of total acids, esters, alcohols, terpenes, carbonyl compounds and 2,4-Di-T-butylphenol than the wine produced with indigenous yeasts. The wines aged in toasted oak barrels contained another volatile phenol, 4-ethylphenol, whose concentration was higher in wines aged in barrels with medium toasting than in barrels with heavy toasting. Further, the highest concentration of total carbonyl compounds was measured in wine fermented with indigenous yeasts and aged in medium toasted barrel, and the highest concentration of total alcohols and esters was measured in wine with *S. bayanus* and aged in medium toasted barrel. The wine with *S. bayanus* that aged in stainless steel tank contained the highest concentrations of total acids, terpenes and 2,4-Di-T-butylphenol. The wines aged in heavy toasted barrels did not stand out in any aroma group. The wine produced with indigenous yeasts and aged in heavy toasted barrel contained the lowest concentration of total acids, carbonyl compounds, terpenes and volatile phenols. The lowest concentration of alcohols and esters was measured in wine produced with indigenous yeasts and aged in stainless steel tank. All aroma compounds were also divided according to their main odour into fruity, fatty, floral and green, citrus, faint and other, and PCA analysis was made. The PCA analysis showed that the aroma profile of all wines differed and that the aging vessel had a higher influence on the aroma profile of wine produced with indigenous yeasts than on the wine with *S. bayanus*.

Keywords: wine aroma, yeast strain, stainless steel tank, wooden barrel, barrel toasting

Functional properties and chemical profile of aged carioca beans (*Phaseolus vulgaris* L) cooked under the steam of autoclave

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Introduction

The common bean is rich in essential nutrients as high protein contents, with high lysine content (essential amino acid), and is used as a complementary protein to the cereal. Thermal treatment of legumes (as cooking) makes the consumption of these foods possible. Although, the thermal treatment of beans can cause considerable changes in nutrients, antinutritional compounds, and chemical profiles [1]. Therefore, this study evaluates the changes caused by cooking pre-soaked aged carioca beans in the autoclave steam, focusing on its bioactive components, antioxidant activity, and nutritional compounds. Additionally, to identify which carioca bean cultivar could preserve the most quantity of bioactive compounds in cooked flour.

Material and methods

Six commercial carioca bean cultivars were selected, aged for three months (at 27.5 ± 1.6 °C and 56.9 ± 10.0% relative humidity), and then soaked in water (6h) and cooked under pressured steam in the autoclave (5 min at 121 °C, 1,1 kgf) as previously described by Bento, Ribeiro, Alexandre e Silva, Alves Filho, Bassinello, de Brito, Caliari and Soares Júnior [1]. The flours were prepared following a 2 x 6 factorial design arrangement (raw bean flour and cooked bean flour x 6 carioca bean cultivars).

Results

The cooked flours from Imperador had the highest antioxidant activity (DDPH: 10.58 µmolTrolox·g-1, ABTS: 18.71 µmolTrolox·g-1), anthocyanins (8.08 µg·g-1) and total phenolic content (TPC) (36.69 mg·g-1). The cultivar Gol also retained part of these compounds before cooking. The Imperador (Im) and Gol cultivars presented the lowest values of phytate (around 0.70%). The phytate content of the common beans increased ($p < 0.05$) with the cooking with the steam in an autoclave after soaking, with the highest values (around 1.2 %) observed for the flours of cooked beans of cultivars Dama. Tannin was not detected in the cultivars Dama and Madreperola, which were expected because these cultivars are slow-darkening beans [2,3].
The LC-MS analysis of phenolic and saponins of the cooked flours revealed a reduction in phenolic compounds as catechin, epicatechin, and kaempferol and an increase in soyasaponin-Ba and Bb. Moreover, the samples Notavel, Dama, and Madreperola, presented the highest amount of soyasaponin-A0.

Conclusion

Cooking the beans in the autoclave steam affected the chemical profile of carioca bean flours, and these changes were dependent on the bean cultivars. The cooked flours from Imperador and Gol stood out due to the retention of part of their bioactive compounds, such as polyphenols and group B saponins. Therefore, they may be suitable to produce cooked flours by autoclave steaming after soaking, as food ingredients.

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Colourful Carrot Snacks Manufacturing by Applying Osmotic Dehydration, Convective Drying and Vacuum Microwave Drying

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Osmotic dehydration (OD) increases the nutritional contents, flavour and aroma of vegetables before the main drying processes. OD is a food process where water transports from fruit or vegetable to the hypertonic solution [1]. Until now, sugar and salt solutions have been utilized for OD, however recently, fruit solutions have been applied as novel materials [2]. Moreover, OD requires low energy consumption and causes low heating damages on food products when applied before main drying technologies [3].
The study aims to define the drying processes of coloured carrot varieties which were dehydrated with fruit juices; polyphenolics, carotenoids and physicochemical characteristics (dry mass, ash, colour, minerals, L-ascorbic acid, organic acid and sugar contents) of dried carrot snacks. Purple, orange, yellow and white carrots were used for snack production with 3 chosen fruit juices (sour cherry, chokeberry and apple juices) due to the colour and health-promoting features of those fruits, sucrose solution was applied as the control group.
According to the study, the moisture contents of carrot materials changed before and after the OD. Solid gains and water losses were observed during the OD. During the main drying processes, drying times and drying kinetics of food products were changed with the applied materials. Moreover, the physicochemical features of colourful carrot snacks demonstrated significant differences. For the polyphenolic contents of carrot snacks, the highest flavonol content was quantified in yellow carrot snack dehydrated with chokeberry solution (59.96 mg-100 g product) and the best outcome of the flavan-3-ols was in orange carrot sour cherry solution (213.07 mg-100 g product).
In conclusion, colourful dried carrots might be consumed as snacking alternatives with high content of polyphenolics and attractive colour features.

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Blue Honeysuckle *(Lonicera caerulea var. caerulea)* extract as potentional natural antioxidant for raw-cooked meat products

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Oxidation of the lipid component of food is a severe problem in the food industry, as it leads to shortened shelf life, deterioration of taste, functional and nutritional properties of food products [1]. Phenolic compounds are a major important group of natural antioxidants with potential beneficial effects on human health. They can be involved in protecting against the harmful effects of reactive oxygen species, especially free oxygen radicals. Free radicals are responsible for many pathological conditions and cell damage [2]. Edible fruits of the honeysuckle *(Lonicera caerulea L.)* are gaining popularity in many European countries, including Russia, Poland, the Czech Republic, and others. The attractiveness of these fruits depends on many factors, including early ripening time (before strawberries in Poland), resistance to spring frost, taste, high content of vitamin C and polyphenolic compounds, and health-related properties. Fruits are a good plant material for the food industry, for the production of juices, jams, purees, and - for the pharmaceutical industry - for the production of nutritional supplements [3]

Extraction of honeysuckle was carried out according to Shirahigue et al. [4]. For the preparation of meat products, we used the following ingredients pork meat (3000 g), water (600 g), salting mixture (60 g), black pepper (15 g), and nutmeg (1.5 g). The first group was made without antioxidants, BHE-3 and BHE-5 groups were incorporated with 3 and 5 mL of blue honeysuckle extract, respectively. All sample groups were vacuum-packed and stored at 4 °C. We evaluated the oxidative stability of a raw-cooked product based on malondialdehyde (MDA) concentration measurements by thiobarbiturate test using 2-thiobarbituric acid (TBA). UV-spectrophotometry measurement was carried out at 532 nm (Jenway 7305, UK).

In our study, we monitored the lipid oxidation of raw-cooked meat products during storage. This experiment aimed to determine the antioxidant effect of blue honeysuckle extract. We observed that one day after making meat product, malondialdehyde levels in all groups were without significant differences ($\alpha = 0.05$). The same condition was repeated after seven days. However, we see higher growth in negative control than in experimental groups. After fourteen days significant differences between the negative and both experimental groups occurred. After another week, on the twenty-first-day measurement, those differences are even more pronounced. We are also noticing the difference, not significant, between experimental groups. Lower MDA values were measured in the BHE-5 group (5 mL extract addition). This is more noticeable when percentage growth of groups is compared: Con 0 (158%) > BHE-3 (58%) > BHE-5 (47%). This also suggests that the antioxidant activity of the extract is, in our experiment, concentration dependent.

However, the ability to reduce lipid oxidation is not the only factor to be observed when testing natural antioxidants. Sensory quality is probably the most important. Natural antioxidants may have colour or taste-altering effects that customers can negatively perceive. Blue honeysuckle shows promising results in its antioxidant ability; further research is, however, recommended.

Fig.1. MDA values in samples over storage time.

Group	MDA [mg.kg-1]			
	Day 1	**Day 7**	**Day 14**	**Day 21**
Con-0	0.161 ± 0.007^{a}	0.175 ± 0.004^{a}	0.225 ± 0.007^{a}	0.415 ± 0.007^{a}
BHE-3	0.158 ± 0.008^{a}	0.164 ± 0.017^{a}	0.197 ± 0.004^{b}	0.250 ± 0.009^{b}
BHE-5	0.156 ± 0.004^{a}	0.160 ± 0.002^{a}	0.185 ± 0.008^{b}	0.229 ± 0.031^{b}

Note: Con-0 – negative control, BHE-3 – 3 $mL.kg^{-1}$ extract addition, BHE-5 – 5 $mL.kg^{-1}$ extract addition.

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Multi-step recovery of antioxidant-rich fractions from *Hierochloe odorata*

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Hierochloe odorata is an aromatic perennial grass widely distributed in West Asia and Europe. This plant's roots and aerial parts have a sweet smell, and their extracts have been shown to have high antioxidant activity and insect repellent properties [1]. In recent years several green extraction methods have been utilized for the isolation of bioactive compounds in order to minimize adverse effects on the environment [2]. The aim of this work was to isolate and characterize high-added-value fractions of *H. odorata* by comparing conventional solid-liquid extraction (SLE) and innovative, sustainable extraction techniques such as supercritical carbon dioxide extraction (SFE-CO_2), pressurized liquid extraction (PLE), and enzyme-assisted extraction (EAE).
The ash, oil, protein, water, and carbohydrate contents of *Hierochloe odorata* were studied using standard methods. A four-step sequential SLE with hexane, acetone, ethanol and water showed that the yield increased with increasing the polarity of solvents, and the collective yield was 26 %. SFE-CO_2 was applied at a pressure of 40MPa, at 60ºC for four hours, and yielded 2.10±0.23 % of the non-polar extract. The defatted, after SFE-CO_2, residues of *H. odorata* were subjected to sequential extraction with increasing polarity solvents and different methods.
A sequential PLE with acetone, ethanol, and water of the SFE-CO_2 residue was performed to assess the extraction yield and total phenolic content. The total extractable content of sequential PLE was 42%, which was higher than the conventional SLE. In an alternative approach, SFE-CO_2 residues were treated with the hydrolytic enzyme Viscozyme L. before the PLE treatment. Moreover, the *in vitro* antioxidant capacity of the extracts obtained with the above methods was evaluated with three assays, namely TPC, $ABTS^+$ $DPPH^+$.
Results indicated that samples treated with both EAE and PLE showed the highest antioxidant capacity in all assays, as well as the highest yield among all treatments.
Furthermore, GC-TOF-MS analysis of the non-polar fraction revealed the presence of phytol and other phytochemicals known for their mosquito repelling properties [3]. On the other hand, polar fractions of *H. odorata* analyzed by HPLC-TOF-MS were characterized by coumarin and 7,8-dihydroxycoumarin, which have shown potent antioxidant capacity and antibacterial activity [4]. Conclusively, high-pressure fractionation combined with EAE was more efficient in obtaining fractions with higher yield and antioxidant capacity than conventional techniques.

Acknowledgments: The authors thank Ona Ragažinskienė (Kaunas Botanical Garden of Vytautas Magnus University) for supplying plant materials.

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Recovery of Valuable Constituents from Hop Residues with Pressurized Solvents: Process Optimization and Extract Characterization

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Supercritical carbon dioxide extraction (SFE-CO_2) is a wide-spread technique for isolation of bitter acids and essential oils from hops. Hop SFE-CO_2 extracts are both valuable aroma containing and highbittering-potential bearing products, offering higher shelf life and bioactive compound stability than hop cones or pellets [1]. However, the residual hop biomass after SFE-CO_2 is commonly considered as waste and mostly discarded together with the valuable higher polarity hop phytochemicals, e.g. prenylflavonoids, exerting numerous beneficial health effects (antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial, neuroprotective, sedative, etc.) [2]. Therefore, optimization of extraction techniques with green, food and pharmaceutical-grade solvents (e.g., ethanol) in order to isolate these hop bioactive compounds from SFE-CO_2 residues could be regarded as a part of a multi-step hop valorisation strategy [3].

The present work is aimed to optimize pressurized liquid extraction with ethanol (PLE-EtOH) process in order to process hop (cv. *Ella*) residues after SFE-CO_2 into valuable functional components. For the purposes of this research, response surface methodology with central composite design (RSM-CCD) were employed to determine the effect of PLE-EtOH temperature (40-100°C) and time (15-45 min) on the extract yield, total phenolic content (TPC), *in vitro* oxygen radical scavenging capacity ($TEAC_{ORAC}$) and xanthohumol (XN) content. Statistically significant and reproducible models were obtained for PLEEtOH yield (11.1-21.8 g/100 g hop residue; F=57.85), TPC (19.7-39.7 mg GAE/g hop residue; F=79.39) and $TEAC_{ORAC}$ (273.5-417.8 mg TE/g hop residue; F=83.25) values, and XN content in extracts (50.683.3 mg/g extract; F=137.42). Model analysis and RSM-CCD plots indicated that extraction temperature was the main parameter that contributed to the observed responses for yield and antioxidant activity indices. Also, PLE-EtOH conditions had a negligible effect on the recovery of XN from the studied hop residue: only ~12% variation between the highest and lowest value (9.1-10.3 mg/g hop residue) were obtained under different experimental conditions and > 90% of the XN was extracted already after 15 min at 40°C. Based on these results, two optimal PLE-EtOH conditions were determined: (1) 40°C and 15 min to obtain xanthohumol-rich *Ella* hop extracts; (2) 85°C and 18 min to increase PLE-EtOH yield and antioxidant constituent recovery. PLE-EtOH at the lowest experimental temperature (40°C) produced Ella hop extract, containing 83.5 mg of XN, while extraction at 85°C allowed to isolate~2-fold higher amount of ethanol-soluble constituents (20.5 g/100 g hop residue), and resulted in 34% and 85% higher $TEAC_{ORAC}$ (397.4 mg TE/g hop residue) and TPC (39.29 mg GAE/g R) values, respectively. Besides the antioxidant potential, xanthohumol and other polar antioxidant-rich hop PLE-EtOH extracts showed inhibitory effect against the *S. aureus* (ATCC 25923). Thus, rich in valuable bioactive constituents, *Ella* hop PLE-EtOH extracts could find multipurpose applications in food, pharmaceutical, nutraceutical and cosmetics industries, for example, to develop antimicrobial and antioxidant phytopreparations for prophylaxis and treatment of various skin infections, which initial stage pathogenesis is primarily conditioned by *S. aureus.*

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Valorization of cranberry pomace by using supercritical fluid and pressurized liquid extraction processes

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Pressing of cranberries for juice generates large amounts of by-products, called pomace. Currently, cranberry pomace, which is a rich in health beneficial polyphenols, are used very inefficiently or even discarded as a waste. Therefore, there is an urgent need of valorizing cranberry pomace for the development of higher added value food grade ingredients. For this purpose, the application of biorefining concept seems to be a preferable approach in developing effective processes for recovery of various valuable substances from cranberry pomace, which may serve as functional ingredients increasing the health benefits of food products. The aim of this work was to develop multi-step biorefining scheme for valorizing cranberry pomace as a source of valuable nutrients by using supercritical fluid (SFE) and pressurized liquid (PLE) extraction processes. The objectives were to determine phytochemical composition, particulalry anthocyanins content, and antioxidant properties of the extracts. The extracts were isolated by using traditional (Soxhlet extraction) and modern (SFE with CO_2 and PLE) techniques. The parameters of SFE-CO_2 (pressure, temperature and time) and PLE with ethanol (temperature and time) were optimized for the highest product yields by using Central Composite Design and Response Surface Methodology modelling. The extracts obtained by different methods were evaluated by determining important composition characteristics and antioxidant potential. The composition of fatty acids in the oil of lipophilic fraction was analyzed by gas chromatography. Oleic (O), linoleic (L) and α-linolenic (Ln) acids were dominating: polyunsaturated fatty acids constituted up to 60% of all fatty acid content. Triacylglycerols (TAGs), tocopherols and phytosterols were determined by ultra-high performance liquid chromatography (UPLC), while β-carotene was quantified by HPLC. The results obtained showed that LLLn, LLnLn and OLnL were the major TAGs, each exceeding 20% of the total TAGs α- and β+γ tocopherols dominated in the tocopherols fraction, while stigmasterol and β-sitosterol in the sterol fraction. The content of β-carotene was 3.48 mg/100g extract. Total carotenoids in lipophilic extract, proanthocyanidins and anthocyanins in hydrophilic extracts were measured by spectrophotometric methods. while individual anthocyanins and other phytochemicals were analyzed by UPLC. Among six in PLE-EtOH extract quantified anthocyanins peonidin-3-galactoside was the major one, followed by peonidin-3-arabinoside. Polyphenols were the most quantitatively important bioactive compounds in defatted by SFE-CO_2 cranberry pomace extract (in mg /100 g extrtact):quinic acid (572.9), chlorogenic acid (88.6) and catechin (60.3) were major ones in ethanol extract Total phenolic content was determined by Folin-Ciocalteu method, while antioxidant capacity of extracts and residues after extractions was measured by ABTS radical cation scavenging and oxygen radical absorbance capacity assays (ORAC). The results showed that higher polarity solvents recovered the extracts with higher antioxidant capacity (AC), while AC of residues gradually decreased after each PLE step. It may be concluded that properly designed biorefining of cranberry pomace enables recovering of valuable products for using as food and nutraceutical ingredients.

Evaluating applicability of wood hemicelluloses as potential wall materials for spray dried microencapsulation of berry juice

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Hemicelluloses account for 20–30% of wood dry mass, but they are currently treated as low-value by-products remaining outside of biorefinery process. By using pressurized hot water extraction (PHWE), spruce galactoglucomannans (GGM) and birch glucuronoxylans (GX) can be effectively extracted from process water and spray dried to powder. Low viscosity and good emulsifying properties of wood hemicelluloses enable them to be used as superior wall materials in spray-dried microencapsulation of food rich in bioactive compounds, but this value-added application of wood hemicelluloses has not been investigated. This study aims to investigate effects of various techniques on physicochemical properties of feed solutions (10-20%, w/w) of either GGM, GX or their mixture with methylcellulose (MC) and carboxymethylcellulose (CMC) with bilberry juice, aiming to produce a highly stable feed solution which can be used for spray-dried microencapsulation. The homogenization techniques including magnetic stirring, ultrasonication and a combination of UltraTurrax mixer-microfluidizer. We found out that bilberry juice highly stabilized the mixture of hemicellulose and cellulose compared to the water by itself. The results of thermal properties determined by differential scanning calorimetry (DSC) revealed that GGM had two glass transition temperatures (85-91°C and 122-135°C); higher than GX (56-64°C), and that both melted at about 170-180ºC. The viscosity and particle size distribution of GGM feed solutions were highly affected by the sonication amplitude compared to the GX. Unlike the GX feed solutions, GGM feed solutions that were homogenized by ultrasonication and microfluidizer formed a gel-like structure, in addition to a reduction in zeta potential values. However, microfluidizer treatment resulted in lower surface charge density for most of the feed solutions. Moreover, the total anthocyanin content (TAC) of all feed solutions decreased significantly with increasing the number of microfluidizer passes. Hence, magnetic stirring resulted in no gel formation, lowest viscosity, and higher zeta potential values of GGM feed solution, in addition to no losses in TAC. Therefore, we found out that magnetic stirring should be used as a method for preparation of high concentration hemicellulose solutions for spray drying; especially, GGM feed solutions. Overall, GGM and GX are envisioned to compete against standard wall materials due to their viscosity, solubility, functionality, cost, and availability in the future.

Keywords: Wood hemicellulose; Microencapsulation; Wall material; Berry juice; Spray drying; Galactoglucomannan; Glucuronoxylan.

High-Resolution Mass Spectrometry Analysis of Melanoidins: The Role of Methylglyoxal in the Formation of MAILLARD Colorants

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The formation of melanoidins in food during of the MAILLARD reaction is still not fully understood. To limit the pathways of this complex reaction, experiments with individual MAILLARD intermediates are commonly used. [1,2] In the present study, the role of methylglyoxal in color formation was investigated by means of high-resolution mass spectrometry (HRMS). Methylglyoxal is known as a pivotal MAILLARD intermediate [3] that is formed by various cleavage reactions from carbohydrates or C6-α-dicarbonyl compounds. In addition, it is among the most abundant 1,2-dicarbonyl compounds in commonly consumed foods. [4]

Methylglyoxal was incubated in absence as well as in presence of L-alanine or L-lysine in aqueous solution at 100 °C and pH 5 for up to 300 min. The color intensity of the reaction mixtures was measured at 420 nm and the molecular weight distribution was analyzed by size exclusion chromatography. The conversion of methylglyoxal was determined by HPLC-DAD after derivatization with *ortho*-phenylendiamine. The structure of the colorants was characterized by ESI-HR-orbitrap-MS.

The browning reaction of methylglyoxal resulted in a fast conversion of the reactant in absence and in presence of amino acids. The reaction mixtures contained colorants with a molecular weight well above 100 kDa. HRMS spectra revealed oligomers of methylglyoxal formed by aldol addition and condensation. Van KREVELEN analysis of the HRMS data indicated varying degrees of dehydration and with the help of KENDRICK mass analysis it could be shown that cleavage, reduction, and STRECKER products of methylglyoxal such as formaldehyde, acetaldehyde, acetol, and aminoacetone became part of the melanoidin backbone.

In conclusion, methylglyoxal as well as its cleavage, reduction, and STRECKER products undergo aldol reactions which yield melanoidins in form of statistical copolymers (Fig. 1). Substructures formed by aldol addition might dehydrate and form conjugated π-systems that act as chromophores. Cross-linking of oligomeric chains is possible via aldol reactions of remaining carbonyl functions in the linear backbones. These reaction mechanisms – as well as the tools used to identify them – can be applied in future investigations to even more complex reactions of carbohydrates and help to unravel the structures of food melanoidins.

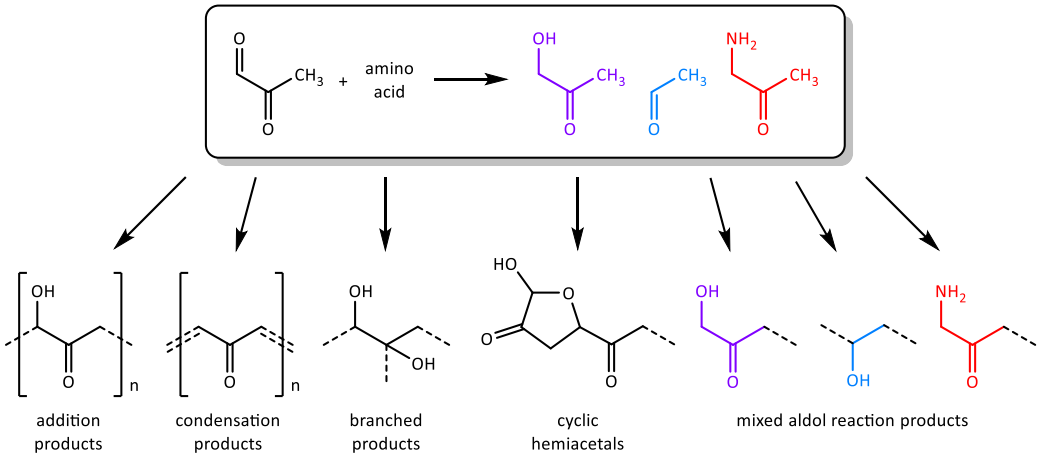

Fig. 1.: Substructures of melanoidins formed from the reaction of methylglyoxal with or without amino acids.

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Association between ultra-processed breakfast cereals and acrylamide

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The NOVA system is a strategy for classifying foods according to their degree of processing and for predicting subsequent risk of non-communicable chronic diseases (NCDs) [1]. The NOVA food framework defines processed foods as simple products manufactured from unprocessed or minimally processed foods alongside salt, sugar, oil, or other substances frequently used as culinary ingredients. NOVA introduced the term ultra-processed foods [2]. Worldwide consumption of ultra-processed foods is increasing significantly, and it represents between 25% and 60% of total daily energy intake in European countries, the US, Canada, New Zealand, and Latin American countries. The Nutrinet-Santé study concluded that various factors related to processing, the nutritional composition of the final product, additives, contact materials, and neoformed contaminants may play a role in the association between ultra-processed food intake, and overall risk of NCDs and cancer [3]. The European Food Safety Agency (EFSA) has identified acrylamide in food as a public health concern due to its relation with the appearance of different types of cancer [4]. The European Commission established benchmark levels (BL) for the presence of acrylamide in breakfast cereals and identified three groups: 1) bran products and wholegrain cereals, and gun puffed grains (BL = 300 µg/kg); 2) wheat- and rye-based products (BL = 300 µg/kg), and 3) maize, oat, spelt, barley and rice-based products (BL = 150 µg/kg) [5]. Breakfast cereals comprise a huge family of cereal-based products, which contribute to dietary acrylamide exposure in humans. This investigation aims to elucidate the association between the NOVA classification of breakfast cereals and the acrylamide content. Fifty-three commercial breakfast cereal samples were obtained from Spanish supermarkets. Samples containing dried fruits, nuts, cocoa, filled cereals and novel grains were intentionally excluded. Samples were classified according to the NOVA system as unprocessed or minimally processed (NOVA-1, n=6), processed culinary ingredients (NOVA-2, not applied), processed (NOVA-3, n=16) and ultra-processed (NOVA-4, n=31). Ultra-processed breakfast cereals have a complex formulation which includes ingredients different from sugars, vitamins, or minerals, such as other sweeteners (starch, maltodextrin, dextrose, honey, caramel, extracts of malted and toasted cereals, glucose syrups, inverted sugar, caramel and maltitol), cinnamon, vegetal oil, salt, lactose, skimmed milk powder, and additives (emulsifying, colouring, flavouring, antioxidant, anti-caking, raising agents). The nutritional composition was recorded from manufacturer declarations. Acrylamide was determined by LC-ESI-MS/MS with isotopic dilution (ISO:EN:16618:2015). A quantitation limit (LOQ) of 20 µg/kg was set. [5]. Statistical analyses were performed using Statgraphics Centurion XV and SPSS v.23.0. Student t-test and analysis of variance (one-way ANOVA) with Bonferroni's multiple comparisons post hoc test was used. The NOVA-1 samples were wholegrain, mainly oat flakes. The NOVA-3 breakfast cereals were from rice, maize, wheat, or a mixture of cereals, and they contained added sugar and vegetable oil. The NOVA-4 breakfast cereals were formulated with oat, maize, wheat, or a mixture of cereals, and not only added sugar and vegetable oil, but also non-culinary ingredients and additives. The acrylamide content varied from < LOQ to 382 µg/kg. The mean was 108 µg/kg and median was 69 µg/kg. All samples with acrylamide content higher than BL in each food group belonged to NOVA-4. Acrylamide correlated significantly with sugar content and the NOVA food classification. No differences were revealed in relation to energy, saturated fat, sodium, fiber and protein content. Results pointed out that the greater occurrence of acrylamide in samples with higher sugar content could contribute to risk factors associated with the overconsumption of sugary foods. Significant differences between NOVA groups were found for acrylamide ($p = 0.0072$). The mean acrylamide content of breakfast cereals was 20, 75 and 142 µg/kg for samples classified as belonging to the NOVA-1, NOVA-3 and NOVA-4 groups, respectively. However, differences between processed and ultra-processed breakfast cereals were not statistically significant. Main conclusions are; a) NOVA classification does not reflect the extent of the processing in terms of thermal intensity, b) the classification of a breakfast cereal as ultra-processed does not predict a significantly higher acrylamide content, c) sugar content was the only nutritional descriptor positively associated with the acrylamide content, regardless the type of grain or complexity of the formulation.

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The reaction of thioglucose and isothiocyanates lead to new transformation products during cooking

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Brassica vegetables like white or red cabbage contain glucosinolates. These can be hydrolyzed by myrosinase (an enzyme) to glucose and an aglycon. This aglycon can be degraded to isothiocyanates like allyl isothiocyanate or sulforaphane, which are valued for their health-promoting properties [1].
The functional group of isothiocyanates is very reactive and many glucosinolate containing vegetables are cooked before consumption. Therefore, a reduction of isothiocyanate levels by hydrolysis during food preparation can be assumed. Additionally, the reaction of isothiocyanates with nucleophilic matrix compounds, like thioles or amines, can lead to a decreasing content of isothiocyanates.
A possible reacting matrix nucleophile could be thioglucose, which can be formed during thermally degradation of glucosinolates like described previously [2]. To test this hypothesis aqueous mixtures of thioglucose and isothiocyanates were treated in different ways like different temperatures and pH values.
New cyclic transformation products were obtained from the reaction of thioglucose and isothiocyanates during cooking. By using mass spectrometry and nuclear magnetic resonance spectroscopy as well as an alternative synthesis route the composition and structure was evaluated. Several derivates of thioglucose and different isotope-labeled reagents were used to unravel the reaction mechanism. These results and the verification of the retro-aldol reaction of thioglucose allows the postulation of the most probable reaction mechanism. The novel transformation products of allyl isothiocyanates respectively sulforaphane and thioglucose were isolated and used to determine their content in boiled conventional red and white cabbages. The presence of the new transformation products in boiled cabbage samples leads to the question of their bioactivity and bioavailability. In cell based assays no acute cytotoxicity was observed up to a concentration which is approximately 1000-fold higher to that taken up with an ordinary meal, while the uptake of both analytes in cells was measureable. Both new cyclic transformation products were able to overcome the gastric and intestinal barrier as shown in model experiments. Additionally, it is was proven that after consumption of boiled red cabbage the new transformation products were detectable and quantifiable in urine.
That underlines the relevance of the thioglucose-isothiocyanate reaction products, but additional experiments regarding their bioactivity and potential to be metabolized should be done.

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Regulation of enzyme activity in spelt flour for breadmaking

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The study was carried out to investigate the effect of supercritical carbon dioxide ($SCCO_2$) on the properties of spelt flour and its staling during storage. Interestingly, enzymes in flour have a significant function since they have a great influence on the processes in flour during storage and in the baking process as they control the baking process in the form of allowing the use of different baking processes, reducing process time, slowing-down staling, compensating for flour variability and substituting chemical additives [1]. Thus, in this work, spelt flour was treated with $SCCO_2$, which has proven appropriate in the past as it offers environmental advantages over chemical solvents and provides enhanced separation and chemical selectivity. The activity of specific enzymes peroxidase, polyphenol oxidase and α-amylase were determined after extraction of proteins and enzymes from flour.
Moreover, the influence of $SCCO_2$ on enzyme activity after exposure was determined with the performed bread baking test. Also, the chemical properties of the flour composition were determined, whereby we found out that the moisture and fat content of the spelt flour decreased after exposure to $SCCO_2$. This further affected the appearance of the baked spelt bread (Figure 1).
Polyphenol oxidase and peroxidase activities were decreased by up to 51% and 74%, respectively, as the time and pressure $SCCO_2$ increased up to 24 hours and 300 bar. However, the activity of α-amylase increased to 114% at the same conditions of $SCCO_2$ exposure. This finding is valuable as α-amylase enhances the fermentation process, improves bread volume and improves crumb texture [2].

The findings showed that $SCCO_2$ affects the activity of individual enzymes in spelt flour without changing the quality of the flour. At the same time, under different exposure conditions, the enzyme activity in spelt flour can be regulated, which is a good starting point for further research to improve flour quality.

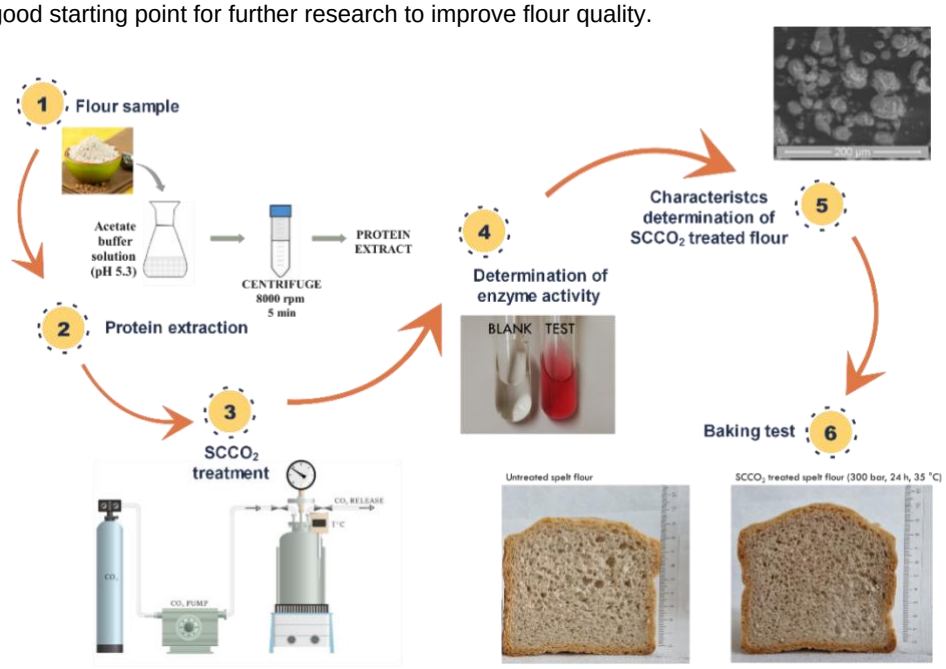

Fig.1. Experimental setup of the research.

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How stable are anthocyanins? A study with elderberry juice

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Anthocyanins are natural compounds that belong to a sub-group of flavanoids characterized by a C_6-C_3-C_6-skeleton and are largely distributed in plants being responsible for the red, blue, or purple color of many fruits, vegetables, and flowers. In addition to their color attributes, anthocyanins are known to present high antioxidant activity and have been associated to several health-promoting properties [1]. Berries as well as their derivative products present high levels of anthocyanins. Many factors such as pH, light, temperature, oxygen, and structure can influence the color and stability of these natural pigments [2]. Many anthocyanin-containing foods are thermally processed before consumption to inhibit microbial growth or inactivate native enzymes in order to ensure the safety and acceptable shelf-life products. The heating can markedly impact on anthocyanin content and color of the final product [3,4]. Anthocyanins present in fruit- and vegetable-based food products can also be degraded to a great extent during long-term storage, especially at room temperature with losses that are usually accompanied by an increase in polymeric pigments [5].

Elderberry (*Sambucus nigra* L.) is one of the richest sources of anthocyanins, almost exclusively cyanidin glycosides, from which cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside have been identified as the major compounds [6]. Due to its limited seasonal availability and for a wider usage, elderberries are often processed to juice concentrates, to be used as food colorants, and in pharmaceutical and nutraceutical applications [7].

This work intends to evaluate the effect of storage on the stability of anthocyanins present in elderberry juice concentrate. The profile and content of anthocyanins in the elderberry juice was accompanied over seven months of storage at 5 ºC and at room temperature. The total monomeric anthocyanins were measured using the pH differential method [8], the individual anthocyanins were monitored by HPLC-DAD and the percent polymeric color was determined according to the method described by Giusti and Wrolstad [8].

The results showed that the storage at room temperature resulted in a strong reduction of anthocyanins content accompanied by an increase in percent polymeric color values, suggesting a possible involvement of anthocyanins in polymerization reactions. The impact of storage was less pronounced at 5 ºC, showing that the refrigerated temperature is important to minimize anthocyanin degradation and maintain the color attributes of the elderberry juice concentrate. Some considerations about potential mechanisms responsible for anthocyanins losses are discussed.

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Extraction and quantification of tropomyosin in selected samples of shellfish

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Food allergies affect up to 10% of the general population and represent an important health problem in the field of food safety in industrialized countries. Hence, developing reliable, specific, and sensitive methods for detecting and quantifying allergens in food products is of high importance. Shellfish have been recognized as one of the eight most common sources of allergens, with tropomyosin (TPM) being considered a major heat-stable allergen, having a highly conserved amino acid sequence among different shellfish species. Allergenicity of TPM may change during food processing, such as cooking. The objective of this study was to develop an enzyme-linked immunosorbent assay (ELISA) for the detection and quantification of shellfish tropomyosin in food samples.

Two different extraction buffers - phosphate-buffered saline (PBS) and PBS containing 1 M sodium-chloride (PBSN), were compared for their ability to recover proteins from pre-cooked frozen Mediterranean mussel (*Mytilus galloprovincialis*) and fresh frozen razor mud shrimp (*Solenocera melantho*). The samples were additionally cooked according to the manufacturer's instruction and analyzed as such. The protein content was quantified using Bradford protein assay, and the protein components of soluble extracts were profiled using SDS-PAGE. TPM presence was confirmed using Western blot. Sandwich ELISA was developed using a monoclonal anti-TPM antibody as a capture antibody, while polyclonal anti-TPM antibody served as a detection antibody and was coupled to the biotinylated secondary antibody and streptavidin-alkaline phosphatase conjugate. Tropomyosin was quantified using highly purified natural shrimp tropomyosin as standard.

The profile of extracted proteins was changed when using PBSN instead of PBS. A higher concentration of proteins was recovered from raw shrimp using PBSN instead of PBS. At the same time, the type of extraction buffer did not affect protein recovery either from heated shrimp or pre-cooked/heated mussels. Significantly fewer proteins were extracted from cooked shrimp sample compared to the raw shrimp, while cooking showed no effect on the extraction of proteins from mussels. Cooking did not affect TPM recognition in Western blot. TPM was quantified in shrimp samples in sandwich ELISA. However, developed ELISA could not quantify mussel's TPM, indicating that this approach may distinguish mussels and shrimp TPM.

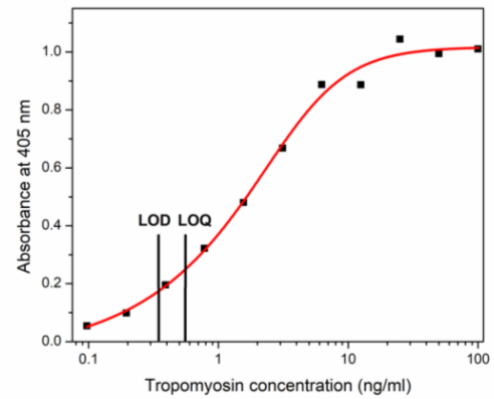

Fig. 1. Quantification of tropomyosin using sandwich ELISA.

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Herbal teas with *Cannabis*: Assessment of potential consumers exposure to THC

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Many phytocannabinoids, secondary bioactive metabolites of *Cannabis* plants, have various positive effects on the human body, such as antioxidant, analgesic or neuroprotective [1]. However, these compounds include also the psychotropic Δ^9-tetrahydrocannabinol (Δ^9-THC), which is considered an addictive substance and therefore its presence in food is undesirable [2,3]. Currently, there is a wide range of *Cannabis* products available on the market, including the increasingly popular '*Cannabis* teas' which are consumed as aqueous infusions. For the regulatory purposes, the possible dietary intake of phytocannabinoids (especially Δ^9-THC) occurring in '*Cannabis* tea' infusions is usually calculated based on their content in the dry material used for infusion preparation. However, this approach is not entirely appropriate, because of their low polarity, the entire amount of phytocannabinoids present in the dry material will not be transferred into the beverage. As the real transfer rate of phytocannabinoids is not yet sufficiently described by scientific studies to allow different approach in the regulatory assessment, the aim of this work is to study and describe this phenomenon in detail [4,5].
In order to study the transfer of phytocannabinoids from the dry material to the aqueous infusion, various preparation conditions were first tested on a model '*Cannabis* tea' sample. Specifically, the following parameters were modified: (i) time and temperature of leaching, (ii) ratio of herb used to water volume, and (iii) type of filtration. Both the dry herbal material and the infusions were analyzed by ultra-performance liquid chromatography coupled with high resolution tandem mass spectrometry (U-HPLC-HRMS/MS) to determine the content of 17 phytocannabinoids. Subsequently, aqueous infusions of a set of various commercially available '*Cannabis* teas' were prepared according to a uniform procedure (1 g of dry material / 200 ml boiling water / 7 min leaching) and analysed to evaluate the effect of different input material on the phytocannabinoids transfer rate. Based on the obtained data, the transfer of phytocannabinoids to the infusions reached units up to tens of percent of their contents in the dried material, with a higher extraction yield for phytocannabinoid acids compared to their decarboxylated neutral forms. To evaluate the real possible intake of psychotropic Δ^9-THC, its content in the prepared infusions was compared with the acute reference dose (ARfD = 1 µg Δ^9-THC/kg b.w.) set by the European Food Safety Authority (EFSA) and additionally also with the German recommended limit for the maximum content of total THC (sum of free Δ^9-THC and Δ^9-THC bound in Δ^9-THCA) in non-alcoholic beverages (5 µg/kg) [6,7].

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Effect of pH on the kinetics of the reaction of gallic acid with methylglyoxal

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In the present study, we investigated the trapping of methylglyoxal (MGO) by gallic acid (GA), a known food grade antioxidant. Methylglyoxal (MGO) is a highly reactive α-dicarbonyl compound that may adversely impact food quality and human health by modifying proteins [1]. The kinetics of the reaction of gallic acid with MGO was studied in the pH 6.5 – 8.5 range at 37 °C by UV–Vis spectroscopy. Our results indicated that the rate of the reaction increases with increasing pH. The reaction rate at pH 8.5 (k_{260nm} =3 x 10^{-3} M^{-1} s^{-1}) was thirty times higher than that at pH 6.5 (k_{260nm} =1 x 10^{-4} M^{-1} s^{-1}). High performance liquid chromatography enabled us to identify the formation of different products at pH 6.5 and 7 compared to pH 8 and 8.5.

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Bio-based pH indicator films for intelligent food packaging applications

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The development of food packaging materials and systems is increasingly attracting research interest as a response to the consumers demand for safe, functional, and convenient packaging and as a quest for solutions to the environmental problems caused by conventional plastic food packaging. This led to more-advanced types of packaging: active and intelligent systems produced from renewable biodegradable materials [1].
Intelligent food packaging systems are designed to monitor and inform on the condition of the food and/or the environment surrounding the food during transport and storage. An indicator incorporated into the matrix of the packaging materials responds to different stimuli usually through a visual change. Color based pH indicators are very useful in food packaging materials because food spoilage is frequently accompanied by pH changes. A pH indicator is generally based on a pH-sensitive dye incorporated into a solid support.
Among natural dyes, which have the benefit of being safe and ecofriendly, anthocyanins are the most researched class. Their antioxidant and antimicrobial properties make them suitable for active food packaging, while their ability to change color upon pH modification enable them to be used as sensing dyes for intelligent food packaging materials [2]. Despite their enormous potential benefits anthocyanins are highly reactive molecules, sensitive to degradation by temperature, light, oxygen, pH which affects their stability and coloring properties [3]. Bio-inspired synthetic dyes are developed to enhance desirable and specific properties and to overcome the disadvantages of natural anthocyanins [4].
In this work a new bio-inspired pH sensitive dye is reported. The synthetic anthocyanidin was characterized by UV-Vis, FT-IR,1D and 2D NMR spectroscopic analysis. The dye exhibits pH dependent photochromic properties which enabled its use as pH sensitive dye for the development of biodegradable intelligent systems based on chitosan blends. The obtained polymeric films were characterized by FT-IR, UV-Vis spectroscopy and their thermal properties were assesed by thermal analysis techniques. Their sensitivity to pH variations was evaluated and visible color changes were observed.

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Effect of sodium nitrite dose on lipid oxidation and colour changes during the shelf-life of refrigerated pork liver pâtés packed in MAP

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Pork liver pâté is highly susceptible to oxidation due to its chemical composition (fat, liver) and process (mincing, thermal treatment). In processed meats, nitrite plays a significant role in the oxidative stability, the microbiological safety and the development of typical colour and flavour, but it may induce neo-formed compounds formation. Removing nitrite remains complex and unsuccessful for some processed meats. This study aims to determine the effect of low sodium nitrite doses ($NaNO_2$) on lipid oxidation and colour during the shelf-life (T) of liver pâtés.
Three experimental pâtés were manufactured in an industrial pilot plant: N80 (conventional dose of $NaNO_2$: 80 mg/kg), N40 (reduced dose of $NaNO_2$: 40 mg/kg) and N0 (control without $NaNO_2$). An identical formula was used with following ingredients: pork fat, pork liver, milk, eggs, salt, spices, herbs, and sodium ascorbate (500 mg/kg). Pâtés were cooked until an internal temperature of 72°C, after cooling, sliced and conditioned under protective atmosphere (70%N_2+30%CO_2), then stored 10 days at 4°C and 20 days at 8°C. Total and nitrosylated heme iron were analysed according to Hornsey[1] method. Lipid oxidation and colour changes were evaluated during storage at day 5 and day 31 post packaging. Lipid oxidation was analysed according to the TBARS[2] method. Colour was measured on the surface immediately after opening the package by CIELAB parameters using a spectrocolorimeter Minolta CM600 (L*: lightness; a*: redness; b*: yellowness). Saturation index and hue angle (h^o: hue = arctang b/a) values were calculated.
Pâtés contained similar amounts of protein (10.5 – 10.8 g/100g), moisture (46.2 – 46.5 g/100g) and fat (39.5 – 40.2 g/100g). $NaNO_2$ dose significantly reduced lipid oxidation with a higher level in N0 compared to N80 and a slight degradation in N40. Lipid degradation during storage was only observed in N0. $NaNO_2$ had a protective effect as previously observed in processed meat[3]. Liver pâtés exhibited different colour characteristics: N80 and N40 were redder and less yellow than N0. Therefore, N0 had higher h° values than N40 and N80, corresponding to the oxidative degradation of lipids. A redness difference between N0 and products with nitrite was noticeable since differences were greater than 2. Furthermore, a*and b* values significantly decreased during storage. A slight effect of $NaNO_2$ dose was observed on L* but this evolution did not follow a defined trend. h° did not varied during storage as described by Estevez et al (2004)[4]. $NaNO_2$ did not affect total heme iron content. Nitrosylated heme increased with level of $NaNO_2$. This effect is known to limit lipid oxidation and improve red colour.
In conclusion, pork liver pâté without $NaNO_2$ is less attractive due to the oxidation that impacts flavour and colour perceived as grey[5] (a* values). 40 mg/kg $NaNO_2$ should be a good compromise in association with polyphenols to improve colour and flavour, if this dose is sufficient to protect against microbiological risks.

Table 1. Effect of $NaNO_2$ on lipid oxidation, colour changes and heme iron composition.

$NaNO_2$ (mg/kg)	Day	N80	N40	N0	*p* $NaNO_2$	*p* T	$NaNO_2$ x T
TBARS (mg/kg)	5	0,71 ± 0.0c	0,88 ± 0.2bc	1.02 ± 0.2b	0.000	0.105	0.111
	31	0,72 ± 0.0c	0,89 ± 0.1bc	1.20 ± 0.1a			
L*	5	64.8 ± 0.4b	65.1 ± 0.6ab	66.1 ± 0.5a	0.274	0.108	0.009
	31	65.8 ± 0.5ab	65.8 ± 0.2ab	65.4 ± 0.4ab			
a*	5	7.2 ± 0.1ab	7.6 ± 0.5a	3.7 ± 0.2d	0.000	0.000	0.147
	31	6.7 ± 0.5bc	6.3 ± 0.3c	2.8 ± 0.3e			
b*	5	18.0 ± 0.1b	17.9 ± 0.6b	19.4 ± 0.9a	0.000	0.000	0.599
	31	15.3 ± 0.3c	15.3 ± 0.3c	17.3 ±0.7b			
h°	5	68.3 ± 0.3b	67.1 ± 0.7b	79.3 ± 0.7a	0.000	0.977	0.011
	31	66.4 ± 1.4b	67.5 ± 0.8b	80.8 ± 0.4a			
Total heme iron(mg/kg)		128.0 ± 8.7a	129.4 ± 27.6a	101.6 ± 14.3a	0.201		
Nitrosylated heme iron (mg/kg)		91.4 ± 7.8a	76.4 ± 3.9b	7.8 ± 4.6c	0.000		

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Miniaturized, green salting-out liquid-liquid microextraction coupled with GC-MS used to evaluate biogenic amines in wine samples

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It is well known, that wine is a source of health-promoting ingredients. However, on the other hand, it can be a source of biogenic amines, which have been shown to have negative effects on human health [1,2]. Biogenic amines are a naturally occurring, organic compounds which are formed as a result of the amino acid decarboxylation process. Their presence can stand as an indicator of microbial contamination. Due to this fact, the evaluation of the presence and the amount of particular biogenic amines in wine can be used to assess the quality of a given beverage [3].
In the available literature, one can find many papers describing the determination of selected compounds in wine samples. Nevertheless, the described processes are based mainly on the use of liquid chromatography. Gas chromatography is employed far less frequently for this purpose. The goal of the study was to develop a method for the biogenic amines determination in wine samples based on salting-out liquid-liquid microextraction (SALLME) coupled with gas chromatography mass spectrometry. The Box-Behnken design was used to optimize three independent factors, these were: amount of NaCl, amount of EtAc and vortexing time. The following validation parameters were obtained: good linearity in the concentration ranges of 0.05 – 1 mg/L and 1 – 10 mg/L, with a correlation coefficient above 0.99; detection limit of 1.5 to 8.1 µg/L and accuracy of 2.3 and 10.4 %RSD for 0.25 mg/L and 2.6 – 11.3 %RSD for 2.5 mg/L.
An additional advantage of the developed method is the simultaneous extraction and derivatization process, which reduces the risk of loss and contamination of the analytes. Moreover, it reduces the amount of waste generated as well as the time needed for the sample preparation. Aforementioned advantages and the use of an alternative extraction solvent (EtAc) are in line with the principles of green analytical chemistry.
An added value of a given research was the application of the biogenic amines index (BAI) for the selected wine quality assessment. Examined wine samples were of high quality. Only two red wine samples exceeded the BAI value of 2.4 mg/L, while the rest were below 2 mg/L. It indicates that given wine can be consumed without the risk of food poisoning from biogenic amines.

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The use of salting-out assisted liquid-liquid microextraction and gas chromatography-mass spectrometry for the determination of biogenic amines in fruit juices

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Biogenic amines (BAs) are low molecular weight organic compounds that are formed in the plant, animal and bacterial cells as a result of metabolic processes. BAs are commonly found in fresh and processed foods, such as fruit, vegetables, wine, beer, cheese, or meat. Consumption of large amounts of biogenic amines can cause a number of side effects, making it important to control their content in food products. Moreover, the concentration of BAs can be a useful indicator of the safety and quality of food. The aim of the study was to develop a simple, rapid, and green procedure for the extraction and derivatization of 14 biogenic amines using salting-out assisted liquid-liquid extraction coupled to gas chromatography and mass spectrometry detection (SALLME-GC-MS). Berry juices not from concentrate (NFC) were tested. Food samples have a complex matrix, so determining substances at trace levels is a challenge. In this research, response surface methodology (RSM) and Box-Behnken Design (BBD) were used to improve extraction and derivatization efficiency and to optimize various experimental conditions. The influence of three independent variables was investigated, namely the sample volume, the addition of NaOH solution, and the addition of the derivatization agent, i.e. ethyl chloroformate. Very good validation parameters were obtained for the developed method. Linearity with determination coefficients greater than 0.99 was obtained for all amines. The detection limits ranged from 1.5 to 8.1 µg/L and the recovery values ranged from 84 to 106%, which proves the high accuracy of the method. SALLME combined with a simultaneous derivatization process is a simple technique that uses small amounts of reagents and solvents for extraction which reduces the negative environmental impact of the sample preparation step. Additionally, the assessment of the method's greenness was performed using two analytical tools: Green Analytical Procedure Index (GAPI) and Analytical Greenness Calculator (AGREE), in both cases, the developed method achieved excellent results compared to other methods for the determination of Bas in juice samples. In the case of berry juice samples, it was found that tyramine, tryptamine, putrescine, and cadaverine were the most abundant amines in the tested juices. However, in each juice, the total amount of biogenic amines was relatively low (<1 mg/L). The obtained results confirmed the usefulness of the SALLME-GC-MS method for the determination of trace amounts of biogenic amines in fruit juice samples. Additionally, it is worth mentioning that the developed method can be used to assess the quality and security of beverages.

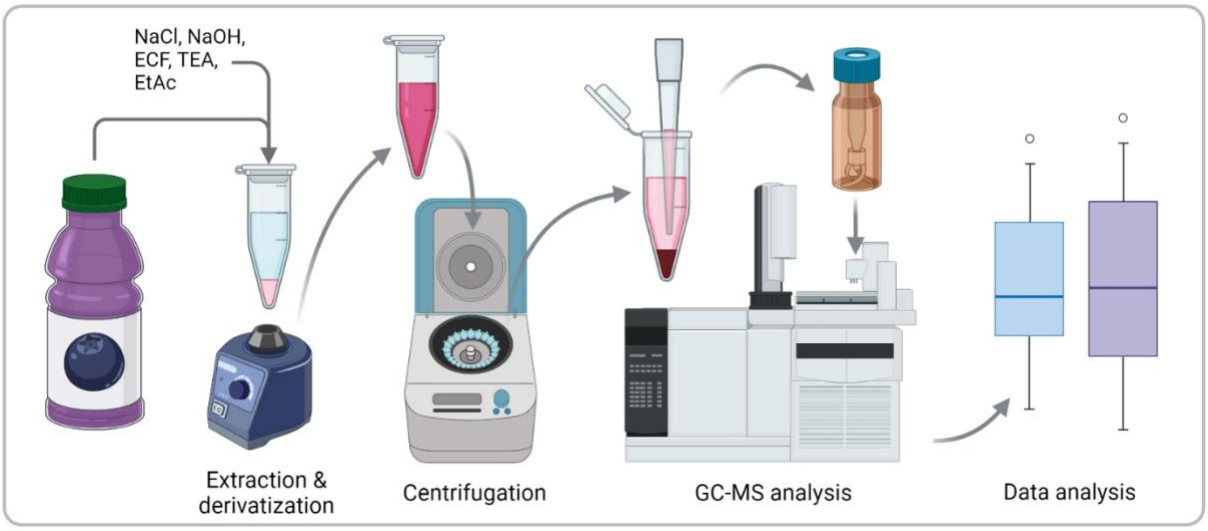

Fig.1. Graphical abstract.

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Determination of organic pollutants in bivalve samples from South Korean markets

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Organic pollutants (OPs) include a variety of substances different chemical structure, which are used as pesticides, dyes, solvents, pharmaceuticals, industrial chemicals, flame-retardants, and heat exchange fluids. Among them, the specific group of compounds are persistent organic pollutants (POPs) like organochlorine pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) and others [1]. The majority of the POPs are halogenated organic compounds which show high stability in the environment where they stay for decades. Because of lipophilic properties, they can bioaccumulate in the fatty tissues of living organisms where they can stay for years. Beside this, they can be transported by wind and water, showing toxic effects on people and wildlife far from the original source. Due to the process of biomagnification, POPs are concentrated in the food chain, so that the organisms at the top of the chain are most exposed to POPs [2]. The most common path of human exposure to POPs is by the intake of food. Bivalves significantly contribute to the seafood consumption in South Korea. Since bivalves are filter feeders and sessile animals, they have tendency to accumulate OPs from the environment. Most of the bivalves on the Korean consumer market are from farms located in the seas around the Korean Peninsula. Some of these farms are located in the proximity some industrial complexes and municipal cities which are the main source of OPs.

The main goal of this study was the determination of pesticides, PCBs and PAHs in bivalve species commonly consumed in Korea, which have different geographical origin. Forty-eight samples of five bivalve species: *Anadara broughtonii* (n=7), *Ruditapes philippinarum* (n=15), *Tegillarca granosa* (n=12), *Mizuhopecten yessoensis* (n=6) and *Argopecten irradians* (n=8) were purchased from different fish markets. Prior to the analysis, the samples were dried by freeze-drying and then homogenized using a mortar and pestle. The obtained powder was further specifically prepared for each type of analysis. For the determination of pesticides, PCBs and PAHs LC-MSD and/or LC-MS/MS were used. The contents of 131 pesticide residues were determined using LC-MS/MS after acetonitrile extraction and purification of the extract using dispersion SPEQuEChERS kit. In all samples the obtained contents of all pesticides were lower than the range of quantification (0.01-0.20 mg/kg) for all specific pesticide. The content of total PCBs, which is given as a sum of content of PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180 was lower than the maximum tolerable limits in all samples. The contents of 16 different PAHs were determined of which six belong to carcinogens or potential carcinogens. Among them, 10 different PAHs were found in more than 5 samples in concentration higher than 0.5 µg/kg fat. Benz[a]anthracene and chrysene as the carcinogens were present in 33 and 26 samples, respectively, but in concentrations safe for human consumptions. Naphthalene was found in all samples, while four PAHs were not found in any sample. Among PAHs found in the samples, the concentrations of naphthalene were the highest (between 21.7 and 190 µg/kg fat). Principal component (PC) analysis was applied to show the differences between species. Two-dimensional PCs did not show a clear separation of most species, except between *Anadara b*. and *Argopecten i.* when each sample (n=48) was initially described by 16 or 8 elements. It was found that PAH 14 has the most negative, while PAH4, PAH11 and PAH9 have the most positive contribution in discriminations between two species.

In all selected samples of bivalve species purchased from the online fish markets or fish markets in Incheon, the content of pesticides, PCBs and PAHs were below or in the safe range for human consumption. The obtained results show that all bivalve (from domestic production or from import) intended for sale on the fish markets pass the quality control by Korean institutions.

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Allergenicity assessment of Cor a 8 from raw and roasted hazelnut upon oral-gastric digestion phase of INFOGEST protocol

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Cor a 8 is a relevant allergen that can cause severe allergic reactions. It is a 115 amino acid protein with a molecular mass of 9 kDa and is a member of the non-specific lipida transfer protein family. This allergen is resistant to high temperatures, pH changes, gastric and intestinal enzymes. The main route of exposure is through ingestion. In order to examine its resistance to digestion, we have applied a popular 1.0 INFOGEST protocol [1], specialized for the complete food, which *in vitro* mimics physiologically relevant conditions of oral-gastric-intestinal digestion. The aim of this study was to compare Cor a 8 resistance to gastric digestion, from both, raw and roasted hazelnuts, before and upon pepsin (gastric) digestion. Stability of the Cor a 8 protein was investigated by simulation of oral and gastric digestion phases, performed with ground raw and roasted hazelnut kernels. Hazelnut proteins were extracted from the digestion mixture and analyzed by 1D and 2D SDS-PAGE, while raw and roasted Cor a 8 western blots were probed with specific anti-Cor a 8 antibodies in 1D and 2D immunoblots. The electrophoretic patterns of the raw and roasted extracts were similar. 1D SDS PAGE profiles demonstrated high stability of Cor a 8 against enzymatic treatments. Control samples of Cor a 8 from raw and roasted hazelnut extracts migrated as a single band at around 12 kDa in 1D immunoblot. However, in case of roasted hazelnut, the protein showed a slightly lower capacity to bind specific anti-Cor a 8 antibody, as compared to raw hazelnut extract. In 2D immunoblot, with higher resolution, specific antibody binding was decting a significant and noticeable smear in the basic region indicating a range of different protein variants. This was more pronounced detectable in the case of roasted sample upon digestion, pointing to a mix of variants in this allergen batch. It has been suggested that the allergenicity of the Cor a 8 is almost insensitive to temperature. The allergen is stable even after digestion and roasting processes up to 140˚C. We hypothesize that a lipid-rich food matrix delays extraction of proteins, thereby delaying their gastrointestinal digestion, which may affect allergen sensitizing capacity and clinical symptoms.

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Investigating the Influence of Brewing Parameters on Coffee Furan & Alkyl-Furan Exposure

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Formed during the thermal processing of food, furan is considered possibly carcinogenic by the International Agency of Research on Cancer [1]. It is now believed that all furans are metabolized through a similar mechanism, contributing to the cumulative furan exposure [2]. While coffee is responsible for 85% of an adult's dietary exposure to furan, this exposure varies significantly based on the brew method [3]. The current study investigated the presence of alkyl-furans; 2-methyl-, 3-methyl-, 2-ethyl-, 2,5-dimethyl- and 2-pentyl-furan, and the influence of grind size, roast degree and brew methods on their levels within the brew. Increasing grind size as well as roast degree were found to increase the levels of all alkyl-furans, with the exception of 2-pentylfuran. Upon extraction furan and alkyl-furans levels were found to be greatest when immersive brew methods were used. Cold brew demonstrating the greatest cumulative furan levels, which was associated with the low extraction temperatures.

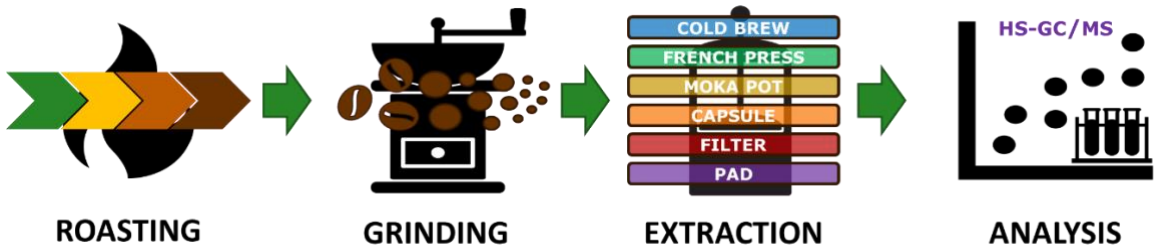

Fig.1. Overview of experimental method.

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Oxidative stability and protein degradation in Vietnamese pig meat during storage

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The decomposition processes of fats and proteins develop in the meat during the maturation period. Oxidation of fats and degradation of proteins during the maturation period takes place in the presence of oxygen, light and by enzymes. Unsaturated and saturated fatty acids contained in meat can undergo oxidation during storage and processing. Oxidation of fats also takes place during cold storage to form malondialdehyde (MDA), which can be considered an indicator of the freshness of the stored product. Protein degradation (proteolysis) of animal origin is caused by native and bacterial enzymes. Proteins are cleaved into their low molecular weight moieties and subsequently into free amino acids, biogenic amines (BA) and total volatile basic nitrogen (TVB-N). The aim of the work was to evaluate the degradation changes during storage in the loin (Musculus longissimus dorsi) and thigh (Musculus semitendinosus) of the meat of Vietnamese pigs. The measurement was performed on days 1 and 7 post mortem. The oxidative stability of the meat was expressed by the content of malondialdehyde (MDA) and total volatile basic nitrogen (TVB-N) v. The experiment included 10 pigs from the same breed and were fed until the age of 120 days. Slaughter pigs were killed at home by the breeder from which they came. Subsequently, the carcasses were cooled within 24 hours to a core temperature of 7 °C and cut into certain cutting parts in accordance with the Decree of the Ministry of Justice of the Slovak Republic no. 423/2012 Coll. After slaughter, loin and thigh samples were taken, stored at 4 °C and further analyzed. The meat was stored at 4°C throughout the experiment. Oxidative stability was analyzed by determining the malondialdehyde content using the TBA (thiobarbiturate) method [1]. TVB-N was analyzed by steam distillation followed by titration of the samples using a Kjeldahl distillation unit [2]. The results obtained from the work were then statistically processed using the program SAS 9.3. Tables were created based on the evaluated statistical data. The most significant difference in MDA content on days 1 and 8 was in the MDA content in the thigh ($P \leq 0.05$). Where on day 1 the values of 0.215±0.028 mg.kg^{-1} were recorded and on day 8 0.311±0.049 mg.kg^{-1}. The measured value of MDA content in the box on the 1st day of storage was 0.265±0.055 mg.kg^{-1} on the 8th day it was 0.300±0.056 mg.kg^{-1}. The content of TVB-N after 8 days of storage was in the loin (13,673±0,805 mg.100g^{-1}) and in the thigh (16,753±1,419 mg.100g^{-1}). A statistically significant increase in MDA content during storage was confirmed only in the thigh. In the loin, the increase in malondialdehyde during storage was inconclusive. In order to maintain the quality of the meat, the degradation of proteins and the oxidation of lipids should be minimized by proper processing of the carcase, storage and processing of the meat.

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Impact of heat treatment of non-wheat flour on acrylamide presence

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Application of wet or dry heat treatment of flours by steaming, extrusion or roasting can improve technological and sensory properties of flours by modification of properties of proteins and starch and removal of bitter taste. However, in presented collaborative study significant formation of processing contaminants, mainly acrylamide, was detected especially in roasted millet, sorghum, barley, oat, rye, and triticale flours, respectively [1]. Acrylamide content in dry thermally treated flours (DTF) was in the range from 160 ± 13 μg/kg up to 1951 ± 4 μg/kg with the lowest presence in sorghum and millet in comparison to barley, triticale, oat and rye. Acrylamide content in hydrothermally treated flours (HTF) using steam extrusion was detected only up to concentration of 28 ± 9 μg/kg in barley, oat and rye. Moreover, dry heat treatment besides acrylamide generated also HMF in the level from 2.0 ± 0.1 mg/kg in sorghum up to 44.3 ± 1.6 mg/kg in oat, that could also contribute to next acrylamide formation during subsequent baking of cereal products. Consideration of safety aspects of heat treatment application of flours is necessary to take into consideration in technological processes of bakery products mainly from oat and rye.

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Investigation on heat-induced chemical indexes in traditional and reformulated biscuits

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Although the traditional-style biscuit made of wheat flour, sugars and fats has a strong presence on the market, the updated recipes mix different grains or include emerging cereals, pseudo-cereals or other ingredients in order to provide consumers with low-sugar, gluten-free alternatives, high fibre or mineral enriched products, among others [1]. Baking is a complex process inducing physical, chemical and biochemical changes in the dough matrix leading to desirable tastes and flavours as well as browning [2]. Starch and sucrose hydrolyse to form reducing sugars, which together with maltose promote non-enzymatic browning reactions, including caramelisation and Maillard reaction [3]. Different heat-induced markers have been proposed to assess the extent of the thermal treatment applied to foods, with technological, nutritional and safety implications [4]. Fructosyl-lysine, chemically measured as furosine after acid hydrolysis of the early Amadori product, is considered a heat-induced indicator of the nutritional quality of the protein, since reflects the impairment of lysine residues of the protein with the extent of the thermal treatment. Furfurals comprise a large family of chemicals formed during thermal treatment of foods with technological and sensorial implications due to many of them are odorants and contribute to the colour in toasted, roasted and baked foods. Particularly, hydroxymethylfurfural (HMF) and furfural, intermediate products of the Maillard reaction and the caramelisation of sugars, have been extensively applied as heat-induced chemical markers to identify the excessive thermal treatments to foods [5]. Although the toxicological relevance of HMF and furfural in humans is still under debate, animal studies have pointed out that HMF can be potentially carcinogenic [6] whereas furfural has shown to induce toxicological effects with diverse magnitude both by inhalation and oral administration [7]. Therefore, HMF and furfural are considered chemical process contaminants that deserve further attention and their content in foods should be reduced as much as possible. In the present study, furosine, HMF and furfural were assessed in biscuits marketed in Spain, comparing traditional biscuit formulations with reformulated ones. They were also classified according to the Nutri-Score scheme considering the factors energy density (kcal/100 g), saturated fat (g/100 g), sugars (g/100 g), sodium (mg/100 g), protein (g/100 g), and dietary fibre (g/100 g). The final purpose was to evaluate if reformulated biscuits recipes are a healthier option than traditional ones in nutritional but also in toxicological terms. Eighty commercial biscuits from 30 different producers were purchased from Spanish supermarkets in January-February 2019. Samples included hard sweet biscuits, short doughs biscuits and cookies. Chocolate biscuits, biscuits filled or coated with dried fruits, nuts, chocolate or jam, and those intended for the infant population were intentionally excluded to avoid bias. Samples were mixed and thinly grinded, placed in a polyethylene container, sealed under vacuum and stored at 4°C until analyses. Nutritional composition was obtained from the manufacturer declarations recorded in the package labelling, except the protein content, which was determined in the laboratory using an automated nitrogen analyser. Furosine was determined by Ion-pairing HPLC and furfurals were measured by HPLC DAD. Average furosine, HMF and furfural contents were 731 mg/100 g protein, 7.32 and 0.64 mg/kg, respectively. Furosine, HMF, and furfural content in traditional compared to reformulated biscuits were 14, 37, and 37 % lower, respectively. The predominant source of protein and the sugar content revealed as key factors influencing the formation of the heat-induced markers studied. Particularly, the replacement of reducing sugars with polyols reduced furosine and furanic compounds. Levels of these compounds among the Nutri-Score groups point to this index as a good tool to help consumers to make healthier choices within this food category. Findings underline the importance of conducting risk/benefit analysis when introducing reformulated recipes in order to reach not only improved nutritional profiles but also controlling toxicological aspects.

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Consumers' decisions on the selection of the end-point in a controlled potato frying process influence the exposure to acrylamide.

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Acrylamide is a chemical process contaminant classified by the International Agency for Research on Cancer as a probable carcinogen for humans [1]. In 2015, the European Food Safety Agency (EFSA) identified acrylamide in food as a public health concern due to its relation with the appearance of different types of cancer [2], and in 2017, the European Regulation 2158/2017 established mitigation measures and benchmark levels for the reduction of the presence of acrylamide in the main sources of exposure to this contaminant, among which are French fries [3]. In French fries, acrylamide is generated due to both the high content of precursors (reducing sugars and asparagine) in the fresh tuber and the intensity of the thermal treatment applied during frying [4] Specifically, European Regulation established a benchmark level of 500 µg/kg for French fries [3]. According to EFSA, preferences in home-cooking may increase the presence of the contaminant by up to 80%, depending on domestic habits and the conditions for potato frying [2]. Although commercial fryers provide user manuals containing tips for proper and healthy frying of potatoes, it is ultimately the consumer who will choose the pre-frying operations (for example washing or soaking), temperature conditions and frying end-point. Thus, the different decisions made by consumers regarding French fry preparation will affect acrylamide formation. Considering all of these consumer-dependent factors, the aim of the present study was to investigate the influence of consumer practices on acrylamide formation during the preparation of French fries, focusing specifically on the selection of the end-point of frying.
A cohort of one hundred volunteers participated in an observational assay under controlled frying conditions in the experimental kitchen at the Institute of Food Science, Technology and Nutrition (ICTAN-CSIC, Madrid, Spain) in May 2019. Members of the research team controlled the main variables pertaining to pre-frying (raw material, peeling, cutting), frying (temperature, potato:oil mass ratio) and post-frying (draining oil excess, cooling) stages of French fry preparation, except consumer decisions regarding the frying end-point. Tubers were previously selected and were peeled and cut into strips. A fixed amount (~45 g) was placed in a domestic deep-fryer with sunflower oil. The initial temperature of the frying oil was set at 175ºC. Once the basket with the potato strips was immersed in the frying oil, volunteers were free to check the progression of frying and decide the end-point according to their preferences. Total frying time (expressed as seconds) was recorded by a timer. Samples were drained to remove excess of oil, weighted and cooled, and stored for further analyses. The acrylamide content was analysed by Liquid Chromatography–Electrospray Ionisation–Tandem Mass Spectrometry (LC-IE-MS/MS) and colour was measured using a HunterLab Spectrophotometer colorimeter.
Results revealed that, although the evaluation of participants' frying habits when preparing French fries was restricted to decisions around frying end-point, such decisions had a great influence on the acrylamide content. Volunteers chose to stop frying after between 45 and 168 s, with mean time being 91.9 s and all of them declared that visual colour appearance was the major criteria for stopping frying. Mean acrylamide content in French fries was 508 µg/kg, with 54% exhibiting values lower than the benchmark value. From the results, it could be deduced that, in the range 4–5 min of frying time to get an acceptable final product for consumers, an increase of 8.9 s could represent approximatively an increment in 150 µg/kg in the final product. Weight loss of potatoes during frying, colour of French fries and frying time showed significant relationships with acrylamide content. Thus, there was a significant relationship between acrylamide content and participants' decisions to end the frying process. The present study reinforces the importance of controlling frying time in order to maintain the acrylamide concentration of French fries prepared in a domestic setting below the benchmark level. In addition, the use of colour charts in the domestic setting would be useful for establishing harmonized criteria for assessing colour and would consequence reduce acrylamide exposure.

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Determination of Etoxazole in the Plum by Gas Chromatography-Mass Spectrometry

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Etoxazole is an acaricide (miticide/ovicide) belonging to the diphenyl-oxazoline group of chemicals and is suitable for use in integrated plant protection. Etoxazole is extremely selective. It has a permit to control red fruit spider, nettle mite and yellow vine spider. It is used in the formulation in the form of a concentrated suspension, where the amount of the active substance etoxazole is in a concentration of 110 g/L. Borneo (Agromarket, RS) formulation of etoxazole insecticide is better known for sale in Serbia. Etoxazole inhibits chitin biosynthesis during larval metabolism. It is used for fruits, primarily for apples, pears, apricots, peaches and plums. Analysis of pesticide residues in food and other environmental products have become an important factor in controlling the quality and safety of food and human health.

The objective of this research is to provide information on the variation of etoxazole levels in the plums. A rapid and accurate method based on modified QuEChERS sample preparation was developed for determination of etoxazole in plums by gas chromatography-mass spectrometry (GC/MS). For the extraction, 10g of sample was weighed in a 50 mL PTFE centrifuge tube. Then, 10 mL of acetonitrile was added and the samples were shaken 3 min. Afterwards, 4 g of magnesium sulfate and 1 g of sodium chloride were added and the extract was then centrifuged for 5 min (4000rpm). In the second extraction phase, magnesium sulfate and PSA were used for clean-up. GC/MS analysis was performed using GC Clarus 680 PerkinElmer system equipped with a MS Clarus SQ8T mass spectrometer. The temperature of injector was 220°C, and sample injection was performed in splitless mode, and the injection volume was 1 µL.

Matrix-matched solutions were also prepared by serially diluting the intermediate solution with blank etoxazole-free plum sample extracts to perform matrix-matched calibration. Matrix-compliant solutions were also prepared by serial dilution of intermediates with blind plum sample extracts to perform matrix-compliant calibration at the same concentrations as in the solvent.

The linearity of the analytical response across the studied range of concentrations (0.01 - 0.10 mg/kg) was excellent, obtaining correlation coefficient higher of 0.999521. The average recovery for fortification levels of 0.01 and 0.1 mg/kg were 98.2% and 106.1% respectively. The precision values associated with the analytical method, expressed as RSD values, were less than 20%. Limit of quantification was 0.01 mg/kg. 20 plum samples were examined. Two samples of plums did not contain etoxazole, while the others contained in the amount of 0.044 to 0.061 mg/kg. The obtained value of the examined plums is above the MRL (maximum residue level) which is 0.04 mg/kg from the Commission Regulation (EU) 2018/686 of 4 May 2018 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament. The obtained validation data has confirmed that QuEChERS extraction ensures satisfactory results for determination etoxazole in plums. Such method showed the advantages of simplicity, rapidness, and sensitivity, and could meet the requirements for the determination of others pesticides residues in various fruits.

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Development of novel colorimetric pyranoflavylium-biopolymer hybrid conjugates

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The conjugation of functional molecules into biopolymers such as marine-origin polysaccharides have been extensively used towards the creation of new biomaterials for applications in food and biomedical areas. There is a huge interest in the design and fabrication of nanostructured functional materials with tuneable compositions, precise layered structures and well-defined control of properties and functions, which found many applications in a wide range of research fields including sensing technologies for food packaging industry [1,2]. Flavylium-based dyes, which comprehends a large family of pigments including pyranoanthocyanins, display different colored species in solution because of their pH-dependent chemical network [3]. This feature makes them very suitable for application in food intelligent packaging as pH-sensors to ensure food quality, safety, monitoring or to detect spoilage at early stages [4]. In this work, two 7-deoxy-pyranoflavylium-based dyes were designed and synthesized and further their equilibrium constants were charcaterized by UV-Vis spectroscopy yielding a great color variation compatible with the food spoilage pH range of many perishable foods (pK_a between 6-7). Afterwards, those pigments were successfully grafted onto chitosan (CHT) and alginate via EDC/NHS carbodiimide coupling chemistry with substitution degree about 54% and fully characterized by FTIR, ^{1}H NMR and DLS techniques. Overall, the new biobased conjugates are promising building blocks for development of multilayer thin-films for application as smart labels to integrate packaging materials solutions for food spoilage sensing in real-time.

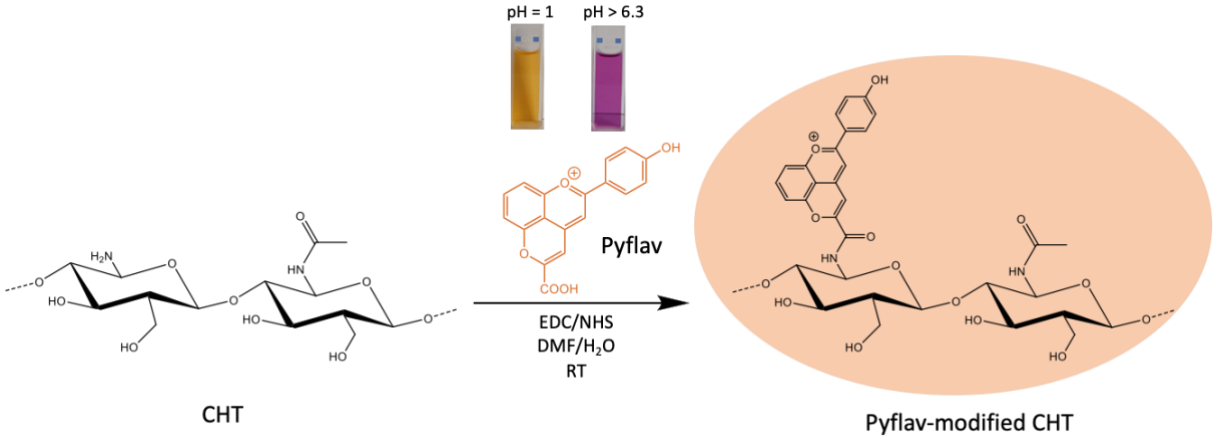

Fig.1. Coupling reaction of pyranoflavylium dye to CHT by EDC/NHS carbodiimide chemistry.

Acknowledgments:
The authors thank Dr. Silvia Maia and Dr. Mariana Andrade for the MS and NMR analysis, respectively. This work was supported by the Associate Laboratory for Sustainable Chemistry, Clean Processes and Technologies LAQV. The latter is financed by national funds from UIDB/50006/2020. This work was also supported by the project PTDC/OCE-ETA/31250/2017 funded by FCT and FEDER. Ana Pires acknowledges the research grant from the project PTDC/OCE-ETA/31250/2017. Luís Cruz acknowledges the FCT research contract (DL 57/2016/CP1334/CT0008). Vânia Gomes and Dina Neves acknowledges the PhD grants from FCT (SFRH/BD/136556/2018 and 2020.065.23.BD, respectively).

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Physico-chemical properties and antioxidant activity of rose (*Rosa damascena* Mill.) petals extracts encapsulated in four hydrocolloids

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A rose water petal extract (*Rosa damascena* Mill.) rich in phenolic compounds was stabilized by using freeze-drying process technology with maltodextrin, inulin, guar gum and sodium alginate, as well as the combination of sodium alginate and inulin as carrier materials. Fourier-transform infrared spectroscopy was performed to show the interactions between hydrocolloids and all components in rose extracts (Fig. 1). All powders demonstrated intermediate cohesiveness and a fair to good flowability according to Carr index and Hausner ratio. Color parameters (L*, a*, b*, hue angle, and chroma) of the *R. damascena* extracts encapsulated with different hydrocolloids by freeze-drying process (Table 1) were shown. The use of inulin and guar gum showed better results for color stabilization in comparison to other hydrocolloids. Moreover, guar gum and inulin stabilized in greater extent the encapsulated anthocyanidins in rose extract (0,64 and 0,56 mg Cya-3 glc/g, respectively). The addition of guar gum was the one that showed higher maintenance of the antioxidant activity of compounds in freeze-dried rose extracts. Guar gum demonstrated the highest values of radical scavenging ability evaluated by the DPPH method (1427 mm Trolox equivalent/g extract) and FRAP method (1227 mM Trolox equivalent/g extract). These results demonstrate the effectiveness of inulin and guar gum in the stabilization of anthocyanidins and higher maintenance of the antioxidant activity freeze-dried petal extract (*Rosa damascena* Mill.). The results from this study reveal the potential application of rose petal extract in the design of new food formula.

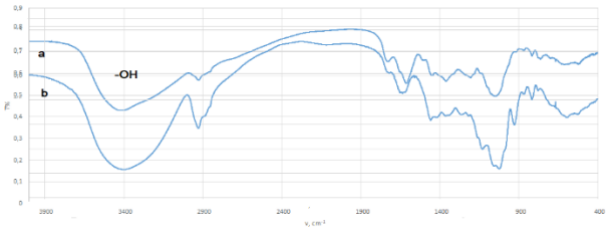

Fig.1. FTIR spectra of freeze-dried rose extracts a) water extract and b) encapsulated with inulin water extract.

Table 1. Color parameters (L*, a*, b*, hue angle, and chroma) of the *R. damascena* extracts encapsulated with different hydrocolloids.

Sample	ΔE	L*	a*	b*	Chroma	Hue
RW	61.07	59.64	13.06	1.69	13.17	7.37
RWI	65.81	64.23	13.77	4.03	14.35	16.31
RWM	75.19	74.00	10.39	8.42	13.37	39.02
RWG	63.32	62.25	11.47	1.42	11.50	7.05
RWA	58.93	48.46	6.78	0.19	6.78	1.61
RWAI	45.03	44.64	5.88	0.19	5.88	1.85

RW – rose extract, RWI – rose extracts with inulin, RWM – rose extracts with maltodextrin, RWG – rose extract with guar gum, RWA – rose extract with calcium-alginate beads, RWA – rose extract with calcium-alginate-inulin beads.

Acknowledgments:
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Halophytes – Future Food? Introducing Alternative Crops for Food Production

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Global warming, scarcity of freshwater resources, reduced availability of agricultural land and increasing urbanisation require a change in food systems. The future food must fulfil various demands: healthy, sustainable, culturally acceptable, affordable and sufficient for all people [1]. Since a plant-based diet is more sustainable than an animal-based diet in terms of land use, water consumption and greenhouse gas emissions, the introduction of alternative vegetables and plant-based products into the future diet is necessary [2]. Sustainable crop production requires among others adaptation to the changing environment, such as reduced freshwater consumption and biodiversity conservation. With 70% of all freshwater used for food production, alternative water uses are urgently requested [3, 4]. Halophytes are saline plants that have great potential to become part of the future food supply, as they can be irrigated with saline water, thus freshwater resources can be conserved and they contribute to increase biodiversity of food. Since most people live in urban areas (76%), vertical indoor cultivation is an emerging field [5, 6]. Our research aims to cultivate halophytes in urban indoor cultivation systems. We evaluated the feasibility of indoor cultivation for selected halophytes by measuring growth and stress parameters. Moreover, we investigated the nutrient profiles and the potential for enrichment of secondary metabolites, such as carotenoids, through altered cultivation conditions. Carotenoids are associated with health-promoting effects, for example β-carotene has provitamin A activity and is potentially protective against ROS-mediated disorders [7]. We could show that the selected halophytes have comparable contents of β-carotene with common green leafy vegetables (Fig. 1) [8]. In summary, halophytes are a diverse group of plants, rich in phytochemicals and have the potential to become part of a future plant-based diet.

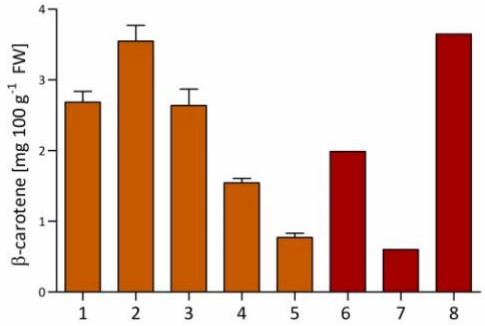

Fig.1. β-carotene content in selected halophytes compared to common green-leafy vegetables. Mean ± SEM. (1)*, *Brassica oleracea* var. *palmifolia*; (2)*, *Cochlearia officinalis*; (3)*, *Chenopodium quinoa*; (4)*, *Atriplex hortensis*; (5)*, *Salicornia europaea*; (6)ɬ, *Lactuca sativa* var. *capitate*; (7)ɬ, *Brassica oleracea* var. *sabauda* (8)ɬ,; *Beta vulgaris* subsp. V*ulgaris*; *data collected through laboratory analysis, ɬ data collected from USDA National Nutrient Database for Standard Reference, Release 27.

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Awareness among Croatian university students about the use, nutritional potential and risks associated with the consumption of hempseed oil

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Approved strains of industrial hemp (*Cannabis sativa* L. subsp. *sativa*) for cultivation are on the variety list of the European Union and are characterised by a Δ^9-tetrahydrocannabinol content of less than 0.2% in the dry matter of the plant [1]. Industrial hemp seeds contain 25 – 35% of oil, which is the most important product of their processing [2]. The mass fraction of polyunsaturated fatty acids in the oil reaches up to 80%. Numerous positive health effects are achieved by the consumption of hempseed oil, mainly due to the favourable ratio of ω-6 and ω-3 fatty acids [3]. The aim of this study was to examine awareness among students of Croatian universities about the use and nutritional and bioactive properties of hempseed oil through an online questionnaire. The study involved 100 students of both sexes aged 19 – 33 years.

As many as 84% of respondents did not consume hempseed oil. Oil consumers mostly inquired about its benefits from friends or acquaintances and preferred to purchase domestic oils. There was a small proportion (6.3%) of those who did not pay attention to the manufacturer and thought that reading the declaration was not important. Almost 70% of respondents considered the price of hempseed oil acceptable. More than half of the respondents consumed oil less than once a month in small doses, most often as part of raw meals. The majority of respondents who were familiar with the fact that hempseed oil is not consumed because of its psychoactive, but because of its antioxidant effect, were students of biotechnical, biomedical and health studies and natural sciences. Half of the respondents did not know that hempseed oil is obtained from industrial hemp seeds, but 78% of students were informed that the use of this oil is legal. Only 13% of respondents were aware of the fact that consumption of hempseed oil can in exceptional cases result in a positive drug test due to the presence of THC. Better legislation is needed for such products on the market to reduce these risks.

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Biochemical characterization of protein fractions extracted from three edible insect species

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With an increase in the world population, climate change and loss of agricultural land production of meat will be challenging in the future. The use of insects as an alternative source of protein is not new; it is environmentally friendly and cost effective with the potential to become a new form of agriculture in the near future worldwide. However, the problem in most developed countries is how to make insects more appealing not only to consumers but also to farmers for animal feeding. As of recently, June 1st 2021, the European Commission authorized for the first time an insect (*Tenebrio molitor*, dried yellow mealworm) as a novel food for human consumption.
Although edible insects have been on the menu of millions of people for many years, very little information from a food science point of view is available on the characteristic of extracted insect proteins, on their allergenicity and cross reactivity with other known food allergen sources. Even less information is available on the impact of different food processing techniques on insect proteins, and food processing is necessary as insect flour is likely the most acceptable form by the consumers in Europe. In this study, we investigated the effect of different protein extraction conditions, such as pH, temperature, sonication and precipitation on the obtained protein fractions extracted from the flour of three edible insects (*Tenebrio molitor, Bombyx mori, Protaetia brevitarsis*) by SDS polyacrilamide gel electrophoresis and Western Blot.
Our data suggest that harsh extraction conditions need to be applied in order to increase the protein yield and that the efficacy of the extraction varies from one insect powder to another. In addition, each edible insect protein extract was different from the other in terms of protein fraction composition and profile. Protein aggregation and denaturation were pronounced in some preparations, showing that insect proteins are both pH and temperature sensitive. Tropomyosin, a well-known invertebrate food allergen is present in insects and represents a health risk for those with documented food allergies. Using shellfish and shrimp extracts as tropomyosin positive controls we tried to estimate tropomyosin content in the protein preparations as well as its cross-reactivity using commercially available antibodies raised to two different tropomyosin sources, house dust mite and shrimp. Tropomyosin from all tested preparations was recognized by both antibodies, suggesting high cross-reactivity between the species, which aligns with the sequence homology, but needs to be further confirmed with allergic patients' sera. Harsh protein extraction conditions reduced tropomyosin content in the protein fractions, suggesting it might be prone to aggregation, and therefore its allergenicity and immunogenicity might be altered.
In conclusion, although insect proteins have high nutritional value, their characterization, techno-functional and immunological properties need to be thoroughly accessed for each insect that is being introduced into the European market. To obtain high quality insect protein isolates different processing techniques need to be applied, but they can easily alter protein structure yielding new possible problems with digestion and immunogenicity.

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Probing the stability of the food colourant R-phycoerythrin from dried Nori flakes

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The high content of vitamins, minerals, antioxidants, and proteins makes red algae *Porphyra* sp. (Nori) superfood with exceptional health-promoting benefits. Its intense colour originates from R-phycoerythrin (R-PE), phycobiliprotein containing covalently attached tetrapyrrole chromophores: red phycoerythrobilin and orange phycourobilin. The present study aims to characterize the stability of R-PE, a natural colourant with a high potential for application in the food, cosmetic, and pharmaceutical industries. We purified R-PE from dried Nori flakes with a high purity ratio (A_{560} /A_{280} ≥5). Far-UV CD spectroscopic showed that α-helix is the dominant secondary structure (75%). The thermal unfolding of α-helix revealed two transitions (T_{m1} and T_{m2} at 56 and 72°C, respectively), ascribed to the different subunits of R-PE. Absorption measurements showed that high pressure (HP) induces dissociation of R-PE into subunits followed by subunit unfolding. Contrary to temperature, HP treatment showed a significant advantage under applied conditions: the protein unfolding is partly reversible, and the R-PE colour bleaching is minimized. Based on the fluorescence quenching approach, R-PE's binding affinities for Cu^{2+} and Zn^{2+} ions were $6.27x10^5$ and $1.71x10^3$ M^{-1}, respectively. Absorption and near-UV/VIS CD spectroscopy suggested conformational changes in protein chromophores upon metal ions binding. Far-UV CD spectroscopy did not reveal that metal binding affects R-PE structure. The obtained results give new insights into the stability of R-PE with a good use-value in replacement of toxic synthetic dyes, preservation of R-PE red colour in fortified food and beverages by HP processing, and as a biosensor for Cu^{2+} in aquatic life systems.

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Fermented and non-fermented *Spirulina* water and ethanol extract treatment effect on yeast at a proteome level

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Filamentous cyanobacteria *Spirulina* (*Arthrospira* spp.) is rich in various nutrients and bioactive compounds with important therapeutic potential [1]. Lactic acid fermentation can be used for enhancing its nutritional and bioactive properties, as lactic acid bacteria can use enzymatic hydrolysis to degrade cyanobacterial cell walls. As a result new metabolites with high antioxidant, anti-inflammatory and immunomodulatory activities are produced from the original large organic compounds [2]. *Saccharomyces cerevisiae* has been treated in this study with fermented and non-fermented *Spirulina* water and ethanol extracts to investigate the effect of lactic acid fermentation on *Spirulina*'s bioactivity, as yeast cells have been proven to be a good model organism for studying metabolic pathways and cellular processes [3].

Spirulina samples were fermented with lactic acid bacteria *Lactobacillus plantarum* and then extracted in water or ethanol. Fermented and non-fermented *Spirulina* extracts were used for yeast cell treatment to obtain yeast treated with non-fermented *Spirulina* water extract (NFV), non-fermented *Spirulina* ethanol extract (NFE), fermented *Spirulina* water extract (SV) and fermented *Spirulina* ethanol extract (SE). Then, cell lysates were prepared and a comparative proteomic study of protein expression alterations between yeast cells receiving different treatments was performed using Q-Exactive HF Orbitrap instrument. To determine protein fold changes between different treatment conditions label-free LC-MS coupled to data dependent acquisition was implemented.

Significant differences were found in protein abundances between yeast treated with non-fermented and fermented *Spirulina* extracts as well as yeast treated with water and ethanol *Spirulina* extracts. SV samples, when compared to NFV samples, showed upregulation of majority of differentially expressed proteins, while in SE samples, when compared to NFE samples, a significant downregulation of the major part of the analysed proteins was found. Furthermore, abundance of stress response related proteins increased in SV compared to NFV samples while their predominant downregulation was observed in SE samples compared to NFE. In this study, analysis of the contribution of lactic acid fermentation to the cell activity and different extract effect were combined for the first time. A better insight into *Spirulina* bioactivity was obtained using proteomic approach and an insight into cells coping mechanisms to maintain metabolic and redox balance in presence of exogenous antioxidants was given.

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Effect of chitosan on encapsulation of chokeberry polyphenols and volatiles in alginate-based hydrogel beads

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Chokeberry polyphenols are known for their various health-promoting properties on human health such as anti-inflammatory, antimutagenic and antioxidant activities [1,2]. In addition, these fruits are known for specific flavor. These high-valuable components are often decomposed and thus their wide utilization is limited. This obstacle can be overcome by the process of microencapsulation i.e. preparation of hydrogel beads [3]. In the present study, hydrogel beads with alginate (A) or alginate/chitosan (A/C) as wall materials for preparation of hydrogel beads were used. Hydrogel beads were prepared by encapsulator under fixed conditions (1000 µm vibrating nozzle, pressure 200 mbar, frequency 200 Hz, electrode 1000 V). Effects of chitosan addition on microencapsulation of chokeberry polyphenols and volatiles were studied. Results revealed that beads prepared with alginate contained higher concentrations of total polyphenols, proanthocyanidins and anthocyanins compared to those prepared with alginate and chitosan. This was proven using spectrophotometric and HPLC methods. Antioxidant activities (FRAP, CURPAC, DPPH and ABTS) of beads followed the same trend obtained for polyphenols. Volatiles were determined by GC-MS and percentage of specific flavor note in overall flavor profile were determined. Hydrogel beads had different flavor profile then chokeberry juice but there were slight differences between A and A/C beads. Our work demonstrated that proper formulation of hydrogel beads is important in order to maximize retention of chokeberry polyphenols and volatiles. Due to the economic and nutritional importance of their preservation, polyphenols and volatiles incorporated inside hydrogel beads can be further used in the food industry for the development of innovative and functional foods.

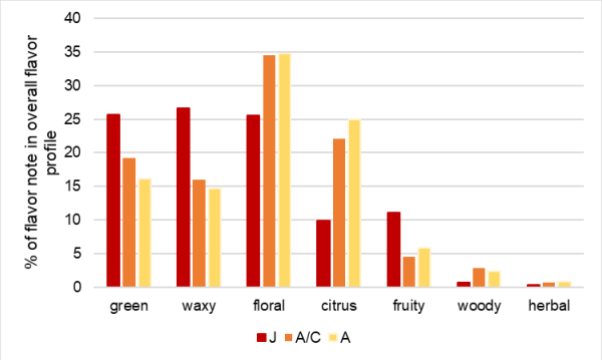

Fig.1. Flavour profile of chockberry juice (J) and hydrogel beads (A – alginate hydrogel beads; A/C – alginate/chitosan hydrogel beads).

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Complexation of quercetin with apple and citrus fibres: Study of quercetin affinities in model systems

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Among the flavonoids, quercetin gained special attention due to its pharmacological activities such as antioxidant, anti-inflammatory, anticancer, antitoxic and immunomodulatory effects [1]. Furthermore, quercetin has disadvantages such as hydrophobic nature, poor solubility and permeability which could be overcome by complexation with different polymers [2]. Dietary fibres are known as "carriers" of polyphenols which can protect them from environmental conditions and thus allow them to be absorbed [3]. In this study, apple and citrus fibres (as applicable food by-products), were used as "carriers" of quercetin. Constant amount of fibres (1%) and different concentrations of quercetin solutions (5 mM, 10 mM and 20 mM) were used for complexation. Obtained complexes were evaluated by HPLC for determination of quercetin concentrations and antioxidant activities (ABTS, DPPH, FRAP, CUPRAC assays). Additionally, IR spectra were recorded to confirm complexation of quercetin. The results of HPLC analysis showed that quercetin had higher affinity for apple fibre than citrus fibre. Also, it was determined that quercetin adsorption is not proportional with initial amount of quercetin (e.g., a twice higher concentration of quercetin in the initial solution did not result in a twice higher concentration of quercetin in the complex). Antioxidant activities, evaluated with four different assays, were higher in apple fibre/quercetin complexes than in citrus fibre/quercetin complexes. FTIR-ATR analysis showed the formation of new and loses of existing bands when quercetin was present, and also changes in band intensities were noticed. These results could contribute to understanding of quercetin behaviour in food matrix. Also, during preparation of food additives based on polyphenols and fibres, it is needed to know influence of different types and sources of fibres on polyphenols preservation.

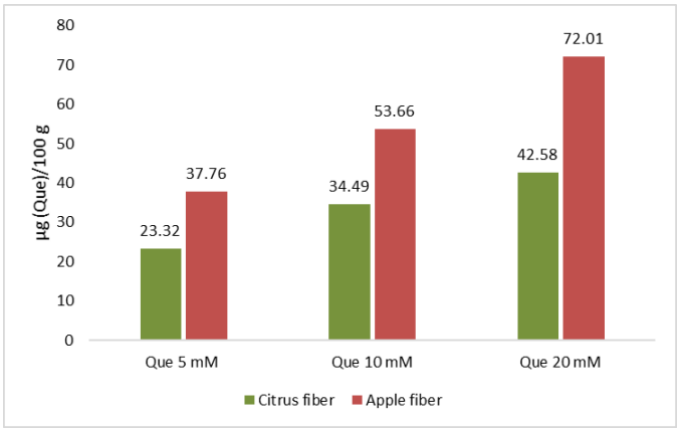

Fig.1. Quercetin concentrations in quercetin/apple fibre and quercetin/citrus fibre complexes.

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Technological changes of wheat-based breads enriched with hemp seed press cakes and hemp seed grit

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Protein is an important macronutrient with a recommended daily intake for healthy adults of 0.75 g / kg body weight [1]. Protein deficiency can lead to a variety of health issue, e.g. growth stunting and impaired development as well as endocrine imbalance, cardiovascular abnormalities and decreased protein synthesis. Consequently, a sufficient protein intake must be ensured [2]. However, a large amount of protein intake stems from animal products, which often have an energy- and resource-intensive production. This is why, it is important to implement other protein rich, plant-based materials into our diets and everyday products of consumption.
Hemp as well as hemp seed press cake – a by-product of hemp oil production – are protein rich, gluten-free raw materials, which are frequently used to enhance the nutritional value of breads. However, the addition of hemp materials often negatively impacts technological parameters of breads [3].
This is why, this study investigated if and how much the addition of 1 % of various by-products of hemp seed press cakes (hemp seed grit (HSG), hemp press cake flour (HPF), hemp press cake flour protein 54 % (HP54), hemp press cake flour protein 46 % (HP46)) to a wheat bread mixture impairs the texture and colour profile. In a second step, more hemp materials were added to reach the EU nutrition claim *high protein* [4] and the texture and colour changes were again recorded. For data interpretation, the particle size of raw materials as well as nutritional parameters according to nutrition lable were also taken into account. The correlation between colour, particle size, texture and nutritional parameters were visualized using principle component analysis (PCA) (see figure 1).
The results showed that the addition of 1 % of some hemp raw materials already causes significant technological changes ($p > 0.05$). Hemp raw materials increase bread hardness and decreases elasticity. The colour of breads containing 1 % hemp was also darker, although the changes were only small ($\Delta E^* < 2.4$).
Breads, which reach the EU nutrition claim *high protein* showed further technological impairments and were visibly darker. The PCA further revealed that colour inversely correlates with chewiness, gumminess and hardness. Elasticity and stickiness on the other hand seem to be mostly independent of the aforementioned parameters. The PCA also shows that breads with 18 % HPF and 14 % HP46 are similar and that the 10 % HP54 bread is most similar to the reference bread WFM.

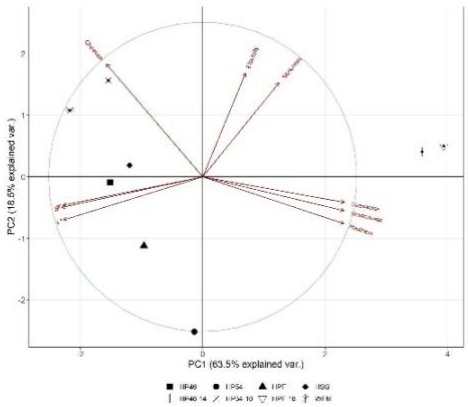

Fig.1. PCA biplot of colour and texture parameters. HSG – Hemp seed grit; HPF – Hemp press cake flour; HP46 – Hemp press cake flour protein (46%); HP54 – Hemp press cake flour protein (54%); WFM – Half-and-half mixture of wheat and whole wheat flour.

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Encapsulation efficiency of spray-dried juniper berry (*Juniperus communis* L.) essential oil microcapsules prepared with different wall materials

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The microencapsulation of essential oils provides their protection from oxidative decomposition and evaporation, flavour retention and masking unpleasant taste. The antioxidant, antimicrobial, antiseptic and antifungal properties of juniper berry essential oil makes it attractive for the preparation of functional food microcapsules, which structure can provide controlled release of active ingredients.

The aim of this study was selection of appropriate wall material used for encapsulation of juniper berry essential oil (JBEO) by spray drying, in terms of the best encapsulation efficiency of oil (EE), followed by complete characterization of obtained microcapsules. Gum arabic (GA) (20% w/v), whey protein concentrate (WPC) (20% w/v) and sodium alginate (ALG) (4% w/v) were used as wall materials. Emulsification procedure involved addition of essential oil into wall material by homogenization at 10000 rpm during 5 min. The core: wall material ratio was 1:4 (w/w). The second step was ultrasound emulsification for 5 min. The feed emulsion was injected into a spray dryer at inlet temperature 120 °C and feed flow rate of 0.192 L/h.

Successful encapsulation of JBEO was performed, with the particle size of obtained powder between 3.97 and 9.59 µm. Microcapsule prepared with GA as a wall material showed the best oil retention (84.67 ± 1.31 %) and EE (64.16 ± 2.77 %). The obtained oil retention was higher compared to EE of rosemary essential oil prepared with similar procedure [1]. The using of WPC enabled oil retention of 52.38 ± 1.61 % and EE of 42.16 ± 7.57 %, while essential oil of ALG microcapsules mainly localized at microcapsule surface, giving low EE (9.71 ± 1.61 %). Thermal stability of GA microcapsules can be defined as the best. Moisture of GA and WPC microcapsule were in the satisfactory range for food processing (4-6%). Hygroscopicity, solubility and dissolution time of GA and WPC powders were in accordance with previous literature allegations [2].

Table 1. Characterisation of juniper berry essential oil microcapsules

Carriers	GA	WPC	ALG
Total oil content (g/100 g)	16.91 ± 0.29	10.48 ± 0.32	4.51 ± 0.56
Surface oil content (g/100g)	6.07 ± 0.56	5.71 ± 0.10	4.08 ± 0.58
Oil retention (%)	84.67 ± 1.31	52.38 ± 1.61	22.72 ± 2.72
EE (%)	64.16 ± 2.77	45.40 ± 2.60	9.71 ± 1.61
Moisture (%)	5.86 ± 0.48	4.50 ± 0.12	8.53 ± 0.25
Hygroscopicity (%)	13.47 ± 0.11	8.0 ± 1.26	25.66 ± 0.21
Dissolution time (min)	5.04 ± 0.41	6.17 ± 0.04	24.74 ± 1.16
Solubility (%)	66.15 ± 1.49	70.20 ± 3.08	84.44 ± 0.94
Bulk density (g cm^{-3})	0.25 ± 0.01	0.16 ± 0.00	0.25 ± 0.03
Tapped density (g cm^{-3})	0.42 ± 0.01	0.32 ± 0.01	0.43 ± 0.04
Particle density (g cm^{-3})	1.69 ± 0.03	1.28 ± 0.05	2.55 ± 0.07
CI (%)	41.85 ± 1.10	49.66 ± 0.60	41.99 ± 1.55
HR	1.72 ± 0.03	1.99 ± 0.02	1.72 ± 0.05
Porosity (%)	75.05 ± 0.9	75.15 ± 1.14	83.04 ± 2.15

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Elderberry wine as a new potential product of functional food

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Elderberry (*Sambucus nigra L.*) is a wild-growing plant species, rich source of protein, amino acids, fatty acids, vitamins, antioxidants, and minerals. Analysis of elderberry showed that it contains high biological activity components, primarily polyphenols, mostly anthocyanins, flavonols, phenolic acids and proanthocyanidins, as well as terpenes and lectins. In folk medicine, elderberry has been used in the treatment of many diseases and ailments [1]. The objective of this research was to investigate chemical and phytochemical composition of two types of elderberry wine. Elderberry wines were prepared according to the standard procedure of wine production in laboratory conditions. Plant species *S. nigra* is characterized by the content of cyanogenic glycosides which are potentially toxic compounds, due to which the obtained wines were treated with different temperature treatments (60 °C during 10 minutes, and 70 °C during 5 minutes), in order to degrade these molecules. Elderberry wines were tested using different oenological methods and the chemical composition was determined, while the phytochemical composition was determined using the HPLC method. The obtained results showed that the wine exposed to temperature 70 °C for 5 minutes was characterized by a higher content of ethanol, volatile acids, while the content of organic acids was similar as of wine with temperature profile 60 °C for 10 minutes. Both types of elderberry wine have been shown to be a very rich source of minerals, especially potassium (for 70 °C, during 5 minutes 5,657.14 mg/L and for 60 °C, during 10 minutes 5,597.45 mg/L). Analyzing the phytochemical composition of elderberry wines, results of this part shown that they had a high content of phenolic acids and flavonoids. The content of phenolic acids (gallic acid 5,36 μg/mL and caffeic acids1,04 μg/mL) was higher in wine that has been exposed to temperature 70 °C for 5 minutes. Also, this wine was very rich in ursolic acid as a triterpene compound (0.70 μg/mL). The dominant flavonoid compounds quercetin and rutin were present in higher concentrations in wine of temperature profile 70 °C, for 5 minutes (158.89 μg/mL and 52.71 μg/mL respectively), while quercetin-*3*-*O*-hexoside was more dominant in wine of temperature profile 60 °C, for 10 minutes (14.35 μg/mL).
Based on the performed analyzes and the obtained results, it is clear that the temperature of 70 °C is more suitable for the isolation of secondary metabolites, which is why this type of wine is recommended for further production and aims at more detailed research with the idea of potential placement on the market.

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Investigation of the effect of a new feed additive formulation on the egg nutritional properties

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The correct feeding of the animals is of great importance for their healthy development. The feed used for laying hens affects not only the health of the animals, but also the composition of their eggs and meat. We participated in the development of a feed supplement for laying hens within the framework of an international EUREKA project at the Department of Foodchemistry and Analytical Chemistry. The feed supplement named "ZINCOPPYEAST" is a mixture of two types of yeast, fresh yeast enriched with zinc and spent brewing yeast with high polyphenol content, which is intended to provide zinc and polyphenol supplementation for animals. The process also recycles spent brewer's yeast as an industrial by-product, which is an additional benefit of the development.
The results presented relate to the different nutritional properties of eggs came from the experiment. According to the experimental design the animals were divided into 3 groups, and they were fed for 2 months. The first group was fed a 2.5% supplemented diet, the second group was fed a 5% supplemented diet, and the third group was the control group, which was fed an unsupplemented diet. The eggs were collected twice during the experiment, after 30 days and after 60 days.
Total zinc content, total polyphenols, protein and fat content of eggs were measured during the research. There were no differences based on zinc, protein and fat contents of the samples from the three test groups, but there were significant differences in polyphenol content. Statistical evaluation of the polyphenol results showed that the polyphenol levels in the eggs of hens fed with ZINCOPPYEAST supplemented diets were significantly higher than those of the control group, and that the 5% eggs showed significantly higher levels compared to the 2.5% eggs. Examining the coherent data sets of eggs collected in two different periods, in case of eggs obtained with 2.5% ZINCOPPYEAST it can be seen that the polyphenol content could be increased applying longer feeding periods. The situation is different for eggs from hens fed with 5% ZINCOPPYEAST supplemented diets, where the 30 and 60 day data sets are not significantly different, namely the elevated polyphenol content had already been reached in the eggs at 30 days, and it did not increase significantly with continued dosing.

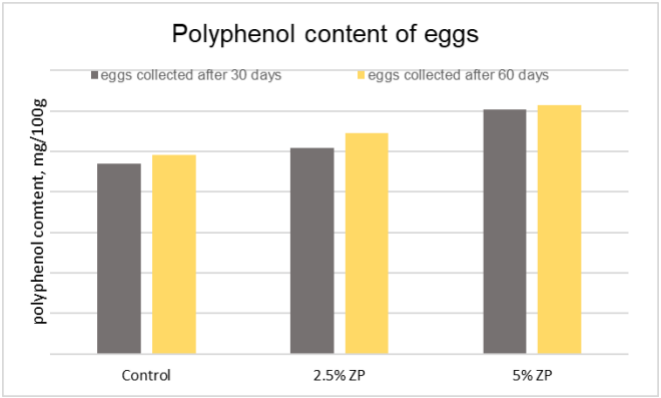

Fig.1. Polyphenol content of egg samples

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Drying of Halophyte Plants: Effect on the Antioxidant Activity

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Salicornia and Sarcocornia belong to the same family and have similar morphological and organoleptic properties. Because of their composition, they have some compounds with antioxidant activity beneficial for consumers' health [1]. The objective of this work was to evaluate the antioxidant activity extracts obtained from the plants in the fresh state and after being dried. Their extracts may represent a valuable source for developing novel food products (antioxidant-enriched foods), and/or table salt substitutes that satisfy the desires of consumers in terms of health benefits and sensorial acceptance.
The plants (*Salicornia macrostachya* Moric. and *Sarcocornia perennis*) were collected from Portuguese salt pans, in the central region of Portugal and the aerial parts were used as raw material. The drying of plants was performed in a pilot tray drier at 40 ºC and air velocity of 1.5 ms^{-1}, for approximately three days. The initial moisture content was 92.30% and 84.24%, respectively, for Salicornia and Sarcocornia. The drying was carried out until reaching a final moisture content of 5%. The antioxidant activity was measured with DPPH method. Regarding the DPPH method, the results showed that the incubation time of 15 minutes is enough to measure the DPPH scavenging activity in halophyte extracts in the tested range of extract concentrations (Figure 1). However, the time defined to measure the DPPH scavenging activity was fixed at 30 minutes, since this was the common time in different laboratorial protocols.

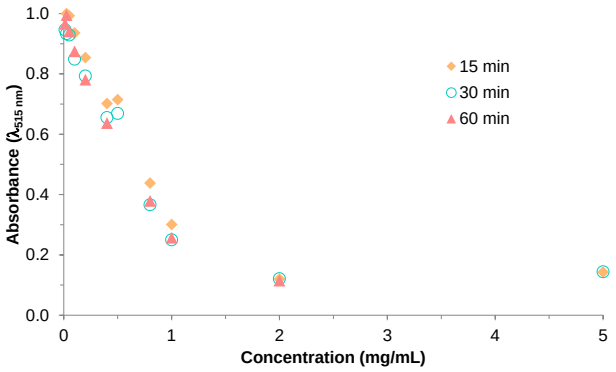

Fig.1. Scavenging of DPPH radical for various concentrations of dried *Sarcocornia perennis* extracts.

For Salicornia, the values of inhibition IC_{50} were 1.09 and 1.12 mg/mL for the fresh and dried samples, respectively. For Sarcocornia, the values of IC_{50} were 1.42 and 1.02 mg/mL for the fresh and dried samples, respectively. The results showed that the convective air-drying process at 40 ºC is adequate to improve the shelf life of the two halophyte plants, since the antioxidant activity was maintained or even improved as compared with the fresh samples. This might be due to a response of the plant to the stress induced by the heat and humidity conditions in the drying chamber. In this way, it was concluded that both studied halophyte plants constitute a valuable source of natural antioxidants when they are consumed as foods either in the fresh or dried states. Additionally, their extracts may represent a valuable source for developing novel antioxidant-enriched food products aimed to meet the desires of consumers who seek for health-beneficial foods.

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Essential oils from *Thymus vulgaris* and *Thymus* x *citriodorus* dose-dependently reduce nitric oxide release from LPS-stimulated macrophages

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Over the years, aromatic and medicinal plants have been used worldwide as the basis of traditional medicine, due to their content in natural bioactive compounds, which make them of high interest to many industries, such as pharmaceutic, cosmetic and food, due to their many bioactive properties. Among medicinal plants, *Thymus* L. genus contains several species that serve as raw material for the extraction of bioactive compounds. *Thymus vulgaris* L. and *Thymus* x *citriodorus* (Pers.) Schreb., have shown high medicinal potential due to their chemical composition and respective biological properties. Essential oils (EOs) of these plants are a rich source of volatile and non-volatile compounds, and have been shown to have antimicrobial, antioxidant and antitussive properties [1].
The aim of this work was to evaluate the potential of EOs obtained from *T. vulgaris* and from *T. x citriodorus* as anti-inflammatory agents, using the macrophage cell model (RAW 264.7 cells) exposed to lipopolysaccharide (LPS), and by the quantification released nitric oxide (NO), using the Griess reagent [2].
Firstly, we assessed cell viability using the Alamar Blue assay. Cells were exposed to different concentrations of EOs for 24 hours to obtain a range of non-cytotoxic concentrations. Then, the anti-inflammatory potential was assessed by exposing RAW 264.7 to a range of non-cytotoxic concentrations of EOs (0.5 to 25 µg/mL), in the presence and in the absence of LPS. After 24 h exposure, the NO released to the supernatant was quantified by Griess reagent, as described [2].
Results showed that EOs of the two *Thymus* species did not reduce cell viability for concentration up to 25 µg/mL. Both EOs showed anti-inflammatory properties as they dose-dependently reduced LPS-stimulated NO released, being *T. x citriodorus* more effective than *T. vulgaris*, at all concentrations tested. In conclusion, the EOs of these two plants have potential to be used as a source of ingredients with anti-inflammatory activity.

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Easy-to-swallow functional foods with potential neuroprotective properties designed for the elderly

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Over the decades the increase in life expectancy has been observed, and this increase also raises questions about the quality of life of senior citizens, since neurodegenerative diseases are very common in this age group [1]. Unfortunately, the existing therapeutic options are not always suitable for all patients, forcing the need to create new products.

Research on macroalgae promises to overcome this problem, since they are known to have neuroprotective and antioxidant properties [2]. In this sense, it was intended to develop functional foods where a seaweed extract was incorporated. These functional foods should consider some of the limitations that people within the silver age group (>65 years old) experience, such as difficulty to swallow, need to be hydrated, etc., and, therefore, gelatine, gummies and jams can be good alternatives. The development of these functional products is based on natural and biological raw materials, where the manufacturing process will be the most environmentally friendly, as well as animal-friendly, and in this way to be able to adapt to the largest number of people regardless of their comorbidities, limitations or eating habits.

Macroalgae from the Portuguese coast, where used to obtain extracts with neuroprotective activity through green methodologies such as subcritical water extraction (SWE).

Functional foods were prepared with the most promising neuroprotective macroalgae extract (*Codium tomentosum* Stackhouse obtained by SWE at 190-250 °C) with was chosen based on its antioxidant activity against several radicals, enzyme inhibition towards enzymes involved in Alzheimer's and Parkinson's disease and depression, and cell neuroprotection. These extracts were applied in gummies, gelatine, and jam. All recipes were developed with seaweeds gelling extracts such as agar-agar and carrageenin, and honey was used as sweetener. Fruits juices prepared immediately before the addition of the gelling agent was obtained from oranges, apples, and red fruits. The seaweed extract was incorporated in the food products. Sensory evaluation was performed to assess the presence of any off flavour related with the seaweed. The gummies were the products that were most optimized, and the best achieved, but it will still be important to test with different gelling mixtures to achieve a more elastic texture, maintaining it chewable for the elderly.

In the future, it will be necessary to optimise the recipes and then carry out a sensory analysis with the target public and develop more products, such as lactose-free yoghurts. Allied to this, a microbiological assessment should be carried out, as well as, looking at the shelf life, also assessing the digestion of the products and evaluating other physico-chemical parameters, with the aim of preventing or delaying neurodegenerative diseases in the elderly.

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The NUTRIBOX project: a HEALTHY & SMART EATHINKING e-commerce platform for vulnerable consumers

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Food has a paramount importance in the daily life since it is among the major factors accounting for a healthy physical state. Recently, the abuse of comfortable foods, the quarantine and physical inactivity, and the poor management of disease risk factors related have summed up to the general privations the population underwent during Covid-19 pandemics; this induced behavior has increased the incidence of Non-Communicable Disease (NCD) [1]. In addition, the constraints applied (social distancing and quarantine) has produced a direct effect on consumer behaviour in moving to online shopping [2,3] and healthier options and personalized nutrition [4]. To facilitate consumers' food selection protecting consumers' health with special regard to vulnerable and people under dietary restrictions, we developed under EITFood scheme the innovative and handy e-commerce platform and NUTRIBOX Eathinking (https://nutribox.store/index.php, fig 1). This platform proposes a "click and collect or delivery home" model, by pointing at the safeguard of food safety and health of the targeted categories consumers (diabetics, allergics to egg or milk, celiacs, fragile patients, obese, and children) and with specific needs or preferences (vegetarians); it promotes the quality of foods and nutritionally balanced food boxes/menu to push towards a healthy food choice and balanced diet. The platform has been developed in accordance with the recent guidelines issued by the World Health Organization (WHO, 2020) alerting consumers on the moderate intake of sugars, salts and cholesterol or saturated fatty acids.

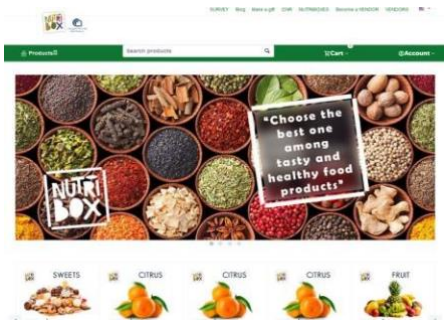

Fig.1. Website: https//www.nutribox.store or https//www.nutribox.it

Each product list was obtained by examining the guidelines on nutritional needs reported by official sources, Departments, agencies, public bodies and societies. Further data were collected by questioning vulnerable subjects on own eating habits (e.g. vegetarians and diabetics), nutrition softwares and with the support of expert in the field (nutritionists, dieticians and doctors). To promote a balanced diet, nutritional values for each listed food were provided by official database (https://fdc.nal.usda.gov/); in addition, nutraceutical properties were highlighted and results with high impact from international peer review scientific papers were summarized to be understood by the different types of customers. Consumers are driven towards the more appropriate NUTRIBOX by a brief survey; alternatively they can select foods on the basis of specific and individual nutritional needs by entering the range of desired values for macro and micronutrients; in this case system will select foods matching with them.
In conclusion NUTRIBOX is a combination of food commodities promoting well being and health advertising innovative and functional foods and food selections mirroring the guidelines of WHO and recent consumers trends; it fill the gap between food industry and healthy benefits during on line shopping by increasing trust of consumer in food system and becoming strategy for disease prevention and management.

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Microwave-assisted extraction of phlorotannins from *Fucus vesiculosus*

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Phlorotannins are a class of phloroglucinol-based phenolic compounds, occurring exclusively on brown macroalgae, that have drawn much attention in the recent years due to their promising bioactive properties. However, the most common protocols used for extraction of these compounds are based on solvent extraction methods which are time consuming and quite environmentally unfriendly [1]. In this context, the aim of this study was to evaluate whether the microwave-assisted extraction (MAE) with an aqueous mixture of ethanol could be used as a greener and more efficient alternative for the extraction of phlorotannins from *Fucus vesiculosus*.
According to the results, the optimal MAE conditions were defined as 57% (v/v) ethanol and 75 ºC during 5 min, which allowed a recovery of phlorotannins from the macroalgae identical to that achieved with a conventional extraction with 70% acetone at room temperature for 3 h. Afterwards the extracts were analysed for their antioxidant activity, via radical scavenging assays, and antidiabetic activity, by evaluating of their ability to inhibit the enzymatic activity of α-glucosidase. Additionally, the phlorotannin profile of both extracts was compared using UHPLC-MS analysis.
Close similarities were found between the MAE and conventional samples, both showing approximate total phlorotannin content and antioxidant activity. In turn, significant differences were found on the inhibitory effects on α-glucosidase, which was stronger on the conventional extract compared to the MAE extract (IC_{50} = 1.73 ± 0.13 versus 6.86 ± 0.70 µg/mL). Nevertheless, the effects of MAE extract on this enzyme were remarkably superior compared with acarbose (IC_{50} = 789.93 ± 41.08 µg/mL), a pharmaceutical drug currently used to treat diabetes mellitus type 2 by targeting α-glucosidase.
According to the UHPLC-MS analysis, many of the phlorotannin compounds were detected on both extracts, which, in general, revealed a phlorotannin profile quite coincident between extracts.
Overall, this study allowed to conclude that the MAE procedure using aqueous ethanol could be adopted as a reliable greener and more efficient technique for phlorotannins extraction maintaining identical yields and phlorotannin constituents, as well as their promising antioxidant and anti-diabetic properties.

Neglected wild edible fruits as potential dietary sources of antioxidants in the Mediterranean Area

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Since ancient times, in the Mediterranean area, wild fruits have provided the human being with a varied diet rich in vitamins, minerals, and other nutrients. However, their consumption decreased with the development of agriculture and industrialisation, which progressively led to an underestimation of their value and potential. Wild fruits are versatile in handling and consumption, presenting a great diversity in colours and flavours, as well as in their chemical and nutritional composition. The hawthorn fruit (*Crataegus monogyna* Jacq.) is a red and fleshy berry like pome with 1 cm in diameter and a single seed. The blackthorn fruit (*Prunus spinosa* L.) is a globular drupe varying from black to blue with a diameter of 10-15mm, with a sour taste that changes to sweet when ripe [1]. Both fruits have been traditionally used in folk medicine to alleviate different types of disorders, as well as in food, either fresh, or in the form of jams, as an ingredient in drinks, etc. [2,3]. The combination of bioactive compounds and rich nutritional composition of these fruits, represent a new valuable source of safe and inexpensive antioxidants with functional potential [4].

In this work, the *in vitro* antioxidant capacity of these wild fruits was determined by Folin-Ciocalteu [5], DPPH and FRAP [6] assays, as well as the amount of total phenolic compounds by Fast blue BB assay [7] and total anthocyanins content by a pH differential method [8]. The determinations were carried out using QUENCHER (Quick, Easy, New, Cheap and Reproducible) extraction methodology, where the solid sample, homogenised to a particle size of 0.037 mm, is subjected to direct contact with the test reagents, which makes it possible to quantify the activity of liquid-liquid (soluble compounds) and solid-liquid (insoluble compounds) interactions, avoiding the extraction process and/or hydrolysis stages, and obtaining a more precise and reliable result.

Both fruits showed a high antioxidant capacity, very similar between them, being the hawthorn the one with the highest activity measured by FRAP (2458.0 mg TE/100g) and Folin-Ciocalteu (640.5 mg GAE/100g) and the blackthorn by DPPH (522.7 mg TE/100g). In addition, blackthorn showed a higher amount of phenolic compounds and total anthocyanins than hawthorns (2725.8 and 1170.5 mg GAE/100g; 1203.9 and 880.0 mg cyanidin-3-glucoside/100g, respectively).

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Bioavailability of Mg and Zn in grain of plant *Amaranthus cruentus* after simulated gastrointestinal digestion

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The ancient civilization of the Incas, Mayas, and Aztecs used the plant species Amaranthus as an analog of wheat and rice. In the 1980s interest increased in exploring these pseudocereals after the US National Academy of Sciences published research of their nutritional value [1]. The amaranth seed (*Amaranthus cruentus*) is perhaps the best known for its hypoallergenic properties (gluten-free product) [2]. In addition, it is important to point out that amaranth seed is characterized by a high content of magnesium, iron, calcium, and other micro and macro elements in comparison with, for example, wheat [3]. These properties make *A. cruentus* a good candidate for use in functional foods production. However, the method of preparation, storage, and processing of food can significantly affect its quality, i.e., the concentration and bioavailability of minerals, vitamins, and other essential nutrients in the food. Following the above claims, this study aims to determine the bioavailability and the influence of mechanical and thermal pretreatment on the bioavailability of magnesium (Mg) and zinc (Zn) from the grain (seed and popcorn) of the plant *Amaranthus cruentus* upon simulated gastrointestinal digestion.

The content of Mg and Zn in samples was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES). The samples were sorted as *A. cruentus* seed (S), and popcorns (P) which were subjected to mechanical (grind (G) and non-grind (NG)) and thermal (boiled (B) and non-boiled (NB)) pretreatment. Then the pretreated samples were subjected to simulated gastrointestinal digestion. The resulting "digesta" samples were centrifuged and in the clear supernatants as well as in the whole grain (WG) the content of Mg and Zn was determined by the ICP-OES method.

The most abundant element in a whole grain was Mg and its concentration was 316.20 ± 1.10 mg per 100 g of WG S and 491.00 ± 40.85 mg per 100 g of WG P. Obtained values for Mg in WG S and WG P are statistically different. This difference is possibly due to losing water molecules during the popping procedure, so even the measured weight of seed and popcorn was the same, the dry weight of seed and popcorn are different so that popcorn has a greater dry weight [4]. Determination of the total amount of Zn in WG revealed that there is no statistically significant difference between S and P. The obtained values were 3.34 ± 0.12 mg in 100 g of WG S and 3.31 ± 0.03 mg in a WG P. The highest achieved bioavailability of Mg was 61.63 ± 1.15 % in digesta of G B S and 47.14 ± 2.12 % in digesta of G NB P. The achieved percentage of bioavailability of Zn was 22.02 ± 1.62 % in digesta of B G S but in popcorn digesta samples amount of Zn was below the limit of detection.

We can conclude that the amaranth grain is a rich source of Mg and although the Mg content was higher in the WG of popcorn, probably due to the higher dry weight of popcorn, the differences in the Mg bioavailability among seed and popcorn digesta were not so high as expected. The content of Zn in WG S and P was negligible compared to Mg content but still beneficial in terms of functional food assets. The method of grain pretreatment strongly influences the bioavailability of metals, a combination of mechanical and thermal treatment results in a higher release of metals. Overall, the amaranth seed can be considered as a good candidate for functional food production.

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The effects of gastrointestinal digestion on antioxidant and anti-inflammatory abilities of phlorotannins from *Himanthalia elongata* and *Laminaria digitata*

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Gastrointestinal digestion is a physiological process that poses extreme conditions capable of affecting the stability, bioaccessibility and bioactivity of most polyphenols [1]. However, when it comes to the phlorotannins, i.e., phenolic compounds specific from brown macroalgae, little is known about how the digestive process affects their stability and biological activity, namely antioxidant and anti-inflammatory properties. In this context, this study aimed to evaluate the stability of phlorotannin-rich extracts from *Himanthalia elongata* and *Laminaria digitata*, commonly known as sea spaghetti and oarweed, respectively, after undergoing a simulated gastrointestinal digestion. In addition, both digested and undigested extracts of each seaweed were evaluated for their antioxidant activity and ability to inhibit the production of nitric oxide ($NO^{\bullet}$) in murine macrophage cells stimulated with lipopolysaccharide (LPS).

The results revealed that, like land plant phenolics [2], phlorotannins exhibit a significant susceptibility to the digestive process, as the total phlorotannin content of the extracts progressively decreased after each step of the simulated gastrointestinal digestion. As expected, a decrease of the antioxidant activity after each digestive step was noticed as well, being correlated to the loss of the total phlorotannin content. In turn, the anti-inflammatory activity, evaluated as the capacity to inhibit the LPS-stimulated $NO^{\bullet}$ production in macrophage cells, was slightly more effective using the digested extracts rather than their undigested pairs. These results suggest that the compounds formed during the digestive process of the phlorotannin-rich extracts may be less effective as radical scavengers, but more prone to interact with the intracellular mechanisms and signalling molecules that regulate the inflammatory response.

Overall, this work provides valuable information on how the digestive process may affect the stability and bioactivity of *H. elongata* and *L. digitata* phlorotannin extracts, showing that although the gastrointestinal digestion may affect the levels of phlorotannins that can reach the intestinal lumen intact, the products of their digestion and metabolization may still exhibit positive bioactive properties and exert interesting health benefits.

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Anti-Inflammatory effects of oleacein and its metabolites

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Epidemiological data show that a Mediterranean diet reduces the incidence of cardiovascular diseases, and it has been suggested that polyphenols in extra virgin olive oil (EVOO), the major source of fats in this diet, may contribute to this health effects [1]. The phenolic composition of EVOO includes the phenolic alcohols hydroxytyrosol and tyrosol and their secoiridoid precursors such as olecanthal and oleacein. Several studies confirm that EVOO polyphenols exert antiatherogenic and anti-inflammatory activity [1]. However, the major protective polyphenol in EVOO is still not defined. Oleacein, the main antioxidant polyphenol in EVOO, is believed to be one of the main responsible for reducing cellular damage and inflammation.
In this work, the anti-inflammatory potential of oleacein, hydroxytyrosol and their main known metabolites was assessed using RAW 264.7 macrophages challenged with lipopolysaccharide (LPS). Oleacein, hydroxytyrosol and hydroxytyrosol acetate at 12.5 μM significantly decreased the •NO and L-citruline cellular levels in macrophages treated with LPL. Despite the lower activity, the hydroxytyrosol acetate sulfate was also able to reduce the cellular levels of •NO. In contrast, hydroxytyrosol sulfate and glucuronide did not show significant anti-inflammatory effect. Additionally, the enzyme inhibition capacity of these compounds was also evaluated using phospholipase A2 (PLA_2) and 5-lipooxygenase (5-LOX), enzymes involved in the biosynthesis of inflammatory mediators. Oleacein showed to significantelly inhibite the PLA_2 + 5-LOX system (IC_{50} = 16,11 μM) and 5-LOX (IC_{50} = 45,02 μM). Hydroxytyrosol acetate was also capable of inhibiting the PLA_2 + 5-LOX system and 5-LOX, although with a IC_{50} values (IC_{50} = 73,53 μM and IC_{50} = 107,28 μM, respectively) higher than those of oleacein. Hydroxytyrosol showed to selectively inhibit the 5-LOX. In contrast, hydroxytyrosol sulfate, hydroxytyrosol glucuronide and hydroxytyrosol acetate sulfate did not show any effect on these enzymes. Since the parental compounds, oleacein and hydroxytyrosol, with the free catecholic moiety, shows better activity, this feature seems to be important for the observed enzyme inhibition. However, it has been described that conjugated metabolites may behave as carriers of bioactive compounds in plasma, which may deconjugate in situ in target tissues, releasing the more bioactive parental compound, which is the final effector. Therefore, further studies are still needed for completely understand the anti-inflammatory activity of EVOO polyphenols in vivo and to relate this bioactivity with the polyphenolic composition of EVOO.

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Antioxidant protection of endangered *Thymus* spp. aqueous extracts on *t*-BHP-induced oxidative damage in an intestinal cell model

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In recent years, the awareness of intestinal homeostasis relevance to the overall wellbeing has increased, accompanying a higher incidence of intestinal diseases such as inflammatory bowel disease, Crohn's disease or colorectal cancer. As a common point between these pathologies is the potential role of oxidative stress in its genesis. And so, as a source of phytochemicals with high antioxidant capacity, medicinal and aromatic plants arise as option to enrich human diet with compounds capable of protecting intestinal epithelium from oxidative damage, and thus preventing the development of such diseases.
Among medicinal plants, and more specifically *Thymus* genus, several species with high market value, such as *Thymus vulgaris* or *Thymus mastichina*, which are currently present in human diet, have an extensive list of health-promoting activities described, such as the antioxidant activity [1,2]. However, other species of this genus are lesser known for their potential bioactivities. As examples, *Thymus carnosus* and *Thymus capitellatus*, both endemic of the Iberian Peninsula, who are listed as endangered species, are still poorly described regarding their potential use in human health, and the validation of new applications could boost a sustainable crop and status change. Thus, we aimed to study their future inclusion in the diet, by evaluating their safety and their ability to protect against oxidative damage at intestinal level.
For this purpose, aqueous decoction extracts were obtained from *T. carnosus* and *T. capitellatus* aerial parts, collected at Arrábida National Park. As medicinal plants are frequently consumed in infusions or condiments, this extraction method was chosen because it mimics natural human consumption [3]. The safety profile of these extracts was evaluated using a well characterized cell line model, Caco-2 (human colorectal epithelium) usually used in studies concerning the intestinal tract. Firstly, and using Alamar Blue® method [3], cells were exposed for 24 hours to a wide range of concentrations (50-500 µg/mL) of both extracts, to obtain information regarding their cytotoxicity. Based on these findings, Caco-2 cells were then exposed for 2, 4 and 8 hours to a non-cytotoxic range of concentrations (2.5-50 µg/mL), followed by the removal of the extract's solutions and the addition of culture media for 16 hours. After this period, the cells were exposed to and oxidative agent (250 µM tert-butyl hydroperoxide; *t*-BHP) for 4 hours. As a final step, Alamar Blue® method was performed to evaluate cell viability, and the results compared to a positive control where culture media was used instead of extracts. This procedure was optimized to simulate pre-exposure conditions and thus study the extracts' ability to sensitize intestinal epithelium protection to oxidative damage.
In Caco-2 cells only exposed to *t*-BHP, a cell viability of 6.0±1.8% was observed, and both species' aqueous extracts efficiently mitigated oxidative damage in a dose- and time-dependent manner. Although concentrations up to 10 µg/mL presented a limited ability to counter *t*-BHP-induced damage, at the highest concentration tested (50 µg/mL), cells pre-exposed to *T. carnosus* and *T. capitellatus* extracts presented a viability of >99% and >91%, respectively, representing an almost complete capacity to counter oxidative damage.
This protective effect should be further studied to unveil the metabolic pathways behind the antioxidant activity. Nevertheless, these findings contribute to increase the value of theses species to both food and pharmaceutical industries. And, as observed for other natural products, a phytochemicals-enriched diet could be a key factor to decrease the incidence of intestinal diseases.

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Plant Matrix Induces Thermal Degradation of Glucosinolates in *Brassica* Vegetable Broth

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Vegetables of the genus *Brassica*, such as cabbage, broccoli or kohlrabi, contain a wide array of different GLSs and therefore are a rich source of Glucosinolates (GLSs) in human nutrition. GLSs are amino-acid derived secondary plant molecules commonly attributed to plant defence systems after hydrolysis by the endogenous enzyme myrosinase [1]. GLS hydrolysis products include isothiocyanates (ITCs), nitriles, and epithionitriles. Importantly, the health-beneficial effects (such as anti-cancerogenic and anti-inflammatory), that are described for the consumption of *Brassica* vegetables, are mainly associated with the presence of ITCs [1,2]. Hence, conversion of GLSs to ITCs is of utmost importance to gain these effects. At the same time, many *Brassica* vegetables undergo thermal processing during food preparation (e.g., cooking or frying), which leads to degradation of myrosinase and therefore terminates GLS hydrolysis. Previous studies showed that in presence of plant matrix, GLSs are thermally degraded and mostly form nitriles over ITCs whereas in model systems using buffers, equimolar amounts of ITCs and nitriles were found [3]. The present study aims to better understand factors influencing GLS degradation during thermal treatment.

Differently concentrated broths from red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra* cv. 'Integro') and kohlrabi (*Brassica oleracea* L. var. *gongylodes* cv. 'Kolibri') were prepared by adding different volumes of water to chopped vegetables and subsequent boiling. The three preparations with matrix concentrations of 1 g/mL, 0.5 g/mL, and 0.25 g/mL were then filtrated and cooled on ice. Finally, the differently concentrated broths were aliquoted and boiled again for different time points (up to 120 min) to assess GLS degradation and formation of GLS degradation products over time. GLSs were analysed using HPLC-DAD while GLS degradation products were determined using GC-MS.

The results showed that plant matrix significantly increases nitrile formation during thermal GLS degradation in a concentration dependent manner. In the most concentrated kohlrabi broth, GLS degradation to the corresponding nitrile was enhanced up to 9-fold after 120 min of boiling. The highest rate of nitrile formation was found for 4-methylthiobutyl GLS (4-MTB) to form 4-MTB-CN. Interestingly, relative GLS degradation rates matched the nitrile formation rates. Strikingly, the same effect was also shown for allyl-GLS (sinigrin), which was added to the broth aliquots artificially before boiling. Comparable results were also shown for red cabbage broth, although they were less pronounced. The highest relative nitrile formation rates in red cabbage broth were found for 4-methylsulfinyl-GLS (4-MSOB), where sulforaphane nitrile (4-MSOB-CN) was formed. Over both matrices and all time points investigated, only trace amounts of ITCs were detected.

In summary, the present study provides further insight on how plant matrix increases thermal GLS degradation to nitriles in a concentration dependent manner. Therefore, GLSs are shown to not only degrade due to thermal energy, but also by interaction with matrix components. Identifying such components could help to shift this trend towards higher ITC formation in thermally processed *Brassica* vegetables. This would help to maintain or even increase the health-promoting properties of these foods in the future.

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A simple high performance liquid chromatographic method for the determination of glucosamine used in the treatment of periodontal disease

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Periodontal disease is one of the most common diseases which can affect the adult population. The treatment of this disease consists on the cleaning of the gum around teeth, in this way it is prevented the damage to the soft tissue and surrounding bone which can lead to loosen teeth. In the incipient part are used different drug and antibiotic to treat this disease and on the advance part the surgical treatment are used [1, 2]. Glucosamine hydrochloride is one of the drugs used to treat the incipient periodontitis and have a higher purity then glucosamine sulphate which led to a smaller quantity is used for treatment [3]. The glucosamine can be obtained usually from the shellfish skeleton, but it can be obtained also from cereals fermentation process using different laboratory procedure [4]. The positive effect of the glucosamine on the periodontal disease is validated with different clinical studies. The determination of glucosamine hydrochloride in pharmaceutical drug and biological fluids can be determined using modern equipment like high-performance liquid chromatography (HPLC) and gas chromatography (GC) [5, 6]. The detectors used for determination of the glucosamine hydrochloride are refractive index detector (RID), corona charged aerosol detector (CAD), evaporative light scattering detector (ELSD) and diode array detector (DAD) [7, 8].
The high-performance liquid chromatography using evaporative light scattering detector (HPLC-ELSD) is a reliable test method to quantify the amount of glucosamine hydrochloride, an active ingredient in the pharmaceutical formulation. Evaporative light scattering detector has a higher sensitivity of the response and it is a well-adapted technique for the determination of the sugar. In the present study a new method using high-performance liquid chromatography coupled with evaporative light scattering detector (HPLC-ELSD) it is developed, method validation and uncertainty measurement were established. The developed and validated method for determination of glucosamine hydrochloride present in pharmaceutical formulation was demonstrated by a good overall recovery and the precision indicated by the percent relative standard deviation. For a good evaluation of the method performance was studied the accuracy, specificity and precision of the developed method. The developed and validated method is simple, rapid and robust, and requires no derivation procedures of the sample, and has no interference with other ingredients present in pharmaceutical formulation.

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Development of high-protein and low saturated fat bread formulations enriched with microalgae

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Bread is one of the most important food products in the world and a staple on the diet and cultural identity of a number of countries worldwide. Nevertheless, the modern times have raised the awareness for the need to develop food products with higher contents of nutrients that are either lost during the industrial production process or are lacking in the person's diet. Microalgae are known to possess a great array of nutrients of interest for the food industry, such as proteins, polyunsaturated fatty acids, vitamins, and minerals [1, 2]. The main objective of this study was the development of a bread enriched with microalgae, with high protein and low saturated fat content. Three formulations with different combinations of white, organic, and wholemeal flours were developed: WSL, with Wheat, Spelt, and Lupin; WRL, with Wheat, Rye, and Lupin; and WBL, with Wheat, Buckwheat, and Lupin. A combination of two types of *Chlorella vulgaris*, Smooth and White, produced by Allma [3] were used at two percentages, 1 and 3 %. The proximate analysis (Table 1) demonstrate that the bread formulations were high in protein, with the SL with 1% of microalgae addition presenting values of 20.39 ± 0.98 g/100g, RL with 3 % of microalgae presenting 18.24 ± 0.63 g/100g and BL presenting 18.69 ± 0.32 g/100g. All formulation, with the exception of BL3 presented values <1.5 g/ 100 g of saturated fat and thus can present the claim of low in saturated fats. Preliminary sensory analysis showed that the breads were palatable with a market potential.

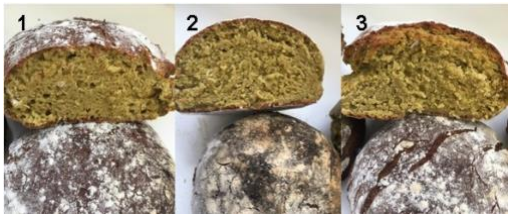

Fig.1. Bread formulations developed. Bread formulated with 1) WSL; 2) WRL; and 3) WBL.

Table 1. Proximate analysis of the different bread formulations developed. WSL – formulation with wheat, spelt, and lupin; WRL – formulation with wheat, rye, and lupin; and WBL – formulation with wheat, buckwheat, and lupin. 1 and 3 represent the percentage (1 and 3 %) of microalgae (White and Smooth *C. vulgaris*) addition.

Formulation	Protein (g/100 g))	Lipids (g/100 g)	Saturated Fats (g/100 g)	Moisture (%)	Ashes (%)
Wheat (Control)	11.37 ± 0.28	1.35 ± 0.33	0.90 ± 0.11	44.59 ± 1.02	1.46 ± 0.05
WSL1	20.39 ± 0.98	3.77 ± 0.17	1.50 ± 0.03	45.84 ± 0.83	1.51 ± 0.02
WSL3	19.47 ± 0.36	3.89 ± 0.17	1.37 ± 0.18	47.93 ± 1.00	1.65 ± 0.04
WRL1	17.96 ± 0.31	3.55 ± 1.35	1.33 ± 0.09	45.65 ± 0.07	1.48 ± 0.00
WRL3	18.24 ± 0.63	3.79 ± 0.85	1.38 ± 0.08	47.99 ± 0.43	1.56 ± 0.04
WBL1	18.61 ± 0.08	3.14 ± 0.31	1.39 ± 0.11	46.34 ± 0.82	1.61 ± 0.13
WBL3	18.69 ± 0.32	3.49 ± 0.08	1.67 ± 0.07	48.01 ± 0.45	1.64 ± 0.02

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Optimization of microwave-assisted digestion prior plasma-based spectrometric techniques for the tissue distribution of gold

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Introduction. Nowadays, the gold nanoparticles are used for a multitude of purposes: diagnostic, therapeutic and research. Gold nanoparticles have potential applications in biomedicine, due to their unique optical, surface and electronic properties, being an attractive candidate in therapy as targeted delivery systems and, in cancer diagnosis due to the considerable higher absorption ability in cancer cells than in normal human cells [1]. Consequently, precise, validated and easily applicable analytical tools are required to evaluate the biodistribution of gold in different tissue samples after gold nanoparticles administration [2]. In this regard, a simple, efficient and cost-effective microwave-assisted (MW) method was developed and optimized for the digestion of mice tissue prior the determination of gold by inductively coupled plasma mass spectrometry (ICP-MS).
Experimental part. Ultrapure HCl 30% and HNO_3 65%, and gold standard solution 1000 mg/L were purchased from Merck. *In vitro* animal study was performed on house mouse (*Mus musculus*). Following single dose gavage administration of different amount of gold nanostructured compound in phosphate-buffered solution, the animals were sacrificed and different tissue specimens (liver, heart, small intestine, lungs, brain and kidneys) were collected. Experimental protocol was carried out with the Institutional Committee approval. The samples were digested using concentrated acids in closed polytetrafluoroethylene (PTFE) vessel using a Speedwave Xpert microwave digestion system (Berghof). The digested samples were quantitatively transferred into volumetric flasks and diluted to the mark with ultrapure water. Three replicate measurements were carried out for each sample. The gold content was measured using a Perkin-Elmer Elan DRC II inductive coupled plasma mass spectrometer (ICP-MS). After the optimization of the microwave-assisted decomposition procedure, the MW/ICP-MS method was used for the digestion of mice tissues and the subsequent gold quantification.
Results and discussion. Since the efficacy of gold nanoparticles medical applications for largely depends on the control of their distribution within the body, it is important to determine their concentration in the organs of interest. A correct choice of the extractant is fundamental for the extraction of analytes from solid, tissue specimens. Among the acids employed for the treatment of the sample matrix with microwave-based instruments, HNO_3 and HCl are the most suitable due to their strong microwave-absorbing properties and interaction with the matrix (leading to efficient extraction), solubility of the analyte and availability at high purity. For the optimization of the microwave-assisted digestion procedure different sample amounts, concentrated acid volumes and digestion times and digestion temperatures were tested. The determination of gold was performed ICP-MS to evaluate the accuracy of the proposed microwaved-assisted method. A ratio of 6:1 HNO_3:HCl (*v/v*) was the most efficient digestion media. Efficient extraction of gold was achieved at a digestion temperature of 180 ^oC for 40 min. The biological matrix effects on the accuracy of ICP-MS measurement were investigated by measuring gold nanoparticles solution at known concentrations mixed with liver. With the optimized conditions, the proposed MW/ICP-MS method provided satisfactory precision and detection limit (LOD, 0.015 µg/g gold in tissue specimens). The low recovery yield of gold at the lower concentration may be due to the absorption of gold ions and/or nanoparticles onto the surface of instrument or vessels. The obtained results demonstrated that the optimized microwave-based approach constitutes a good alternative sample preparation methodology for the determination of gold in mice tissue samples.
Conclusions. Considering the obtained results, the optimized microwave-assisted digestion method demonstrates robustness, efficiency and reliability. Furthermore, the proposed microwave-assisted method offers the following advantages: (*i*) less extractant (acid) volume, (*ii*) quantitative recoveries under optimal conditions, (*iii*) the possibility of performing multiple, simultaneously extractions and (*iv*) rapid turn-around time. Therefore, the optimized microwave-assisted digestion procedure prior plasma-based spectrometric technique (ICP-MS) is an important tool for the determination of gold in biological tissues after gold nanoparticles administration.

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A novel ultra-high pressure liquid chromatographic method for the determination of vitamin D3 in simulated human gastrointestinal tract

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Introduction. Cholecalciferol (CHL), also known as vitamin D3, is a fat-soluble micronutrient that plays an important role in maintaining bone, teeth and cartilage health, as well as in the proper function of the immune system [1]. CHL is involved in calcium and phosphorus homeostasis and skeletal mineralization, its deficiency being associated with osteoporosis and osteomalacia in adults and rickets in children. The determination of the vitamin D3 levels can provide valuable information regarding the vitamin D absorption rate, other diagnostics and scientific purposes [2]. However, the relatively poor stability and low-accessibility of vitamin D3 contributes to the vitamin D3 deficiency. In this regard, the oil-in-water emulsions is a good vehicle for the delivery of vitamin D3 in order to enhance its stability and bioaccessibility in food supplements [3]. This study aims to develop a simple, rapid, sensitive, robust and effective ultra-high pressure liquid chromatography (UHPLC) method to explore the stability and bioaccessibility of vitamin D3 (CHL) encapsulated within oil-in-water emulsions in a simulated human gastrointestinal tract (GIT) model.

Experimental. Materials. All chemicals were of analytical grade (Merck), excepting crystalline vitamin D3 standard 99.9% (Thermo Scientific) and were used as received without further purification. Human salivary α-amylase (300-1500 U/mg), pepsin from porcine gastric mucosa ($\geq$2500 units/mg), porcine pancreas lipase (100−500 units/mg) and bovine bile from Sigma-Aldrich and rabbit gastric extract for gastric lipase ($\geq$500 units/mg) from Lipolytech were used. Corn and flaxseed oils were purchased from a commercial food supplier. Ultrapure water from an Evoqua (Siemens) system was used for all dilutions and for the preparation of standard solutions. Emulsion preparation. Oil-in-water emulsions were prepared using 10 w/w oil phase (1 w/w vitamin D3 + 9 w/w flaxseed oil) and 90 w/w aqueous phase (5 mM phosphate buffer solution, pH=7). Emulsions containing 4 wt % oil phase (0.4 wt % vitamin D3) were obtained by diluting stock emulsion in phosphate buffer solution. Simulated GIT model. In vitro simulated GIT included mouth, gastric and small intestinal phases. The test samples were passed through the simulated GIT to simulate the passage upon mouth (simulated salivary fluid, SSF, pH=6.8, 2 min), gastric (simulated gastric fluid, SGF, pH=3.0, 2 h) and intestinal phases (simulated intestinal fluid, SIF, pH=7.0, 2 h). Vitamin D3 bioaccessibility. was calculated as the fraction dissolved within the mixed micelle phase after small intestine digestion. Vitamin D3 extraction and HPLC analysis. The stock solution of vitamin D3 standard was prepared (1 mg/mL) in methanol and stored at -20°C. The calibration solutions were prepared by diluting the stock solution with methanol. 1 mL of simulated GIT was diluted using 9 mL methanol. The sample was then filtered through 0.45 µm cellulose filter and injected into the ultra-high pressure liquid chromatograph (UHPLC, Vanquish, Dionex Softron GmbH) equipped with an Acclaim C30 150x46 µm, 5 µm (Thermo Scientific) separation column. The column oven temperature was set at 30 °C and the injection volume was 5 µL. A mobile phase consisting in a mixture of methanol (98%) and ultrapure water (2%) at a flow of 1 mL/min was used.

Results and discussion. The calibration curve was found to be linear in the range 25-200 µg/mL, with a correlation coefficient (r^2) of 0.99955. The detection limit (LOD) was 0.03 µg/mL and the quantification limit (LOQ) was 0.1 µg/mL. The method proved to be accurate, with mean recoveries ranging between 84.1 to 95.4%. The influence of mono-unsaturated corn oil and polyunsaturated flaxseed oil on the bioaccessibility of the vitamin D3 was also investigated using a three-stage static *in vitro* GIT model. The vitamin bioaccessibility was significantly ($p<0.05$) higher for the corn oil-in-water emulsion than for the flaxseed oil-in-water emulsion, due to the different extent of digestion of the carrier oil and nature of the formed mixed micelles.

Conclusions. LOD and LOQ were relatively low in comparison to other existing HPLC methods. The developed method is accurate and simple, can achieve high throughput and may be used to monitor vitamin D3 status in clinical practice and research. Also, the current study offers a better insight of how carrier oil type impacts the bioaccessibility of vitamin D3 encapsulated in plant-based emulsions (flaxseed and corn oils). The obtained results indicated that the digestion and bioaccessibility of the vitamin D3 is dependent on the carrier oil type.

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Seasonal variations in the fatty acid profile of unexploited and low commercial value fish species from the Portuguese coast

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Fish discards are a widely recognized problem resulting from fisheries worldwide. The valorisation of discarded fish is important not only for the fishery sector, but also for marine systems sustainability [1]. However, there is a lack of information regarding nutritional profiles and seasonal changes of non-target species. In this study, the fatty acid profile of two unexploited (*Serranus cabrilla*, *Capros aper*) and three low-commercial value (*Trachurus picturatus*, *Spondyliosoma cantharus* and *Trigla lyra*) fish species captured on the Portuguese coast over one year were analysed. The main fatty acids present in the five species were palmitic acid (C16:09, oleic acid (C18:1), EPA (C20:5ω3), and DHA (C22:6ω3), except for *C. aper*, that presented lower contents of EPA and higher contents of erucic acid (C22:1ω9) instead. All the species presented good amounts of omega-3 FAs (70-90% of PUFAs). Seasonal variations in the total FA amount and in the FA profile were observed for all the species, although the highest variations were obtained for *C. aper* (maximum peak in May/Jun) and *T. picturatus* (peak from Mai/Jun to Sep/Oct). The peak in the FA content could be associated to the spawning season of the species and/or food availability. Therefore, these unexploited and low-commercial value fish species demonstrate a great potential to become more commonly consumed fish species. The nutritional information on these less consumed species will be valuable to consumers wishing to make a more varied fish diet and a more conscious fish consumption, as well as for the fish industry that may use these less exploited species in the development of nutritious marine-based food products [2,3].

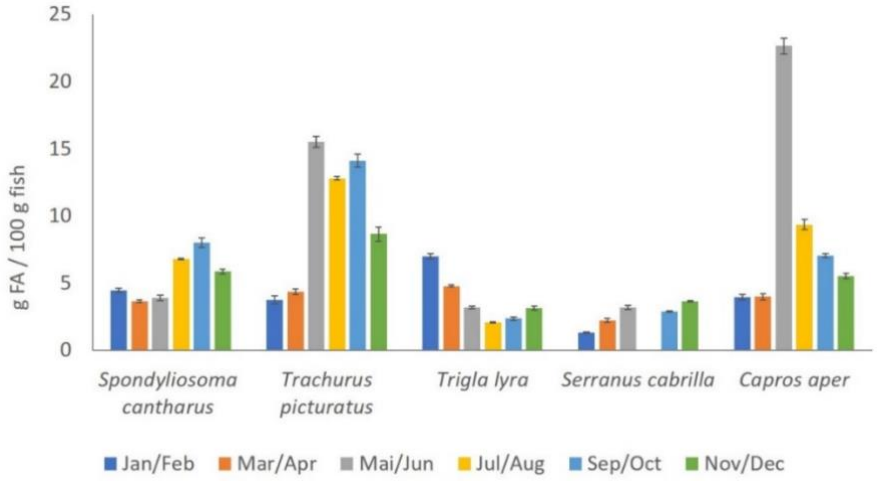

Fig.1. Annual variations in the fatty acids content for the five fish species studied.

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Potential use of edible flowers as substitutes of synthetic antioxidants

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The consumption of flowers as food is reported in various cultures around the world as part of traditional cuisine or alternative medicine, in addition to their wide use as ornaments. In this scenario, flowers represent an important segment to expand food market, due to their suitable sensory and nutritional characteristics, as well as by the presence of bioactive compounds with antioxidant capacity, which can be used to replace synthetic antioxidants [1–3].
Therefore, the present study determine the phenolic composition and antioxidant capacity of five different edible flowers, namely *Viola tricolor, Rosa, Pelargonium graveolens* and two different species of *Calendula officinalis* L. For each edible flower, the goal was to evaluate the phenolic composition and antioxidant capacity, and for that we performed the determination of the total polyphenolic, *ortho*-diphenols and flavonoids contents, as well as an accurate quantitative and qualitative determination of phenolic compounds by HPLC-DAD.
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 GA/g; between 0.89 ± 0.00 and 222.67 ± 0.02 mg GA/g, and from 5.53 ± 0.38 to 12.97 ± 0.71 mg CAT/g, respectively. For the antioxidant capacity, using the FRAP methodology, the results ranged from 0.08 ± 0.00 to 0.84 ± 0.02 mmol trolox/g, for the ABTS•+ methodology the values obtained ranged from 0.14 ± 0.01 to 0.93 ± 0.02 mmol trolox/g, and for the DPPH• methodology results ranged between 0.05 ± 0.00 and 0.87 ± 0.00 mmol trolox/g. Regarding the determination of phenolic compounds by HPLC-DAD, 17 compounds belonging to flavonoids (flavonols and anthocyanins) and non-flavonoids (phenolic acids) groups were identified. Thus, this study revealed the possibility to use edible flowers with objective to replace synthetic antioxidants.

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Assessment of the possibility of enclosing chokeberry powder inside a polysaccharide capsule using microencapsulation by extrusion

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Microencapsulation has been used in various industries for many years [1-2]. In the food manufacturing industry, its initial purpose has changed. Originally, the main purpose of its use was to introduce additives into food in order to improve taste and smell as well as to extend product shelf life. Currently, it is increasingly used as a way to protect bioactive compounds such as: anthocyanins, vitamins and polyphenols [3]. The microencapsulation process consists in enclosing the encapsulated material inside the shell by creating a thin film layer on the surface of the substance inside. The described phenomenon prevents the encapsulated substance from coming into contact with its surroundings, thus the material gains protection from harmful external factors such as high temperature, unfavorable environmental conditions, and UV radiation [4]. The microencapsulation technique also allows for a more controlled release of the encapsulated material inside the body by extending the release time [5].
The aim of this study was to evaluate the feasibility of using microencapsulation to enclose isolated bioactive compounds from chokeberry inside a polysaccharide capsule using an extrusion method and to determine the effect of the coating material used on the stability of polyphenolic compounds quantified by ultraperformance liquid chromatography (UPLC-PDA) [6], antioxidant activity (measured by ABTS [7], FRAP [8] and ORAC [9] methods) and ability to inhibit α-amylase and α-glucosidase [10]. The test material was chokeberry microspheres, obtained by adding a powdered preparation of isolated chokeberry bioactive compounds to a polymer solution. A solution of sodium alginate (1% w/v) was mixed with 0.8 g of chokeberry powder and then infused into a 1.5% (w/v) sodium chloride solution. In order to determine the potential effect of the encapsulating substance on the content and stability of the bioactive compounds, guar gum and chitosan were added to the basic coating material, which was a sodium alginate solution, resulting in four different variants of the encapsulating material, including a ternary coating. Finally, the type of coating material used was shown to be significant in shaping the content of polyphenolic compounds in the capsule and the antioxidant activity. The presence of guar gum in the coating solution, increases the stability of anthocyanins during storage. The combination of three polysaccharide materials (sodium alginate, guar gum and chitosan) is the most advantageous option from the point of view of preservation of polyphenols in microspheres (maintenance of approximately 87% initial content of polyphenolic compounds after four weeks of storage at 4°C). Chokeberry microspheres composed of a solution of sodium alginate and guar gum showed the highest potency of ABTS cation radical scavenging (0.840 mmol Trolox/100 g product) and the highest ability to reduce FRAP iron ions (0.771 mmol Trolox/100 g product). This variant, also showed the highest ORAC free radical adsorption capacity in freshly obtained microspheres (2.154 mmol Trolox/100 g). However, the variant of the sodium alginate-chitosan capsule showed the strongest α-amylase and α-glucosidase inhibition properties.

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Interaction of SARS-CoV-2 Spike protein with phycocyanobilin

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Algae have been consumed as food and medicine for centuries. Their benefits are so pronounced, due to high concentrations of vitamins, minerals, antioxidants and proteins that they are commonly referred to as superfoods. Phycocyanobilin (PCB) is a bioactive compound of microalga *Spirulina platensis*. It is a blue tetrapyrrole chromophore of C-phycocyanin (C-PC), the major chromoprotein of this microalga. It is covalently attached to cysteine residues of C-PC via thioether bond. The outbreak of Coronavirus Disease 2019 (COVID-19) has posed a serious threat to global public health, calling for the development of safe and effective prophylactics and therapeutics against infection of its causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The SARS-CoV-2 spike (S) protein plays the most important roles in viral attachment, fusion and entry, and it serves as a target for development of antibodies, entry inhibitors and vaccines. It mediates viral entry into host cells by first binding to a host angiotensin-converting enzyme 2 (ACE2) receptor through the receptor-binding domain (RBD) and then fusing the viral and host membranes. This study aimed to investigate interaction of bioactive PCB with S protein and RBD respectively.

Combination of electrophoretic techniques and fluorescence spectroscopy was employed in order to examine interactions of PCB and S protein, as well as interactions of PCB and RBD, while the effects of PCB binding on RBD structure were studied by CD spectroscopy.

SDS-PAGE with Zn^{2+} staining has revealed that PCB covalently binds to both S protein and RBD, via free cysteine residues. Binding constants determined by fluorescence quenching method were: 2.1×10^7 M^{-1} for PCB and S protein, and 8.4×10^4 M^{-1} for PCB and RBD. Far-UV circular dichroism spectra showed that PCB influences RBD structure.

Our results support the importance of further research on covalent binding of PCB to S protein and RBD and its implications. Due to its interaction with S protein and RBD, PCB may exert one of its many bioactive effects via impact on S protein binding to ACE2 receptor.

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CBD-based food supplements: Are phytocannabinoids transferred to the brain? (case study)

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Phytocannabinoids are biologically active substances occurring in cannabis plants (*Cannabis sativa* L.). These substances have wide therapeutic potential. The most important phytocannabinoids include the psychotropic delta-9-tetrahydrocannabinol (Δ^9-THC) and its nonpsychotropic isomer cannabidiol (CBD) with antioxidant, analgesic, and neuroprotective effects. Phytocannabinoids are widely used to alleviate the symptoms of various diseases, they are administered either in a form of isolated substances or as cannabis extracts. The metabolites of individual phytocannabinoids are also biologically active. The potential therapeutic effect of the complex of these substances depends on their bioavailability, i.e., transfer to the target tissues, which is affected by dose, route of administration, physiological factors (absorption, metabolic rate, excretion), and of course the vehicle. The number of publications describing the transfer of phytocannabinoids and their metabolites to target organs such as the brain (the target tissues for the treatment of certain diseases with a large number of cannabinoid receptors) is still very limited [1-4].

Cannabinoids, especially CBD, are components of various, nowadays very popular dietary supplements represented mostly by CBD oils. However, this route of oral administration has a worse bioavailability of CBD compared to the intravenous or inhalation route. Although CBD oils are still preferred by consumers, the market is currently evolving toward more sophisticated products, exemplified by various liposomal forms of phytocannabinoids [4-8].

The aim of this case study was to evaluate the transfer rate of CBD to the brain tissue of rats fed through gavage by various forms of CBD. Five different CBD carriers were tested compared to CBD oil as a reference. Experimental animals were sacrificed at regular intervals (0, 30, 60, 120, 240, 360 min) after exposure. Subsequently, brain tissue samples from experimental animals were analyzed by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) using a newly developed method for the determination of 17 phytocannabinoids, three Δ^9-THC metabolites, and two CBD metabolites.

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Potential of Natural Flavonoids in skin photoprotection

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The skin is the main organ that protects the human body from external factors, however it is also necessary to protect against them. One of the main external stresses to the skin is exposure to ultraviolet radiation (UVR) from the sun, causing several biological effects to skin cells, such as oxidative damage from reactive oxygen species (ROS), inflammatory processes, photoaging, mutation, cell cycle dysregulation and cancer. A form of skin cell's self-protection, when exposed to UVR, is melanin (synthesized from tyrosine), which is produced by melanocytes and then transferred to neighbouring keratinocytes, forming a photoprotective barrier. However, this photoprotection is not always effective enough against UVR, being necessary an external barrier to reduce the amount of ROS produced by UVR exposure. Antioxidants demonstrate high UVR absorbing power and high scavenging activity against harmful ROS. Flavonoids are among the compounds with high antioxidant power. This group of natural compounds is produced by the secondary metabolism of plants, being their synthesis modulated by stress, aiming plant protection, e.g. protecting them from UVR, showing therefore great interest in the application as skin photoprotector agents.
The main objective of this study is to understand and classify the photoprotective capacity of some flavonoids.
To fulfill the objectives, a survey of some characteristics of three different flavonoids was carried out, Epigallocatechin-gallate (EGCG), Apigenin and Naringenin. The research focused mainly on factors that lead to the perception of how these compounds modulate the protective capacity of skin cells against UVR, such as UVR absorption power (sun protective factor (SPF)), cytotoxicity against cells (melanocytes), intracellular tyrosinase activity (melanocytes) and intracellular melanin content (melanocytes) by different flavonoids.
The SPF is the capacity of each compound to absorb UVR, Anwar and Rizkamiarty [1] demonstrated that EGCG has a high SPF value, following a dose dependent pattern, Apigenin also presents a dose dependent SPF, however with values relatively lower than EGCG [2]. On the other hand, Naringenin does not have a dose-dependent factor and its SPF value is much lower compared to EGCG and Apigenin [2].
However, the exposure of melanocytes to different flavonoids demonstrates different toxicities, all of them with a dose dependent trend, in cancer cell lines the order of toxicity was Naringenin > Apigenin > EGCG (for the cell lines B16F10, A375 and B16F10) [3-5].
The tyrosinase enzyme activity may directly correlates with the amount of melanin present in melanocytes. The effect of flavonoids on tyrosine activity depends on the flavonoid, EGCG dose-dependently reduces the enzyme activity, and the same is verified in the melanin content[3]. However, Apigenin and Naringenin showed a tendency to increase the tyrosinase enzyme activity, and consequently an increase in the melanin content with the increase in a concentration-dependent manner, showing a biological effect at the molecular level [5-7].
We conclude that EGCG, Apigenin and Naringenin show photoprotection, but EGCG show greater activity in UVR absorption, while Apigenin and Naringenin present higher bioactivity at the cellular level. Apigenin is the one that stands out among of all the characteristics and bioactivities.

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Insight into the interaction of NaCl with sugars from computer simulations

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Thermodynamic and transport properties of aqueous solutions containing carbohydrates has proved to be very useful in several fields, such as food technology, chemical, biological and biochemical phenomena.
Electrolytes play a crucial role in living beings and among these electrolytes, NaCl is essential for living organisms and is omni-present in them, conditioning all their vital processes. In turn, sodium chloride is a well-known flavouring agent and a food preservative, which usually accompanies sugar in food.
Although binary diffusion coefficients of lactose, sucrose and sucralose in aqueous solutions have been reported [1-3], no data on the effect of sodium chloride on the diffusion, in ternary systems, have been reported so far. In this poster we present the results obtained from Molecular Dynamics and comparison with experimental results. We generated the forcefield for each sugar and calculated the diffusion coefficients using best-practices [4]. GROMACS [5] was used for all Molecular Dynamics calculations.

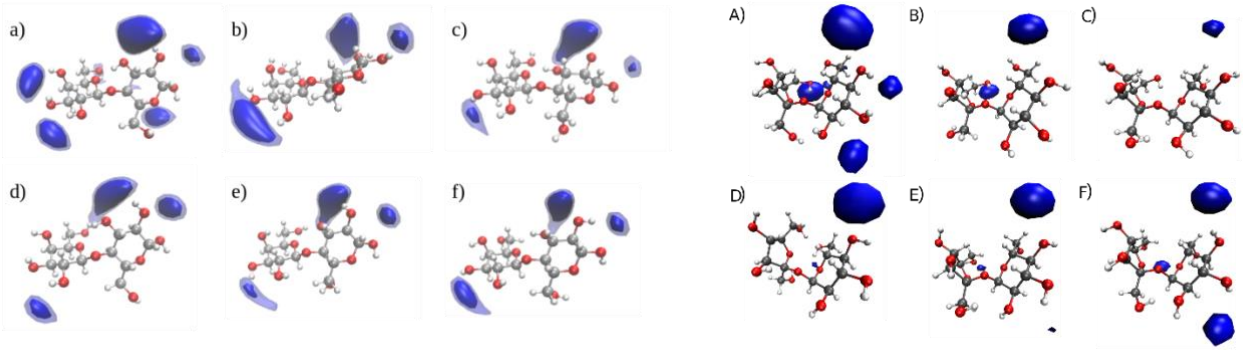

Fig.1. Spatial distribution functions (SDF) calculated from the MD simulations of lactose with NaCl. Plots take the lactose molecule as reference. The particle density along the dark (light) blue iso- surface is 2.8 (2.35). Six NaCl concentrations were considered for both figures: a/A) 0.01 mol dm³ , b/B) 0.05 mol dm³ , c/C) 0.1 mol dm³ , d/D) 0.2 mol dm³ , e/E) 1.0 mol dm³ , and f/F) 2.0 mol dm³. Plots on the right take the sucrose molecule as reference. The particle density along the blue iso-surface is 2.2.

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Determination of mineral content in extracts of Centaurium *erythraea*

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Common centaury (*C. erythraea*) belongs to the Gentianaceae family. It grows in Europe, North Africa, and Southwest Asia. *C. erythraea* is a medicinal plant, which extracts have been traditionally used for treating gastrointestinal disorders, dyspepsia, constipation, fever, anemia, acute jaundice, chronic active hepatitis anorexia, rheumatism, wounds, and sores, to stimulate appetite, and to cleanse blood and kidneys. *C. erythraea* extracts are rich in secoiridoid glycosides, sterols, phenolic acids, coumarins, flavonoids, and xanthones.
In order to determine the mineral content of *C. erythraea* aerial herb, the concentrations of Ag, B, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, Zn, Si, P, and S were determined in three different extracts of *C. erythraea* (tea infusion, ethanol, and propylene glycol). *C. erythraea* was harvested from the Old Mountain during the flowering stage and dried to the moisture content of 7.89 (*w*/*w*) in a dark place. Elements content was analyzed by ICP-OES method (Inductively Coupled Plasma - Optical Emission Spectrometry, ARCOS FHE12, SPECTRO, Germany). Samples were prepared by wet digestion procedure before ICP-OES analysis.
The analysis showed the absence of Ag, Cd, Co, Ni, and Tl. Of all analyzed macro elements, the highest concentration in all three samples was observed for potassium (92.3 for infusion, 270.475 for propylene glycol, and 461.625 *μ*g/g for ethanol extract). Propylene extract contains the highest concentrations of calcium (852.95 *μ*g/g), while the concentrations of sulfur and phosphorus were higher in ethanol extract (55.825 and 322.55 *μ*g/g, respectively). The highest concentration of zinc, as a macro element, was found in tea infusion (2.675 *μ*g/g). Heavy metals were detected in almost negligible amounts.
*C. erythraea*can be found in forest meadows, glades, or sandy places. This species is utilized for its medicinal properties. Natural habitats of this species may be influenced by industrial pollution, which can lead to the contamination of plants by metals. Also, the diversity and content of the identified elements are conditioned by the solvent used for extraction. Further studies should be focused on the examination of *C. erythraea* as an accumulator or excluder of metals to ensure consumer safety.

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Impact of antifogging food packaging material on phenolic compounds in green and red lettuce cultivars

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Lettuce is a vegetable rich in health beneficial compounds [1]. Storage conditions are important to prevent post-harvest quality losses. To increase shelf life and protect the vegetables, plastic films are used for food packaging. Antifogging additives are not necessarily associated with our daily food. However, their application in plastic films for food packaging brings them into contact with it. They are used in such films to prevent the formation of water droplets on the packaging surface, as they are typically not aesthetically attractive and may increase food spoilage [2]. Information is lacking on the impact of such additives in food packaging materials on bioactive compounds like polyphenols in the packaged vegetables. In this study, we investigated the effect of antifogging additives in packaged green and red lettuce on flavonoid glycosides, caffeic acid derivatives, and anthocyanins during storage.

Lettuce was stored for 10 days at 7.5 °C and 64 % relative humidity in darkness. 50 g of each lettuce cultivar was packed in perforated polypropylene: half of the bags contained an antifogging additive (Atmer 1440), and the other half was additive-free. Weight loss and oxygen concentration in the food bags were measured. Phenolic compounds were determined via HPLC-MS^n.

Red lettuce contains higher amounts of phenolic compounds. No anthocyanins were detected in green lettuce. A low water loss of 0.51 % for green lettuce and 0.48 % for red lettuce was observed after 10 days. Our results show that oxygen concentration in the food bags as well as the total flavonoid glycosides, caffeic acid derivatives and anthocyanins were rather constant during storage of both cultivars. In addition, no differences were found due to the use of additives in the packaging with exception of the anthocyanins. Lettuce in packages with additives, showed significantly higher amounts of total anthocyanins compared to the package without additives at day 10. We assume that water-soluble anthocyanins probably tend to leach into the water droplets on the packaging surface.

Packaging materials provide conditions to protect the vegetable quality. Though, antifogging additives have a negligible effect on the bioactive compounds examined here. Their functions are therefore more characterized by consumer preferences and prevention of spoilage.

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Fatty Acid Composition of Mother Milk

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'Breastfeeding is one of the most effective ways to ensure child health and survival' – which is shown on the official webpage of WHO. Breast milk provides nutrition for infants and also contains a variety of bioactive factors that influence the development of the infant [1]. The nutritional quality of breast milk is constant within certain limits, but its composition may be affected by the eating habits or health problems of the mother. Fat is the most variable macronutrient in mother milk. The fatty acid profile of breast milk varies depending on the maternal diet, and some clinical trials show that obese or diabetic patients produce breast milk with an altered fatty acid composition [2].
The aim of our study was to provide information on the differences in the fatty acid composition of the breast milk of obese, non-obese gestational diabetic mothers, and obese gestational diabetic mothers.
Breast milk samples were collected from 15 mothers in Ukraine, represented four groups: Normal B*ody Mass Index* (BMI); Obese; Normal BMI with Gestational Diabetes (GD); Obese with GD. Twelve Hungarian mothers had normal BMI. The fatty acid composition of breast milk was determined by gas chromatography with a flame ionization detector.
The main fatty acids found in breast milk samples were palmitic acid (C16:0; 26-28%), oleic acid (C18:1, n-9; 23-28%), and linoleic acid (C18:2, n-9; 15-17%), followed by myristic acid (C14:0; 3-8%), lauric acid (C12:0; 4-6%) and stearic acid (C18:0; 4-5%). Monounsaturated fatty acids (MUFA) accounted for 29-38%. The amount of *polyunsaturated fatty acids (PUFA) was* less than 1%. Significant differences were found between groups for some anti-inflammatory saturated fatty acids such as caproic (C6:0), caprylic (C8:0), lauric (C12:0) and myristic acid (C14:0). Ratio of saturated fatty acids showed slight decrease in obese or obese with GD milk samples, at the same time the ratio of MUFA and PUFA showed slight increase compared to normal BMI milk samples.
The main fatty acid composition (palmitic acid, oleic acid, linoleic acid) showed only modest differences among the breast milk samples. In contrast, minor fatty acids (caproic, caprylic, lauric, myristic acid) showed greater changes according to the types of breast milk.
In conclusion, it is important to examine the nutritional and health factors that influence the fatty acid composition of breast milk and the role of fatty acids in strengthening the immune system and preventing disease.

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Enzymatic synthesis and biological evaluation of crude fructooligosaccharide preparations

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Over the last years, short-chain fructooligosaccharides (FOS) have gained significant attention as valuable components of food and dietary supplements [1]. Apart from implications as dietary fibers, sweeteners, and humectants, oligosaccharides are hailed for their prebiotic properties [2]. Moreover, a significant amount of research is dedicated to their role as anticancer agents. Despite the ongoing interest, both technical aspects, challenges of FOS production, and their anticancer role necessitate more in-depth investigations to understand their healthcare role better [3].

This study aimed to optimize critical FOS synthesis parameters using a commercial enzyme, Viscozyme L, to obtain a preparation with a high FOS content. Moreover, we evaluated the *in vitro* antioxidant capacity of the obtained preparations. The cytotoxicity of FOS has been tested in 2D and 3D tumor models using two different types of colorectal carcinoma cell lines HT-29 and HCT116. In addition, the FOS sensitization effect on cancer cell treatment with anticancer drugs 5-fluorouracil and doxorubicin has been studied. Towards this, a central composite design and response surface methodology evaluating the effect of pH, temperature, synthesis time, substrate and enzyme load on multiple responses, chosen as independent variables, were utilized to define optimal conditions. The individual mono- di- and oligosaccharide contents as well as the total FOS content as measured by HPLC-RI, were chosen as responses for the models.

Multi-response optimization indicated a pH of 5.5, the temperature of 56 °C, 5,5 hours of synthesis, and an enzyme load of 2,5% as optimal conditions. Under these conditions, a crude preparation with a total FOS content of 50% was obtained. The crude preparation showed potent capacity in four in vitro antioxidant assays: the total phenolic content, ABTS●+ scavenging assay, and CUPRAC, ORAC assays.

Cytotoxicity of FOS against tested cell lines was different. It did not reduce HT-29 cell viability even at the concentration of 2.5 mg/mL, whereas FOS inhibited the proliferation of HCT116 cells by 50% at 2.35 mg/mL. Also, the sensitization effect of FOS on HCT116 cell treatment with doxorubicin has been revealed.

Overall, this study shows the potential utilization of a commercial enzyme to obtain a preparation with a high FOS content and promising *in vitro* biological properties.

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Production of fructooligosaccharides by *Aspergillus welwitschiae* inulinase enzyme complex, obtained on natural substrate

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Fructooligosaccharides (FOS) are used in the human diet thanks to their prebiotic effect and they are generally regarded as safe [1]. The increase in the functional food that contains prebiotics, has been tremendous over the last two decades. The most suitable process for FOS synthesis is by enzymes [2], particularly the one produced by filamentous fungi from the genus *Aspergillus* [2,3]. *Aspergillus welwitschiae* FAW1 strain, isolated from the environment, has proven to be non-toxigenic and safe for use in food production. Moreover, the absence of ochratoxins and fumonisins production capability was molecularly confirmed by the absence of complete or critical parts of biosynthetic gene clusters. Growing fungi on the natural substrate, triticale (*Triticosecale* sp), led to the production of various enzymes from inulinase complex (InuA, InuE, FTase, FFase). Production of FOS has been tested in two possible ways with the obtained inulinase enzyme complex: (1) synthesis from sucrose – fructosyltransferase activity and (2) hydrolysis of inulin – endoinulinase and exoinulinase activity. The obtained FOS were detected by TLC and HPLC methods and characterized by examination of antioxidant capacity. Produced FOS showed significant antioxidant potential according to ABTS and ORAC which classifies them as important additives in functional food. These findings open up opportunities for an easy approach for FOS production by fungal inulinase enzymes, without their prior separation and purification.

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Resveratrol interaction with starch: implications on digestibility?

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Understanding the interactions of dietary compounds on our food and organism is a crucial step to developing non-pharmacological, diet-based strategies for disease prevention. Resveratrol (RSV) is a bioactive phenolic compound known for its numerous bioactive properties. Among them, its anti-diabetic properties are partly due to its inhibitory activity towards enzymes involved in carbohydrate digestion, such as α-amylase and αglucosidase [1,2]. Incorporating RSV on a broadly-consumed everyday product, such as bread, is a strategy to promote its intake. In this particular case, since bread has a high postprandial glycaemic response due to its high content in starch, the presence of RSV could represent a strategy to decrease starch digestibility, as seen in other interactions studies between starch and phenols [3,4]. Based on this, this study aimed to formulate added-value bread using RSV as a new ingredient and to evaluate the anti-diabetic properties of RSV in a starch matrix regarding digestibility and levels of glucose provided.

Wheat bread was formulated with 0.5% of RSV, baked, freeze-dried, milled and used for RSV extraction with 70% ethanol for 15 minutes. RSV in the extracts was analyzed by high-performance liquid chromatography (HPLC) as previously described [5]. In parallel, starch complexes with RSV were prepared by mixing wheat starch in 30% ethanol with 0 to 5% RSV (in a weighted base) for 20 minutes, at 70ºC to gelatinize [3]. The resulted precipitate was freeze-dried, ground, sieved through a steel 224-mesh sieve, and characterized by FT-IR Spectroscopy (FTIR) and Powder X-ray diffraction (PDRX). The supernatant was also freeze-dried and resuspended in 70% ethanol for RSV quantification by HPLC to estimate the binding amount and loading efficiency. Starch digestibility was evaluated for Englyst starch fractions, namely rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [6], through glucose amount of digested starch complexes with α-amylase (1300U) and α-amyloglucosidase (25U), quantified by gas chromatography after samples acetylation [7].

The amount of RSV recovered in the bread extracts represented 89.0% of the added amount. Regarding starch complexes, data from FTIR and PDRX allowed confirming the formation of an amorphous structure of starch with 2% of RSV (Starch-2%RSV), with 9.09±0.78 mg/g RSV bound and 46.99±4.78 % of loading efficiency. Complex with 1% of RSV was also formed, but with a lower binding amount, while complexes with 3, 4 and 5% were excluded due to the presence of free RSV in PDRX spectra. Regarding starch digestibility, no differences were found between Starch-2%RSV and starch with 0% of RSV. Indeed, Englyst parameters for these samples were respectively 17.3 and 16.3% for RDS; 38.7 and 37.8% for SDS; 44.2 and 44.7% for RS and glucose levels after 2 hours of digestion were also the same between samples. These observations were contrary to the expectation that phenolic compounds would modify starch digestibility [3]. It is possible that the starch matrix serves as a shield for RSV, obstructing its ability to act as an enzyme inhibitor. Overall, this work suggests that bread with RSV works properly as delivery of RSV in our daily diet, although RSV may not serve as an anti-diabetic agent under these conditions.

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Impact of phenolic acid derivatives on β-lactoglobulin stabilized oil-water-interfaces

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In dispersed food systems such as emulsions, oil-water interfaces are often stabilized by protein films. The inter- and intramolecular interactions of the adsorbed proteins determine the properties of the protein film and, thus, the stability of the emulsion. In aqueous model solutions, it was already studied that other food constituents such as phenolic acid derivatives interact with the proteins by non-covalent (at acidic conditions) or covalent (at alkaline conditions) interactions. However, the extent to which intermolecular interactions with phenolic acid derivatives affect the interfacial properties of a protein film has not yet been clarified.
The aim of this study was to investigate how non-covalent and covalent interactions with interfacial-active phenolic acid derivatives affect the stability of a protein film against mechanical stress and, consequently, the stability of the emulsion. For this approach, the whey protein β-lactoglobulin was chosen as model and the interfacial film with and without intermolecular interactions with phenolic acid derivatives was investigated at weak acidic (pH 6.0) and weak alkaline reaction conditions (pH 9) at hand of dilatational rheology (using oscillation of a droplet). In addition, phenolic acid derivatives were added to β-lactoglobulin-stabilized emulsions and the stability of the emulsions was characterized by optical microscopy and oil droplet size measurements.
The results showed that phenolic acid derivatives reduce the elasticity and, thus, the viscoelasticity of an interfacial protein film. The reduced elasticity most probably results from a lower extent of intermolecular interactions within the protein film, as protein-phenol interactions compete with protein-protein interactions at the interfacial protein film. While emulsions at weak alkaline reaction conditions with mostly covalently bound phenolic acid derivatives exhibited a high storage stability, non-covalently interacting phenolic acid derivatives at weak acidic reaction conditions led to an increased flocculation and creaming of the oil droplets. At weak acidic reaction conditions, intermolecular electrostatic repulsive forces (due to proximity to the isoelectric point) between proteins and between proteins and phenolic acid derivatives are low due to a lower net charge. The lower repulsion favours intermolecular interactions, both between proteins within the interfacial film and between interfacial films of two approaching oil droplets (Fig. 1, left side). At weak alkaline reaction conditions, the molecules involved are negatively charged, they repulse each other, and intermolecular interactions are sterically hindered (Fig. 1, right side).
The results of this work contribute to the systematic elucidation of the interactions between phenolic acid derivatives and proteins at interfaces in order to be able to specifically influence the stability of protein-stabilized emulsions and, thus, that of dispersed food systems in the future.

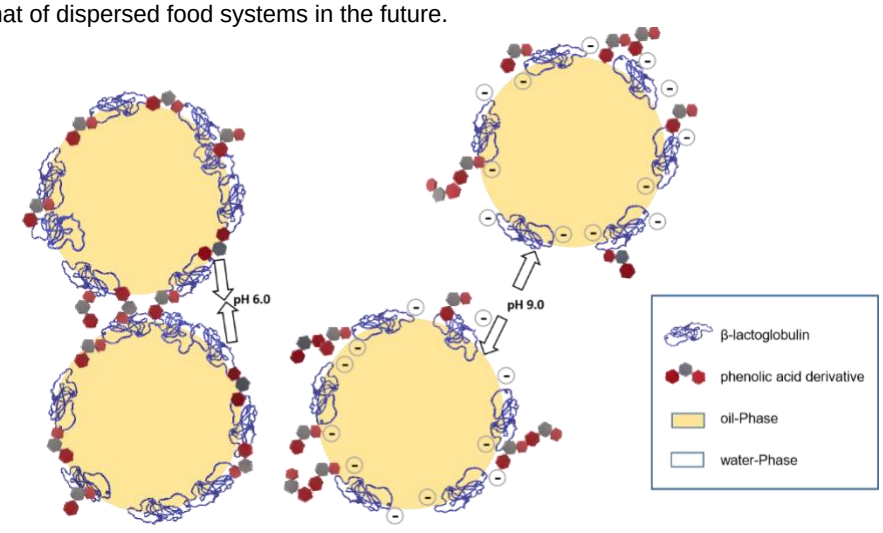

Fig.1. Approaching oil droplets, stabilized by proteins and including phenolic acid derivatives. Left side: pH 6.0, low electrostatic repulsive forces result in bridging flocculation through the phenolic acid derivatives; right side: pH 9.0, high electrostatic repulsive forces inhibit bridging flocculation through phenolic acid derivatives.

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Application of raw and defatted hempseed press-cake and sweetgrass antioxidant extract in pork burger patties

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During storage and processing various (bio)chemical reactions, including oxidation of lipids and proteins, and microbiological processes occur in the meat and may adversely affect its quality. These undesirable events may be controlled by various physical means and plant origin ingredients; some of which are good sources of natural antioxidants and antimicrobial agents [1]. In addition, plant ingredients may increase the overall nutritional value of meat products by enriching them with bioactive health beneficial compounds and other valuable nutrients, such as vitamins, dietary fiber, and minerals. Finally, some plants have become popular as cheaper substitutes for animal origin proteins [2]. Hemp seeds (*Cannabis sativa* L.) are good source of various nutrients. Recently the interest in hemp seeds as an excellent source of high-value oil and proteins has remarkably increased [3]. In addition, sweet grass (*Hierochloe odorata*) extract, which has demonstrated very strong antioxidant potential [4], was used. The aim of the study was to evaluate the physicochemical properties and oxidative stability of pork burger patties produced with the addition of dried mechanically pressed hemp seeds (2%), fully defatted by supercritical CO_2 extraction hemp seed (2%), sweet grass extract (2%) and sweet grass extract with dried pressed hemp seed additive (0.5 and 1.5% respectively). The patties were compared with the control sample (without additives) during storage on days 0, 4, 8, 15, and 21 at 4 °C in modified atmosphere conditions.
Hemp seed press-cake (1.5–2.0%) and sweet grass (0.5%) had insignificant effects on the majority of the measured physicochemical characteristics of pork meat patties, both after the addition and during storage, except for lightness L* value. Grilling losses were lowest in patties with fully defatted hemp seed flour, 14.3% (24.2% in control). The highest grilling loss (26.2%) was in patties with sweet grass extract, which indicates to the decreased water-binding capacity. pH values of grilled patties ranged within 6.1 – 6.3. Slightly higher pH values were in patties with hemp press-cake, most likely due to the addition of a small amount of buffer-type compounds present in hemp [5,6]. There were no negative effects on the sensory characteristics of pork meat patties with hemp seed press-cake, while patties with sweet grass extract received lowest scores due to its dark green colour and bitter taste notes. Raw (with residual oil) hemp press-cake increased the formation of oxidation products in meat patties, while the application of sweet grass extract as natural antioxidant effectively inhibited the oxidation process, which was determined by measuring the content of malondialdehyde.
Current research showed that hemp seed press-cake ingredients may be used in the production of pork patties; the combination of raw hemp press-cake with sweet grass extract may substantially mitigate the pro-oxidative effects of residual and highly unsaturated hemp seed oil during storage. The use of selected plant-based ingredients in meat products in the study revealed their potential to improve shelf life and the yield of pork burger patties during thermal treatment.

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Influence of thickness on mechanical properties of composite biopolymer films based on sunflower oil cake

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The oilseed industry creates significant quantities of by-products that are currently underused. Application of by-products could be not only for animal feed but also for a wide range of food products, as well as biopolymer-based materials production. The use of biopolymers derived from agriculture products appears as an interesting alternative to synthetic polymers, that have been seriously damaging environment. Of particular importance is the use of various oilseed cakes obtained in the processing of pressing. Among five major oilseed plants cultivated (soybean, rapeseed, cotton, sunflower and groundnut), sunflower oil cake (SuOC) occupies the main part of the oil industry market in Serbia. Seed oil cake chemical composition is affected by different factors, primarily by the plant variety and growth conditions. Sunflower oil cake is rich in dietary fiber and proteins (roughly 500 g of protein/Kg of SuOC) [1,2]. Therefore, some authors [3] produced protein-based films with protein concentrates obtained from SuOC and others [4] produced biodegradable composite films based on whole SuOC. The application of biopolymer films as packaging materials is influenced by many film characteristics: mechanical, barrier, structural, optical, thermal, biological, etc. Mechanical properties are of great importance due to the preservation of packaged product integrity. The mechanical properties of biopolymer films depend on the structure of the polymer chains, the coherence of the polymer matrix, the interaction of the additive and the matrix, the mode of film production and the presence of plasticizers, additives used to improve film elasticity, with glycerol as representative and most commonly used plasticizer. The basic mechanical properties of packaging films are their elongation at break, tensile strength, and modulus of elasticity. [2] Film thickness was measured using a micrometer Digico 1 with sensitivity of 0.001 mm (Tesa, Renens, Switzerland), since it has a certain influence on tensile strength (TS) and elongation at break (EB), which were measured on the Instron Universal Testing Instrument Model No 4301 (Instron Engineering, Canton, Massachu- setts, USA), according to ASTM standard method D882-10. With this aim, composite sunflower oil cake films with three different thickness are produced and the influence of thickness on mechanical properties was examined. Results presented in the Table 1 show increase in tensile strength values, as well as in elongation at break values with increasement in values for thickness. Those results give important introductory research in finding optimal film properties, that can be potentialy used as packaging material in the food industry.

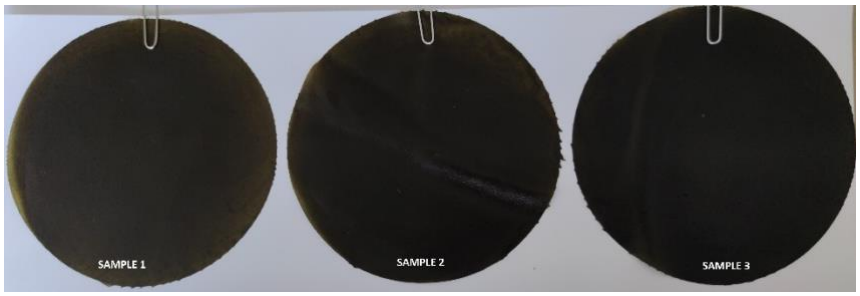

Fig.1. SuOC biopolymer films with different thickness (trend of increasing thickness from left to the right)

Table 1. Sunflower oil cake SuOC based biopolymer films mechanical properties with different thickness (mean values)

Biopolymer film sample	Tickness (μm)	Tensile strength (MPa)	Elongation at break (%)
1	99.44	22.63	19.52
2	111.47	30.30	27.54
3	136.32	32.63	35.17

Acknowledgments: This study was supported by the Ministry of Science and Technological Development of the Republic of Serbia, Project No. 451-03-9/2021-14/200134.

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Formation of epithionitriles in vegetables and metabolism in humans

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In presence of the epithiospecifier protein (ESP), which occurs in many *Brassica* vegetables, glucosinolates degrade enzymatically mainly to nitriles or epithionitriles [1]. So far only one ESP isoform was characterized in *Brassica oleracea* and the metabolism of epithionitriles in humans was unknown. Understanding the distribution and regulation of ESP will help to find strategies to shift the glucosinolate degradation to isothiocyanate formation will increase health beneficial potential of these vegetables.
We functionally characterized three ESP isoforms expressed in *Brassica oleracea* and studied their substrate specificity. Transcript and protein of BoESP1 and BoESP2 were expressed mainly in shoots, while BoESP3 was abundant in roots. Recombinant ESPs showed an isoform-specific substrate preference towards selected glucosinolates, a differing sensitivity to pH changes and all ESPs were functional *in vivo* as shown via expression in *Arabidopsis thaliana* [2].
Moreover, the human metabolism of the epithionitrile 1-cyano-2,3-epithiopropane (CETP) was studied after consumption of CETP-rich *Brassica* vegetables using UHPLC-ESI-QToF-MS in a first pilot study. Regarding the metabolism of epithionitriles, N-acetyl-S-(3-cyano-2-(methylsulfanyl)propyl-cysteine was identified to be the main and only CETP-metabolite detectable in human urine. Already three hours after consumption of CETP-rich *Brassica* vegetables the highest urinary concentrations were detected and declined thereafter, suggesting a high bioavailability and metabolism [3].
Understanding the regulation of glucosinolate metabolism *in planta* and bioavailability of products and human metabolism is a key to explore the full potential of these plant metabolites for plant and human health.

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Binding affinity ovalbumin on different type of microplastics using Langmuir isotherm

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Microplastics are plastic fibers, particles or films with diameters smaller than 5 mm and they have shown different effects on proteins [1]. Microplastics (MPs) are small in size, have low densities, can exist in the atmosphere for a long time and can easily be spread by wind [2]. The objective of this study was to investigate adsorption affinity of different types of MPs (polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC)) with ovalbumin. In this study ovalbumin, isolated from chicken egg white, was used. Plastics were mixed with ovalbumin for 1,2,4 and 19 h and then the absorbance of the remaining protein in the solution was measured at 280nm. In addition, Langmuir isotherm mathematical model to calculate the adsorption affinity of ovalbumin for MPs was used. We determined affinity constants by using Langmuir isotherm models for different particle size (PS 120 µm and PS 500 µm), different types of plastics (PET, PS and PVC) and pH values (3 and 7,2). Adsorption experiment results showed that adsorption depends on type of plastics. Our results showed that PVC did not adsorb ovalbumin, however, PET and PS have interacted with protein. Adsorption capacities of all analysed MPs increase with pH of solution. Under different pH values, MPs and protein have different charges that may affect adsorption characteristics. With increase of pH from 3 to 7, the affinity for protein adsorption increased 1.4 times for PS (smaller in size) while for PS (bigger size) and PET protein affinity was two times higher at pH 3.

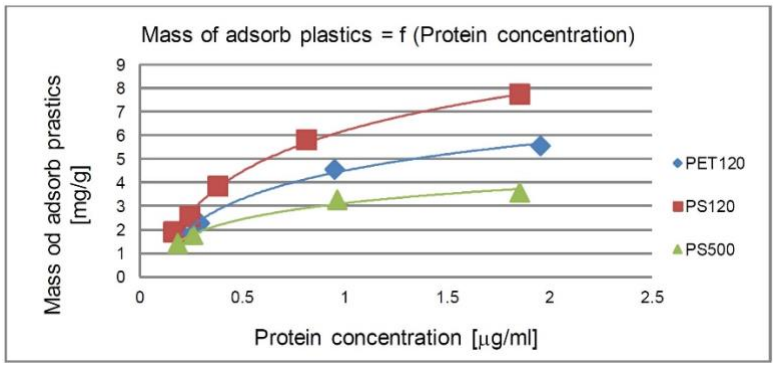

Fig.1. Langmur isotherm model; Mass of all type adsorb plastic in function of protein concentration, pH 7,2.

Table 1. Values of determined affinity constants, K_L, by using Langmuir isotherm models and ratio between different pH levels.

Plastics type and size	K_L pH 3,0	K_L pH 7,2	K_L pH 3,0/ K_L pH 7,2
PS 120µm	3,423 E+04	4,888 E+04	0,7
PS 500µm	4,293 E+04	2,285 E+04	1,9
PET 120µM	6,940 E+04	3,483 E+04	2,0

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Toxicological and anti-tumoral potential of pomegranate (*Punica granutum* L.) leaf infusion in HPV16-transgenic mouse model

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Punica granutum L. (pomegranate) is a well-known deciduous shrub, which has been developed into different functional foods due to its various beneficial effects [1]. However, knowledge of the *in vivo* biological properties regarding its leaf infusion remains scarce [2]. Thus, this work aimed to characterize the therapeutic capacity of the pomegranate leaf infusion (PLI) in HPV16-transgenic male mice for four consecutive weeks.

The polyphenolic profile of PLI was identified and quantified by RP-HPLC-DAD system. The stability of infusions was spectrophotometrically evaluated on the content of total phenols, *ortho*-diphenols, and flavonoids, as well as the level of free radical scavenging capacity during three days. The animal experiments were authorized by the Ethics committee of the University of Trás-os-Montes and Alto Douro (approval NO. 10/2013) and the Portuguese Veterinary Authorities (approval NO. 0421/000/000/2014), following the national legislation (Decree-Law 113/2013, August 7) and European Directive 2010/63/EU for the purpose of animal protection. Twenty wild-type (homozygous HPV16-/-) and eighteen transgenic (hemizygotic HPV16+/-) mice, with the age of 20–22 weeks, were divided into six groups according to different phenotypes and treatments: two groups were exposed to 0.5% PLI (0.5 mg dry leaf powder/100 ml water): WT 0.5% PLI (n = 7) and HPV 0.5% PLI (n = 6), two groups were exposed to 1.0% PLI: WT 1% PLI (n = 6) and HPV 1% PLI (n = 7), and two groups were exposed to cooled boiled water: WT (n = 7) and HPV (n = 5). After 30 days, all animals were sacrificed by intraperitoneal injection of xylazine-ketamine overdose. Blood samples were collected through intracardiac puncture in accordance with the Federation of European Laboratory Animal Science Association (FELASA) guidelines. Complete necropsies were conducted. Organs and skins were collected, weighed, and fixed in 10% neutral buffered formalin for histological analysis.

Results indicated that ellagitannin IV, followed by ellagitannin VII, were the most abundant components among the 18 identified polyphenolics. Phenolic classes and antioxidant activities of the infusion maintained high stability within 3 days, less than 10% degradation. Significant differences were observed between experimental groups concerning the mean body weight variation, mean daily consumption of drink and food, relative weight of internal organs, and different ratios of weight and length. The transgenic groups consumed more drink and food than the wild-type groups. Interestingly, the lower drink intake in the HPV 1.0% PLI group than other HPV groups can be possibly related to the taste of infusion. The HPV 0.5% PLI group showed the highest mean relative weight on all collected organs. Groups exposed to 0.5% PLI and WT 1.0% PLI presented lower risk of obesity. There were no statistical changes regarding haematological and biochemical parameters. Statistical alterations in the histological analysis of liver, kidney, ear skin, and chest skin were also found between different test groups. Double dose of infusion (1.0%) exhibited beneficial effects on nephritic and splenic histology.

The consumption of pomegranate leaf infusion showed anti-tumoral potential on the HPV16-transgenic mice, which deserves further studies on its functional profile and on its utilizations in food-pharma and nutraceutical industries.

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Methylglyoxal trapping ability of phenolic compounds from *Sambucus nigra* L.

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Sequential chemical reactions between reducing sugars and amino acid residues (e.g. lysine and arginine), lipids and nucleic acids, lead to the formation of complex and heterogeneous compounds named advanced glycation end-products (AGEs). The accumulation of AGEs are implicated in numerous diseases, such as neurodegenerative diseases, diabetes *mellitus* and its complications, however AGEs also accumulate with normal aging [1-2]. Due to their complexity, a potential strategy to reduce AGEs' impact in the organism might be acting at an early stage of glycation. Mainly, through trapping methylglyoxal (MGO) one of the most reactive precursor of AGEs [1-2]. Bioactive compounds, as polyphenols, may play an important role due to their properties as antioxidant, antiradical, metal chelation, and acting as natural inhibitors of MGO and/or AGEs [2]. *Sambucus nigra* L. or European elderberry is known to possess such bioactivities [3]. Thus, the main aim of this work was to evaluate the anti-glycative potential of elderberry polyphenols by evaluating their capacity to trap MGO. For this purpose, elderberry extracts and polyphenol standards were incubated with MGO under physiological conditions and the formation of adducts was analysed by chromatography (HPLC-DAD, ESI-MS and UHLC-MS). The results obtained demonstrated that the major phenolic compounds present in elderberries, as cyanidin-3-glucoside and cyanidin-3-sambubioside, are capable to trap efficiently and rapidly MGO, forming several adducts (Fig.1). Whereas, quercetin-derivatives reacted slowly with MGO, but also formed adducts. Thus, elderberries present anti-glycative potential by trapping directly MGO, which might be a promising strategy to reduce the impact of MGO and AGEs in the organism.

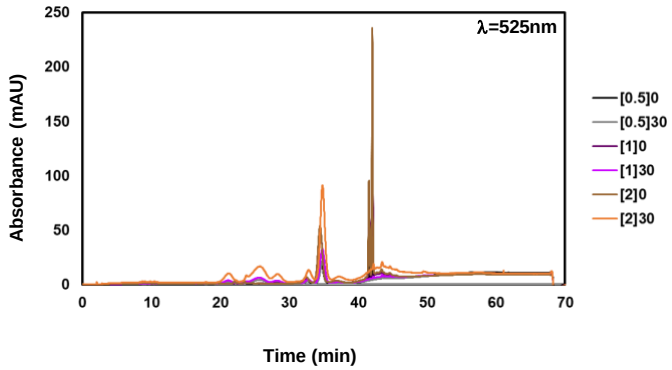

Fig.1. Anthocyanins profile of elderberry extracts, before (0) and after (30) the trapping experiment, obtained by HPLC-DAD.

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Optimization of the extraction method for determination of total phenolic content (TPC) of bee pollen

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Bee pollen is an apicultural product, which contains high amounts of phenolic compounds. The Folin-Ciocalteu method is commonly used for the determination of total phenolic content (TPC) of pollens. The results of the analysis are influenced by the plant origin [1], as well as by extraction conditions, including the nature of the solvent. Since the pollen wall is complex and resistant, sonication is widely applied for assisting the extraction of antioxidant constituents [2]. The aim of our research was to investigate the effect of extraction solvent type (methanol, ethanol, 70% aqueous methanol, 70% aqueous ethanol) and sonication treatment on the total phenolic content of bee pollens of different botanical origin. Six plant species were selected for the experiment, the pollen of which can be characterized with different morphological properties and pollen wall structure. Our results indicate that pollen of lacy phacelia (*Phacelia tanacetifolia*) contains the highest amount, while pollen of sunflower (*Helianthus annuus*) contains the lowest amount of phenolic compounds compared to the other samples. Based on our results, 70% aqueous ethanol seems to be the most effective solvent for polyphenol extraction. Sonication increased the results of TPC determination by 5-161%. No relation was found between the effectiveness of sonication and morphological characteristics of pollen grains, though, further research is needed involving other plant species.

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