12º ENCONTRO NACIONAL

CROMA-TOGRAFIA

6 › 8 dez'22

Aveiro | Portugal

universidade de aveiro

Sociedade Portuguesa de Química

XIV WARPA

TÍTULO:

Livro de Resumos do 12º Encontro Nacional de Cromatografia & XIV WARPA

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INITIAL MESSAGE

CHROMATOGRAPHY: DO THE CURRENT DEVELOPMENTS RESPOND TO FUTURE CHALLENGES?

After a break, caused by the pandemic, we are happy to organize the 12th National Chromatography Meeting (12ENC) that took place from the 6th to the 8th of December 2022 at the University of Aveiro. This is the only national congress focused on the topic of chromatography, and it brings together the Portuguese chromatography community, including experts from academia, companies, and a wide set of people from public and private institutions. Also, it provides an atmosphere to share experiences and knowledge and trigger networking between young researchers, namely undergraduate, master's and doctoral students, and seniors. This community only is complete with the presence of exhibitors and sponsors who have an exclusive space.

This event is intended to address the latest instrumental and methodological developments, as well as conceptual innovations and applications to discuss how chromatography and related techniques play a central role to respond to current societal challenges, namely safety, health and well-being, sustainability, healthy eating and new ingredients, climate change, management of environmental contaminants, crisis management, and space exploration, among others.

The event program was also designed to bring together the Portuguese chromatography community in Portugal with experts from other countries. Namely, in the program of this 12th edition we are honoured to include an afternoon dedicated to XIV WARPA - *Workshop in recent advances in sample preparation*. In this way, we can strengthen relations with the internationally recognized Brazilian community in the area of sample preparation and chromatography. In this event, a Portuguese researcher will also be awarded with Prof. Janusz Pawliszyn medal.

The scientific program includes plenary lectures (5) and keynotes (9) presented by recognized experts, and oral (37) and flash presentations (10) mainly attributed to researchers. A significant number of poster presentations were also submitted (84).

All the best to all participants!

Sílvia M. Rocha, President of the SPQ Chromatography Group

PAST SYMPOSIA

EDITION	YEAR	PLACE
1	1999	Fundação Calouste Gulbenkian, Lisboa
2	2001	Torre do Tombo, Lisboa
3	2003	Torre do Tombo, Lisboa
4	2005	Universdade de Évora, Évora
5	2007	Universidade de Aveiro, Aveiro
6	2009	Universidade da Madeira, Funchal
7	2012	Universidade do Porto, Porto
8	2013	Universdade da Beira Interior, Covilhã
9	2016	Universidade de Lisboa, Lisboa
10	2017	Instituto Politécnico de Bragança, Bragança
11	2019	Universidade Nova de Lisboa, Caparica
Current Edition		
12	2022	Universidade de Aveiro, Aveiro

PROF. JANUSZ PAWLISZYN MEDAL

This medal was created by the scientific committee of the "Workshop in recent advances in sample preparation" to honor Prof. JANUSZ PAWLISZYN, from Waterloo University, Canada, and a researcher of the South America or other countries where Portuguese is spoken for their relevant contribution in the area of the sample preparation.

The 2022 winner was announced during this 12ENC – XIV WARPA event: Prof. Sílvia M. Rocha, from Department of Chemistry & LAQV-REQUIMTE, University of Aveiro, Portugal.

The Scientific Committee is composed by:

Fernando M Lanças - Universidade de São Paulo, Brasil
Maria Eugênia Queiroz - Universidade de São Paulo, Brasil
Fabio Augusto - Universidade Estadual de Campinas, Brasil
José M F Nogueira - Universidade de Lisboa, Portugal
Eduardo Carasek da Rocha - Universidade Federal de Santa Catarina, Brasil
Eduardo Figueiredo - Universidade Federal de Alfenas, Brasil
Renato Zanella - Universidade Federal de Santa Maria, Brasil

SHORT COURSE

5 | December | 2022

Since the first ENC symposia, short courses were provided and the 2022 meeting continue this long-standing tradition.

Participants will receive a certificate of participation, in each will be included the program and number of hours of the short course.

MULTIDIMENSIONAL CHROMATOGRAPHY AND MASS SPECTROMETRY: TOOLS FOR FUTURE CHALLENGES

Regina Duarte, Department of Chemistry & CESAM, University of Aveiro
Sónia Santos, Department of Chemistry & CICECO, University of Aveiro
Cátia Matins, Department of Chemistry & LAQV/REQUIMTE, University of Aveiro

BRIEF SUMMARY

The course will include an overview of multidimensional techniques, namely GC×GC, LC×LC and tandem mass spectrometry.

The different techniques will be introduced, the cases for which each one can be applied and how the data processing is a key strategy in the analysis and characterization of different compounds and/or different matrices (food, environmental, natural products) will be discussed.

Participants will have the opportunity to put hands-on and get closed to the equipments. In a case-study the same matrix will be analysed in GC×GC and LC×LC. Key strategies for the optimization of separation will be discussed. Identification of compounds by MS^n will be addressed to proceed in a final stage to the quantification of compounds.

PROGRAM

11h00-12h30: Theory
12h30-14h00: Lunch break
14h00-18h00: Hands-on, including on (LC×LC, MS^n and GC×GC)

WHO SHOULD ATTEND

All chromatography and mass spectrometry users, specially who need to analyze samples of increasing complexity.

SCIENTIFIC AND ORGANIZING COMMITTEES

SCIENTIFIC COMMITTEE

Ana Maria Loureiro da Seca - Universidade dos Açores

António da Silva Ferreira - Universidade Católica, Porto

Armando Silvestre - Universidade de Aveiro

Cristina Delerue Matos - Instituto Politécnico do Porto

Cristina Barrocas Dias - Universidade de Évora

Eduardo Carasek da Rocha - Universidade Federal de Santa Catarina, Brasil

Eduardo Figueiredo - Universidade Federal de Alfenas, Brasil

Eduardo Mateus - Universidade Nova de Lisboa

Eugenia Gallardo - Universidade da Beira Interior

Fabio Augusto - Universidade Estadual de Campinas, Brasil

Fernanda Cosme - Universidade de Trás-os-Montes e Alto Douro

Fernando M. Lanças - Universidade de São Paulo, Brasil

Fernando Nunes - Universidade de Trás-os-Montes e Alto Douro

João Queiroz - Universidade da Beira Interior

José S. Câmara - Universidade da Madeira

José Manuel F. Nogueira - Universidade de Lisboa

José Maria Oliveira - Universidade do Minho

Lígia Salgueiro - Universidade de Coimbra

Lilian Barros - Instituto Politécnico de Bragança

Luísa Custódio - Universidade do Algarve

Manuel António Coimbra - Universidade de Aveiro

Marcela Segundo - Universidade do Porto

Maria Eugênia Queiroz - Universidade de São Paulo, Brasil

Marco Gomes da Silva - Universidade Nova de Lisboa

Maria João Cabrita - Universidade de Évora

Maria Rosário Bronze - Universidade de Lisboa

Nuno Mateus - Universidade do Porto

Ofélia Anjos - Instituto Politécnico de Castelo Branco

Regina Duarte - Universidade de Aveiro

Renato Zanella - Universidade Federal de Santa Maria, Brasil

Sílvia M. Rocha - Universidade de Aveiro

CHAIRPERSON

Sílvia M. Rocha - Universidade de Aveiro

ORGANIZING COMMITTEE

Armando Silvestre - Universidade de Aveiro

Cátia Martins - Universidade de Aveiro

José Manuel F. Nogueira - Universidade de Lisboa

Manuel António Coimbra - Universidade de Aveiro

Maria Eugênia Queiroz - Universidade de São Paulo, Brasil

Regina Duarte - Universidade de Aveiro

Sílvia M. Rocha - Universidade de Aveiro

Sónia Santos - Universidade de Aveiro

LOCAL ORGANIZING COMMITTEE SUPPORT

Alexandre Fonseca - Universidade de Aveiro

Samuel Patinha - Universidade de Aveiro

Sónia Ribeiro - Universidade de Aveiro

CONGRESS GENERAL INFO

Congress Office

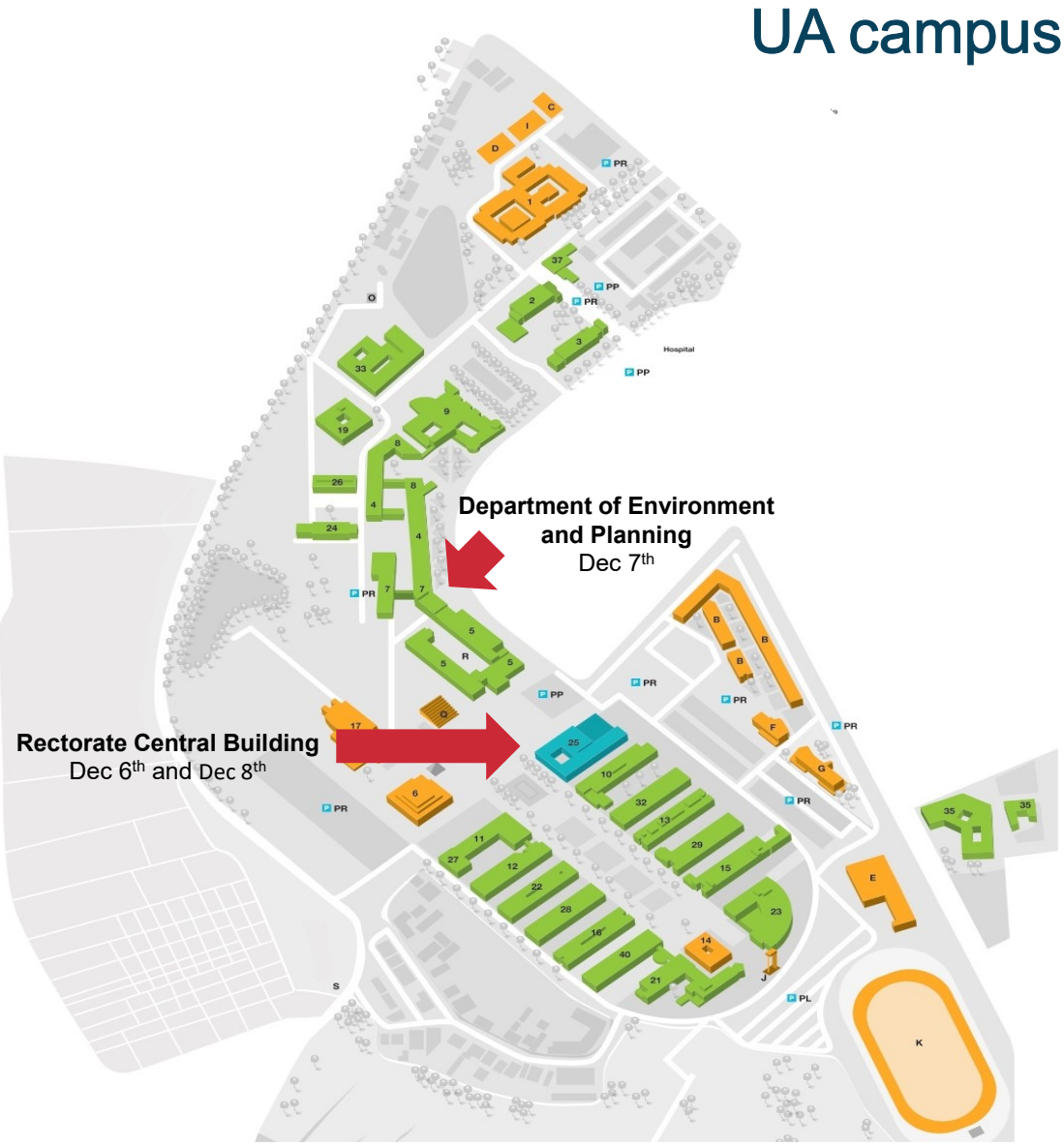
December 6th and 8th: The Congress Office is located at the entrance of the Rectorate Building – building 25 at the UA campus map.

December 7th: The Congress Office is located at the entrance of the Department of Environment and Planning – building 7 at the UA campus map.

Opening hours

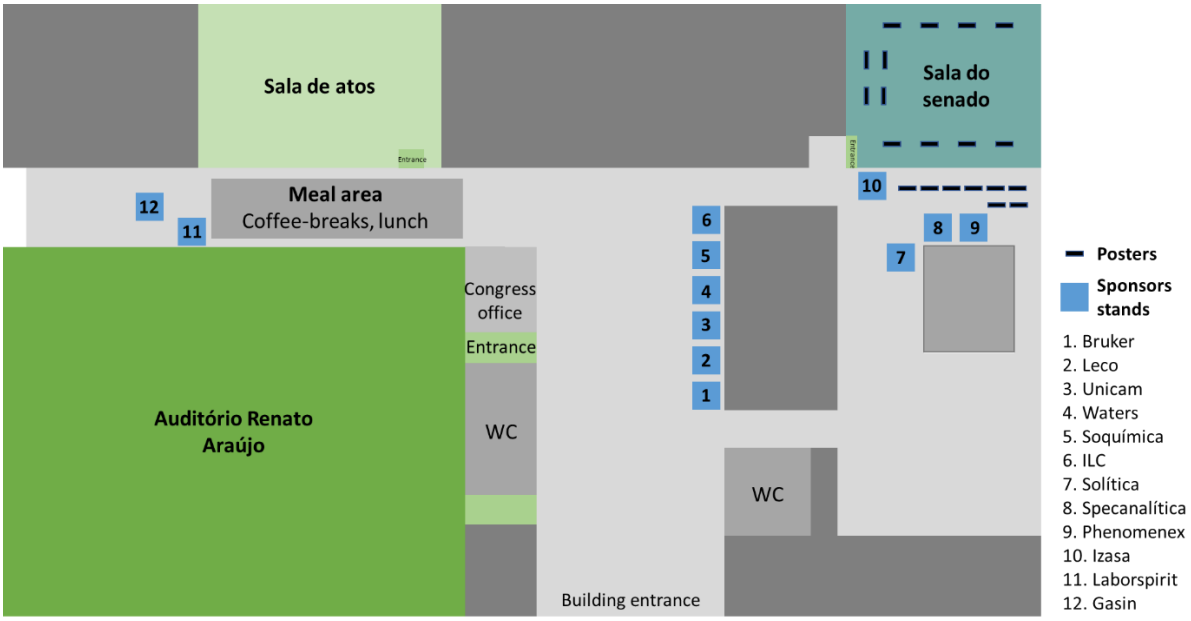
December 6th and 7th: From 8h00 to 18h30

December 8th: From 8h00 to 16h30



Main congress area

December 6th to 8th: Location of the coffee-breaks, lunches, sponsors and posters exhibitions during all the three days of the conference.



SCIENTIFIC AND SOCIAL PROGRAM

December 6th

Rectorate Building
(Building 25 - UA campus map)

8h00	**Registration** Hall of the Rectorate Building	
	Auditório Renato Araújo	
9h00	**Opening session**	
9h15	PL1	
10h00	KN1	
	Auditório Renato Araújo	**Sala do Senado**
10h30	OC1	OC6
10h45	OC2	OC7
	11h00 – 11h30: Coffee break & Poster session Hall of the Rectorate Building	
11h30	OC3	OC8
11h45	OC4	OC9
12h00	OC5	OC10
12h15	Sponsor 1	
	12h45 – 14h15: Lunch break Hall of the Rectorate Building	
	Auditório Renato Araújo	
14h15	PL2	
15h00	KN2	
	Auditório Renato Araújo	**Sala do Senado**
15h30	OC11	OC14
15h45	OC12	OC15
16h00	OC13	OC16
16h15	Sponsor 2	Sponsor 3
	16h45 – 17h15: Coffee break & Poster session Hall of the Rectorate Building	
17h15	**Round table**: Chromatography: do the current developments respond to future challenges?	
18h30	**Welcome Reception: Port Wine and Ovos Moles de Aveiro degustation under a particular musical moment** Hall of the Rectorate Building	

December 7th

Department of Environment and Planning
(Building 7 - UA campus map)

8h00	**Registration** Entrance of the Department of Environment and Planning (Building 7 - UA campus map)
	Auditório Carlos Borrego
9h00	PL3
9h45	KN3
10h15	OC17
10h30	OC18
10h45	OC19
	11h00 – 11h45: Coffee break & Poster session Hall of the Rectorate Building
11h45	OC20
12h00	OC21
12h30	Sponsor 4
	12h45 – 14h15: Lunch break Hall of the Rectorate Building
	Auditório Carlos Borrego
	XIV WARPA *Recent advances on sample preparation*
14h15	Opening session and announcement of the award Janusz Pawliszyn medal
14h30	KN4
15h00	KN5
15h30	KN6
	16h00 – 16h30: Coffee break & Poster session Hall of the Rectorate Building
16h30	KN7
17h00	KN8
17h30	OC22
17h45	OC23
18h00	**Closing session of XIV WARPA**
18h00	**Meeting of the Chromatography Group members – SPQ** Auditório Carlos Borrego
20h30	**Gala Dinner with a special entertainment moment** Meliá Ria Hotel & Spa

December 8th

Rectorate Building
(Building 25 - UA campus map)

8h00	**Registration** Hall of the Rectorate Building	
	Auditório Renato Araújo	
9h00	PL4	
9h45	KN9	
	Auditório Renato Araújo	**Sala de Atos**
10h15	OC24	OC27
10h30	OC25	OC28
10h45	OC26	OC29
	11h00 – 11h30: Coffee break & Poster session Hall of the Rectorate Building	
11h30	FC01	FC06
11h35	FC02	FC07
11h40	FC03	FC08
11h45	FC04	FC09
11h50	FC05	FC10
11h55	Discussion on Flash Communications	Discussion on Flash Communications
12h10	Sponsor 5	Sponsor 6
	12h45 – 14h15: Lunch break Hall of the Rectorate Building	
	Auditório Renato Araújo	
14h15	PL5	
15h00	Sponsor 7	
	Auditório Renato Araújo	**Sala de Atos**
15h30	OC30	OC34
15h45	OC31	OC35
16h00	OC32	OC36
16h15	OC33	OC37
16h30	**Closing session and announcement of the award communications and the next 13ENC**	

SCIENTIFIC AND SOCIAL PROGRAM

December 6th

Auditório Renato Araújo (25 Rectorate Building)

8h00	**Registration**
9h00	**Opening session** Artur M. S. Silva *President of the Chemical Portuguese Society* *Vice-rector for Research, Innovation and Training of the 3rd Cycle, University of Aveiro* *Coordinator of the LAQV/REQUIMTE (University of Aveiro)* Armando J. D. Silvestre *Head of the Chemistry Department, University of Aveiro* Sílvia M. Rocha *Chair of the 12º ENC, LAQV/REQUIMTE & Department of Chemistry, University of Aveiro*
Session 1: Chairs Sílvia M. Rocha & Maria do Rosário Bronze	
9h15	**PL1 - Are the "applications of the future" the key to unlocking the deserved success of GC×GC?** Chiara Cordero *Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Italy*
10h00	**KN1 - Bioactive compounds from natural resources: from bioprospection to innovative processes and applications** Sónia Santos *CICECO-Aveiro Institute of Materials, University of Aveiro, Portugal*
10h30	**OC1 - How hops or blended hops are related to beer terpenic volatile composition?** Cátia Martins *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*
10h45	**OC2 - The potential of comprehensive two-dimensional gas chromatography in the disclosure of broas volatile composition** Andreia Leonor Vieira Bento da Silva *Faculdade de Farmácia, Universidade de Lisboa, Portugal*
11h00 – 11h30: Coffee break & Poster session	
Session 2: Chair Manuel António Coimbra	
11h30	**OC3 - Assessment of the volatile profile of Alentejo varietal wines by HS-GC/ToFMS with multivariate chemometric analysis** Catarina Correia da Cruz Pereira Mendes *MED - Mediterranean Institute for Agriculture, Environment and Development & Institute for Advanced Studies and Research, Universidade de Évora, Portugal*
11h45	**OC4 - *Thymbra capitata* L. essential oil and hydrodistillation residual water: Phytochemical characterization by GC-MS and HPLC-PDA-ESI-MSn and evaluation of the antioxidant, anti-inflammatory and wound healing properties** Sónia Raquel Jorge Pedreiro *Faculty of Pharmacy, University of Coimbra, Portugal*
12h00	**OC5 - Anti-glycative effect of phenolic compounds from *Sambucus nigra* L. by trapping methylglyoxal** Sandrine dos Santos Ferreira *CQ-VR & University of Trás-os-Montes and Alto Douro, Portugal*

12h15	***Sponsor 1* - Bruker timsTOF: New 4D-applications by Trapped Ion Mobility High Resolution MS** Rui Rocha *Bruker Portugal*

Sala do Senado (25 Rectorate Building)

Session 3: Chair Maria João Cabrita	
10h30	**OC6 - Analysis of volatile compounds of sparkling wines: the effect of using free and immobilized yeasts combined with traditional and Charmat methodologies** Davide Miguel da Silva Correia Mendes *Departamento de Química & LAQV-REQUIMTE, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal*
10h45	**OC7 - Determination of selected carbonyl compounds in Port wines by reductive amination of aldehyde 2,4-dinitrophenylhydrazones using cyanoborohydride** Juliana Milheiro Ferreira *CQ-VR - Chemistry Research Centre, Portugal*
11h00 – 11h30: Coffee break & Poster session	
Session 4: Chair Maria João Cabrita	
11h30	**OC8 - Gas chromatography and chemical sensors as reliable tools for the assessment of adulterations in coffee** Maria Carolina Pinho Loura *Departament of Chemistry & LAQV/REQUIMTE & CESAM, University of Aveiro, Portugal*
11h45	**OC9 - Liquid and gas chromatography as a tool for the characterization of *Fucus vesiculosus*-rich extracts as potential food ingredients** Ana Rita Sousa Circuncisão *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*
12h00	**OC10 - Development of statin-like and ergosterol enriched extracts from mushroom bio residues** Jonata M. Ueda *CIMO & SusTEC, Instituto Politécnico de Bragança, Portugal*

12h45 – 14h15: Lunch break

Auditório Renato Araújo (25 Rectorate Building)

Session 5: Chairs José S. Câmara & Ana Maria Loureiro da Seca	
14h15	**PL 2 - *Quo Vadis* Indoor Air Quality?** Agapios Agapiou *Department of Chemistry, University of Cyprus, Nicosia, Cyprus*
15h00	**KN2 - "Catch me if you can!!" – Illicit substances detection for forensic purposes** André Lobo Castro *Forensic Chemistry and Toxicology Service, National Institute of Legal Medicine and Forensic Sciences - North Delegation, Porto, Portugal*
15h30	**OC11 - Headspace-solid phase microextraction coupled with gas-chromatography as a useful tool to detect volatile compounds in a pulmonary arterial hypertension pre-clinical model** Jorge Miguel Alves-Silva *Faculty of Pharmacy, University of Coimbra, Portugal*

15h45	**OC12 - Chromatographic-mass spectrometry methods for risk evaluation of anthropogenic and natural contaminants in raw milk** Marta Sofia Carvalho Ferreira Malheiro Leite *Faculty of Pharmacy, University of Coimbra & INIAV & REQUIMTE/LAQV, Portugal*
16h00	**OC13 - Treatment of wastewater effluents using nanofiltration and low pressure UV treatment to produce high quality water that can be reused for irrigation for food production** Vanessa Jorge Pereira *iBET, Instituto de Biologia Experimental e Tecnológica, Portugal*
16h15	***Sponsor 2* - Low Adsorption UPLC Systems and Columns to solve challenges in HPLC separations of metal-sensitive analytes** Sandra Cachopo *Waters Technologies LCMS Portugal*

Sala do Senado (25 Rectorate Building)

Session 6: Chair Ofélia Anjos	
15h30	**OC14 - Development of a solid phase extraction methodology for pharmaceuticals quantification by HPLC using waste-based sorbents** Alexandr Stratulat *Department of Chemistry, University of Aveiro, Portugal*
15h45	**OC15 - Removal of estrogens from water using activated carbon from olive stone** Eduardo Candido Milani *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal*
16h00	**OC16 - Bioaccessibility determination of omega-3 and conjugated linolenic acid using an *in vitro* standardized digestion model (INFOGEST) by GC-FID** Ana Sofia Salsinha *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Portugal*
16h15	***Sponsor 3* - Analysis of flavour compounds in milk flavourings by SPME-GC-MS** João Maria Lopes da Cunha Cappelle Teixeira *Specanalítica*

16h30 – 17h00: Coffee break & Poster session

Auditório Renato Araújo (25 Rectorate Building)

17h15	**Roundtable: *Chromatography: do the current developments respond to future challenges?*** **André Lobo Castro**, Forensic Chemistry and Toxicology Service, National Institute of Legal Medicine and Forensic Sciences - North Delegation **Cristina Fernandes**, Sogrape Vinhos, S.A. **Hugo Rocha**, Newborn Screening, Metabolism and Genetics Unit Human Genetics Department, National Institute of Health Doutor Ricardo Jorge **Regina Duarte,** CESAM & Departament of Chemistry, University of Aveiro **Vítor Vale Cardoso,** Organic Chemistry Area of the EPAL Water Analysis Laboratory **Moderator:** Journalist Elsa Silva Santos, University of Aveiro

18h30 - Welcome Reception: Port Wine and Ovos Moles de Aveiro degustation under a particular musical moment

December 7th

Auditório Carlos Borrego (7 Department of Environment and Planning)

8h00	**Registration**
Session 7: Chairs Marco Gomes da Silva & Renato Zanella	
9h00	**PL3 - From LC to LC×LC in environmental analysis: A timeline overview to identify bottlenecks and trends** Armando da Costa Duarte *Department of Chemistry & CESAM, University of Aveiro, Portugal*
9h45	**KN3 - Organic compounds in primitive extraterrestrial bodies – challenges in sampling and analysis** Zita Martins *Instituto Superior Técnico (IST), Portugal*
10h15	**OC17 - Chromatography for the analysis and purification of phenolic compounds** Joana Oliveira *LAQV-REQUIMTE, Faculdade de Ciências, Universidade do Porto, Portugal*
10h30	**OC18 - Modified capillary column for comprehensive two-dimensional liquid chromatographic separation of complex environmental matrices** Pedro Francisco da Silva Brandão *Department of Chemistry & CESAM, University of Aveiro, Portugal*
10h45	**OC19 - Unraveling the metabolites of N-ethylpentylone in mice serum and urine** Andreia Leonor Vieira Bento da Silva *Faculdade de Farmácia, Universidade de Lisboa, Portugal*
11h00 – 11h45: Coffee break & Poster session	
Session 8: Chair Cristina Barrocas Dias	
11h45	**OC20 - Development of a GDME-HPLC-DAD-MS/MS methodology for the extraction and identification of volatile carbonyl compounds in wood-based panels** Rui Miguel Castro Ramos *Departamento de Química e Bioquímica & LAQV-REQUIMTE, Faculdade de Ciências, Universidade do Porto, Portugal*
12h00	**OC21 - A fast analytical approach based on µSPEed/UHPLC-PDA for the simultaneous determination of pesticides in wastewaters** Jorge Augusto Machado Pereira *CQM-UMa, Centro de Química da Madeira, Portugal*
12h15	***Sponsor 4* - Two-dimensional gas chromatography for food analysis. The ultimate challenge: MOSH&MOAH determination** Julio Lluch *LECO Instrumentos*

12h45 – 14h15: Lunch break

Auditório Carlos Borrego (7 Department of Environment and Planning)

XIV WARPA	
Session 9	
14h15	**Opening session and announcement of the award *Janusz Pawliszyn medal*** Maria Eugénia C. Queiroz & José Manuel Nogueira *WARPA Scientific Committee* Artur M. S. Silva *President of the Chemical Portuguese Society* *Vice-rector for Research, Innovation and Training of the 3rd Cycle, University of Aveiro* *Coordinator of the LAQV/REQUIMTE (University of Aveiro)* Armando J. D. Silvestre *Head of the Chemistry Department, University of Aveiro*
Chair Marco Gomes da Silva	
14h30	**KN4 - Bioanalysis of biomarkers for neurodegenerative diseases by In-tube SPME-LC-MS/MS** Maria Eugénia C. Queiroz *Department of Chemistry, Faculty of Philosophy Sciences and Letters, University of São Paulo - Ribeirão Preto, Brazil*
15h00	**KN5 - Complete automation for complex online sample preparation approaches using robotic and non-robotic systems** Fernando Mauro Lanças *Institute of Chemistry of the University of São Paulo - São Carlos, Brazil*
15h30	**KN6 - Future challenges for passive microextraction techniques** José Manuel Nogueira *Department of Chemistry and Biochemistry, Faculty of Science, University of Lisbon, Portugal*
16h00 – 16h30: Coffee break & Poster session	
Session 10: Chair Regina Duarte	
16h30	**KN7 - Sample treatment and bead injection: *Quo vadis*?** Marcela Segundo *Faculty of Pharmacy & LAQV/REQUIMTE, University of Porto, Portugal*
17h00	**KN8 - Analytical challenges for the determination of pesticide residues in complex matrices** Renato Zanella *Department of Chemistry, Federal University of Santa Maria, Brazil*
17h30	**OC22 - Monitoring of Bladder Cancer Patients Through the Urinary Proteome** Hugo M. Santos *BIOSCOPE Research Group, LAQV-REQUIMTE, Chemistry Department, FCT-NOVA, Portugal*
17h45	**OC23 - Bioanalytical method using DLLME and LC-MS/MS to determine doxycycline residues in fish** Marina Alves Damaceno *Department of biomolecular Sciences, School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Brazil*
18h00	**Closing session of XIV WARPA**
18h15	Meeting of the Cromatography Group members – SPQ (Auditório Carlos Borrego)
20h30	Gala dinner: Meliá Ria Hotel & Spa

December 8th

Auditório Renato Araújo (25 Rectorate Building)

8h00	**Registration**
	Session 11: Chairs José Manuel Nogueira & Regina Duarte
9h00	**PL4 - Prescriptomics: where do we stand now?** José Luis Capelo Martínez *BIOSCOPE Research Group, LAQV-REQUIMTE, Chemistry Department, FCT-NOVA, Portugal*
9h45	**KN9 - Development and optimization of a targeted GC-MS metabolomics-based approach to study dendritic cells deficient of the subunit A of Succinate Dehydrogenase** Annalaura Mastrangelo *Spanish National Center for Cardiovascular research, Spain*
10h15	**OC24 - Encapsulation efficiency measurements on polymeric and lipid drug-delivery nanocarriers: are we there yet?** Sara Cristina da Silva Marques *LAQV/REQUIMTE & Chemical Sciences Department, Faculty of Pharmacy, University of Porto, Portugal*
10h30	**OC25 - Semi-preparative enantioseparation of cathinone derivatives on amylose-derived LC columns and binding studies to human serum albumin by HPALC** Carla Sofia Garcia Fernandes *CIIMAR & Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal*
10h45	**OC26 - Anticancer drugs in the aquatic environment: where should we act and is membrane filtration a solution to this problem?** Maria do Rosário Bronze iBET & Faculdade de Farmácia, Universidade de Lisboa, Portugal
	11h00 – 11h30: Coffee break & Poster session
	Session 12: Chair Maria Eugênia Queiroz
11h30	**FC01 - Multi-detection and quantification of pharmaceuticals residues in seafood by a liquid- chromatography tandem mass spectrometry method** Sara Cunha *LAQV/REQUIMTE, Faculty of Pharmacy, University of Porto, Portugal*
11h35	**FC02 - Multi-detection of pharmaceuticals in environment matrices by UHPLC-ToF-MS** Andreia Alexandra Ribeiro Freitas *INIAV & LAQV/REQUIMTE, Portugal*
11h40	**FC03 - Advances in the extraction of antibiotics from hen eggs** Érica Cortez de Lima *INIAV & Faculty of Pharmacy, University of Porto, Portugal*
11h45	**FC04 - Development and validation of a method for sugar analysis by HPLC-ELSD** Pedro Filipe Rodrigues Lopes *Super Bock Group & LAQV/REQUIMTE, Departamento de Química e Bioquímica, Universidade do Porto, Portugal*
11h50	**FC05 - Comparison of microwave assisted and conventional solid-liquid techniques for the chlorogenic acids extraction from silverskin: an analysis by HPLC-DAD** Marlene Conceição Pereira Machado *REQUIMTE/LAQV, Faculty of Pharmacy, University of Porto, Portugal*

11h55	**Discussion on Flash Communications**
12h10	***Sponsor 5* - Polar Pesticides Anions in water and food using a UHPLC or Ion Chromatography Hypehenated with Orbitrap HRMSMS or Multiplexing HR-SIM** Daniel Ettlin *Unicam Sistemas Analíticos*

Sala de Atos (25 Rectorate Building)

Session 13: Chair José Maria Oliveira	
10h15	**OC27 - Profiling the phlorotannin composition from *Laminaria digitata* before and after gastrointestinal digestion** Marcelo Dias Catarino *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*
10h30	**OC28 - Regimes de fertilização por medida como estratégias para o incremento da composição fenólica: estudo de caso em *Cichorium spinosum* L.** Maria Inês Moreira Figueiredo Dias *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal*
10h45	**OC29 - HPAEC-PAD as a tool towards the characterization and design of novel carbohydrate-based sweeteners** Pedro António Rodrigues Fernandes *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*
11h00 – 11h30: Coffee break & Poster session	
Session 14: Chair José Maria Oliveira	
11h30	**FC06 - Comprehensive two-dimensional gas chromatography as a tool to unveil the volatile profile of grape spirits used in Port wine fortification** Sónia Ribeiro *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*
11h35	**FC07 - Phenolic profile and bioactive potential of cardoon (*Cynara cardunculus* L.) inflorescences: selection of the best genotype for food application** Ana Filipa Mandim Pires *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal*
11h40	**FC08 - Salt pan brine water glycans: complementary characterization by GC-FID and HPAEC-PAD** Sónia dos Santos Ferreira *Departament of Chemistry & CICECO, University of Aveiro, Portugal*
11h45	**FC09 - Perfil lipídico de óleos de subprodutos de peixe obtidos por extração assistida por micro-ondas e Soxhlet** José Virgílio Santulhão Pinela *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal*
11h50	**FC10 - GC×GC-ToFMS analysis of passion fruit volatile profile: a step to unveiling consumers aroma perception** Alexandre Miguel Alves Fonseca *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*
11h55	**Discussion on Flash Communications**
12h10	***Sponsor 6* - A importância dos gases BIP na cromatografia** Fernando Lourenço *Gasin, Gases Industriais*

12h40 – 14h15: Lunch break

Auditório Renato Araújo (25 Rectorate Building)

Session 15: Chairs Armando Silvestre & Marcela Segundo	
14h15	**PL 5 - Down to Earth** Geraint Morgan *Department of Physical Sciences, The Open University, UK*
15h00	**Sponsor 7 - Analytical tools to discover more in complex samples – from high-capacity sorptive extraction to GC×GC** Carlos Gil Zapico *Schauenburg Analytics*
15h30	**OC30 - Digestion of a phenolic-rich extract from extra virgin olive oil using a dynamic *in vitro* gastrointestinal model: exploring the metabolomic profile** Elsa Velez Mecha *ITQB & iBET, Instituto de Biologia Experimental e Tecnológica, Portugal*
15h45	**OC31 - Brewer's spent yeast gluco-oligosaccharide profiling by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)** Elisabete Verde Martins Coelho *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*
16h00	**OC32 - Use of shotgun proteomics for the assessment of the chemical composition of biscuits melanoidins** João Rafael Rodrigues Siopa *CQ-VR & University of Trás-os-Montes and Alto Douro, Portugal*
16h15	**OC33 - Chromatographic approaches to study pine nut skin: exploitation of its composition and bioactivities** Ana Soraia Pires Silva *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*
16h30	**Closing session and announcement of the award communications and the next 13ENC**

Sala de Atos (25 Rectorate Building)

Session 16: Chair Sónia Santos	
15h30	**OC34 - Fig (*Ficus carica* L.) bioresidues: A chromatographic study of five varieties for its valorization** Carlos Seiti Shiraishi *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal*
15h45	**OC35 - Impact of growth medium salinity on galactoxylan exopolysaccharides of *Porphyridium purpureum*** Andreia Filipa Sousa Ferreira *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*
16h00	**OC36 - How do extraction methodologies influence the biological properties of pomegranate leaves?** Sandra Andreia Gonçalves Marcelino *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal*
16h15	**OC37 - Establishment of the volatile fingerprint of the PDO pear "Pera Rocha do Oeste" by HS-SPME/GC×GC-ToFMS** Ana Maria Simões da Costa *Departament of Chemistry & LAQV/REQUIMTE, University of Aveiro, Portugal*

LIST OF THE PLENARIES

Are the "applications of the future" the key to unlocking the deserved success of GC×GC?

Chiara Cordero
Dipartimento di Scienza e Tecnologia del Farmaco, University of Torino, Turin, ITALY

Chiara Cordero (orcid.org/0000-0003-3201-0775) since July 2021 is Full Professor of Food Chemistry (CHIM/10 - Food Chemistry) at the Dipartimento di Scienza e Tecnologia del Farmaco of the University of Torino (Turin, Italy). In 2008 Prof. Cordero was Research Fellow the Deutsche Forschungsanstalt für Lebensmittelchemie - DFA (German Research Center for Food Chemistry) at the Lehrstuhls für Lebensmittelchemie der Technischen Universität München - Garching (Germany) under the supervision of Prof. Peter Schieberle; in 2019 Visiting Professor at Poznan University of Life Sciences, Poznan, Poland and in 2020 Visiting Professor at Stellenbosch University, Stellenbosch, South Africa.

Her research interests focus on: (a) development of innovative and advanced instrumental configurations for comprehensive two-dimensional gas chromatography (GC×GC) coupled to mass spectrometry (MS) for food-omics investigations (profiling and fingerprinting approaches); (b) discovery of food intake markers (food metabolomics, nutrimetabolomics and volatilomics) in bio-fluids after diet-induced derangements; (c) development and application of miniaturized, fully automated and solvent-free sample preparation approaches (Solid Phase Microextraction SPME, Stir Bar Sorptive Extraction SBSE) for sensomic characterization of food. In 2008, Chiara received the "Leslie S. Ettre" Award for "original research in capillary gas chromatography with an emphasis on environmental and food safety" and in 2014 received the "John B. Phillips Award" for her research activity in the field of comprehensive two-dimensional gas chromatography. In 2016 Chiara was included by The Analytical Scientist in the "Women Power List" as one of the 50 most influencing women in the analytical sciences. In 2022 she received the Scientific Achievement Award for her research in comprehensive two-dimensional gas chromatography (https://www.gcxgc-symposium.com/saa-2022).

PL1 Are the "applications of the future" the key to unlocking the deserved success of GC×GC?

Cordero C,[1] Caratti A,[1] Squara S,[1] Liberto E,[1] Bicchi C,[1] Tao Q,[2] Reichanbach SE[2,3]

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Modern "omics" strategies applied to food and nutrition domains require a "comprehensive" approach to capture the compositional complexity of samples (*i.e.*, chemical code – metabolome, volatilome) and to establish robust correlations with the complex biological phenomena behind them. Comprehensive two-dimensional gas chromatography combined with mass spectrometry (GC×GC-MS) has the potential to tackle compositional challenges in terms of high chemical dimensionality and a large dynamic range of concentrations of food and biological samples. When implemented by artificial intelligence (AI) algorithms and concepts, it provides a consistent basis for hypothesis generation.
Undoubtedly, GC×GC-MS is a key-analytical platform in food metabolomics/food volatilomics and its widespread has boosted research opening new and concrete perspectives for a more comprehensive understanding of food quality, nutritional value, and functional properties.
The industrial request for straightforward analytical workflows and solutions to solve practical problems does not prevent the adoption of multidimensional chromatography platforms and omics concepts even in an industrial research or quality control laboratory framework. The larger the breadth of an investigation, the better the understanding of the impact of processing, storage, fermentation, and biotransformation on the overall quality and safety of a product.
The full potential of GC×GC was probably not clear at its introduction, but its widespread adoption in different areas and the infusion of strategies and concepts from other disciplines, have definitely highlighted its central role of missing technique: "*from a technique that did not exist… to a technique that was missing*"[1].
The potential of GC×GC in food research will be presented from the perspective of the author's experience; future trajectories will also be delineated based on food industry requirements. Artificial Intelligence smelling, computer vision, food *identitation*, quality prediction, are all intriguing topics where GC×GC has no equal.

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Quo Vadis Indoor Air Quality?

Agapios Agapiou
Department of Chemistry, University of Cyprus, Nicosia, CYPRUS

Dr. Agapios Agapiou is an Assistant Professor at the Department of Chemistry of the University of Cyprus (UCY). He received a Diploma and a Ph.D. in Chemical Engineering from the National Technical University of Athens (NTUA), Greece in 2001 and 2006, respectively. From 2007 to 2013 he worked at the Laboratory of Inorganic and Analytical Chemistry of the School of Chemical Engineering, NTUA. In September 2014 he was elected Lecturer at the Department of Chemistry of UCY. His research focuses on hyphenated analytical techniques (e.g., GC-MS) for the analysis of complex mixtures (e.g., Volatile Organic Compounds) in biomedical, environmental, food, forensic, safety, and security applications. Dr. Agapiou is currently involved in the EU projects 'ONELAB' and 'intoDBP'. He has published more than 70 peer-reviewed journal articles. He also serves in several editorial boards of analytical chemistry journals.

PL2 Quo Vadis Indoor Air Quality?

Agapiou A[1]

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"Your air, my air": indoor air quality (IAQ) is an emerging and challenging global issue with direct implications for human health. This lesson was learned during the Covid-19 pandemic. Many people spend days indoors, or move and work in crowded and confined spaces as part of their daily routine. As early as the 1970s, scientists used the term "sick building syndrome" (SBS) after residents complained about their health and well-being. The composition of indoor environments affects human health, well-being, and performance; inorganic gases (*e.g.,* CO_2, O_3, *etc.*), particulate matter, microorganisms, and volatile organic compounds (VOCs) co-occur. Some VOCs are precursors for the formation of ozone (O_3) and secondary organic aerosols (SOA). Researchers use either passive or active samplers to measure indoor VOCs released from a variety of sources, including building materials, floor coverings, decorations, cleaning and personal care products, and human activities such as cooking, smoking, *etc.* Sample preparation includes the steps of trap cleaning, sample extraction and collection, pre-concentration, desorption, data analysis, and correlation. Understanding the interactions of surface chemistry and indoor oxidants with humans is still in its infancy[1]. Both online, near real-time instruments *e.g.,* proton transfer reaction mass spectrometry (PTR-MS), selected ion flow tube mass spectrometry (SIFT-MS), gas chromatography-ion mobility spectrometry (GC-IMS), *etc.* and bench-top systems exist; *e.g.,* thermo-desorption gas chromatography-mass spectrometry (TD-GC-MS). However, each method has its advantages and disadvantages. Examples of selected indoor workplaces (*e.g.*, offices, hospitals, hair and beauty salons[2], schools, libraries[3], museums, *etc*.) are presented and discussed, highlighting current and future challenges for chromatography.

Acknowledgements: The author would like to thank the President of the 2022 SPQ Chromatography Group for the kind invitation.

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From LC to LC×LC in environmental analysis: A timeline overview to identify bottlenecks and trends

Armando da Costa Duarte
Department of Chemistry & CESAM University of Aveiro, Aveiro, PORTUGAL

Armando da Costa Duarte graduated in Chemical Engineering at University of Oporto (1977) and obtained a PhD in Public Health Engineering at University of Newcastle-upon-Tyne (1981).

In 1977 started working at the University of Aveiro and in 1995 was appointed as Professor of Environmental & Analytical Chemistry lecturing on the quality of data, provided by a plethora of methods based on Analytical Chemistry, for supporting decisions on environmental protection, and sustainable development.

In 2006, the Portuguese Foundation for Science and Technology (FCT) awarded him a prize for Scientific Excellence, and from 2013 to 2016 was a Member of the FCT Scientific Council for Natural and Environmental Sciences.

As a Researcher, he has been particularly interested in the application of fit-for-purpose analytical methods for decoding complex samples and characterization of the environmental quality, with more than 500 scientific publications with more than 21000 citations and reaching an h-index of 72 (Scopus).

PL3 From LC to LC×LC in environmental analysis: A timeline overview to identify bottlenecks and trends

Duarte AC[1]

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A critical view of developments and limitations in the field of Liquid Chromatography (LC) up to two-dimensional liquid chromatography (2D-LC), namely Comprehensive 2D-LC (LC×LC) applied to the analysis of complex environmental matrices, is thoroughly discussed considering the contributions of our research group to this research field. Basically, the timeline evolution from LC to LC×LC is overviewed to identify bottlenecks, critical issues, and trends leading to the actual state of the art in this field of sciences of separation.

Firstly, the clarification of Analytical Chemistry concepts and specific operational details is of the essence for a clear understanding of the scope for the application of LC and LC×LC in the production of analytical data with quality for supporting decisions on food safety, health and environmental protection, and sustainable development[1,2].

Secondly, the aspects leading to the development of fit for purpose LC×LC instrumentation with components available from different manufactures will also be highlighted to show how this type of laboratorial work could lead to analytical developments of interest when aid by suitable data processing tools[3].

Finally, the results obtained with LC×LC instrumentation developed in our laboratory and published in high-impact journals will be highlighted and used to demonstrate how suitable such analytical methods are to provide researchers with unique information to decode the chemical nature of complex environmental matrices[4-6].

Acknowledgements: Thanks are due to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020), through national funds. This work was also supported by projects ORGANOSOL (PTDC/CTE-ATM/118551/2010), CN-LinkAIR (PTDC/AAG-MAA/2584/2012), and AMBIEnCE (PTDC/CTA-AMB/28582/2017) funded by FEDER, through COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE) through FCT/MCTES.

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Prescriptomics: where do we stand now?

José Luis Capelo Martínez
BIOSCOPE Research Group, LAQV-REQUIMTE, Chemistry Department, FCT-NOVA, Lisbon, PORTUGAL

José-Luis Capelo is co-head of the bioscopegroup (www.bioscopegroup.org). His CV comprises (up to August 2022): 280 manuscripts; 220 congress communications (orals and posters); 23 projects; 2 Patents, 1 license agreement and 3 books (1 authored and 2 edited). He has organized and chaired 60 international conferences and presently is involved in the direction of 7. He was member of the advisory board of Talanta from 2006 to 2014, and he is Editor in Chief of the on-line Journal JIOMICS (www.JIOMICS.com) since its creation in 2011. He is presently mentoring or co-mentoring a total of 3 doctoral theses and he has mentored 3 post-doctoral grants, 10 doctoral grants, 9 masters and 8 final projects. His current research interest is devoted to developing new methodological approaches in personalized medicine using new proteomics approaches as well as to unravel bacterial resistance to antibiotics using nanotechnology. H index 42 (Google Scholar). Citations about 6700. He is considered worldwide within the best 2% of researchers of his area of expertise (Stanford University, USA). J. L. Capelo, PhD, gets his bachelor's degree in chemistry by the University of Santiago de Compostela (Spain), his doctorate in Analytical Chemistry by University of Vigo, UVIGO (Spain, award to the best doctoral thesis 2002) and his Post-Doc from the Instituto Superior Técnico de Lisboa (Portugal). His academic career comprises assistant to staff and lecturer at the UVIGO; research fellow at the Chemistry Department of the New University of Lisbon, CD-FCT-UNL, research fellow at the CD-UVIGO, and assistant professor at the CD-FCT-UNL. Currently he is Associate Professor at the CD-FCT-UNL.

PL4 Prescriptomics

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Analysis of proteins has been an integral part of clinical chemistry for decades. However, technological advances have opened new opportunities for the large-scale analysis of proteins for clinical diagnostic purposes and personalised medicine.
First, the development of mass spectrometers with significantly higher resolution and more extensive dynamic range has allowed generating of high-quality quantitative data from complex sample matrices such as serum, plasma, urine, and tissue biopsies without the need for isotopic labelling. Instruments like TOF, Orbitrap, FT-ICR and the most recent timsTOF were a stepping-stone towards proteomic-based personalised medicine by facilitating the detection of patient-specific protein signatures that reorganise over time due to genetic, environmental, and treatment constraints.
Second, developing high-throughput robust chromatographic-based sample preparation methods in conjunction with mass spectrometry has allowed the processing of clinical specimens in an efficient and reproducible manner in tens of minutes. Parallel to such achievements, the exciting advances done by bioinformatics have been a cornerstone in translating quantitative proteomics data into actionable clinical information.
We are what our genetic code express. And what we express can now be quantified. By quantifying the proteome expressed by a patient, any disease can be diagnosed, prognosed and followed either using a solid or a liquid biopsy.
Within this talk, I will introduce the analytical chemistry-driven concept of prescriptomics by describing how the advances in sample treatment, mass spectrometry, chromatography and bioinformatics have led to the advent of truly personalised medical treatment [1].

References

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Down to Earth

Geraint Morgan
Department of Physical Sciences, The Open University, UK

Geraint Morgan is highly active in analytical chemistry and the space technology translation agenda at The Open University, having developed instruments for the Rosetta and Beagle2 space missions. Since successfully analysing a comet, he has since led research teams to develop a wide range of bespoke, high impact, sector disruptive solutions to terrestrial challenges, including developing the award-winning air quality monitoring system for use on all future UK submarines and instruments for the world's largest flavours and fragrance company. Funding from academia-industry programmes, has allowed him to work with end-users to develop novel sniffing solutions (mainly GC-MS and GCxGC-MS) for a diverse range of commercial partners. As a result, he has recently signed a formal Collaboration Agreement with LECO Instruments. In partnership with the Scotch Whisky Research Institute (SWRI) and IBM Research UK his team is developing methods to authenticate Scotch whisky and with funding from the Partnership for Clean Competition his team are developing sports anti-doping screening tests. Taff is a Founder/Director of 4 start-up companies and is a named inventor on two patents. He is a Fellow of the Royal Society of Chemistry, a Fellow of the Royal Society for the Encouragement of Arts, Manufactures and Commerce and a Fellow of the Royal Astronomical Society.

PL5 Down to Earth

Morgan G[1]

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Taff is highly active in analytical chemistry and the space technology translation agenda at The Open University. The talk will describe how he spent the first half of his career developing instruments for the Rosetta and Beagle2 space missions. Having successfully analysed a comet, using a miniaturised ion-trap GC-MS, he has since led research teams to develop a wide range of bespoke, high impact, sector disruptive solutions to terrestrial challenges, including developing the award-winning air quality monitoring system for use on all future UK submarines and robotic instruments for the world's largest flavours and fragrance company.

Funding from academia-industry programmes, such as SPRINT, STFC Impact Accelerator Account, the STFC Food Network +, and internal OU programmes has allowed him to work with end-users to develop novel sniffing solutions (mainly GC-MS and GCxGC-MS) for a diverse range of commercial and academic partners, including: determining the shelf-life of rocket salad, identifying avocados damaged or infected with fungi and earlier detection of Campylobacter in chicken farms. In partnership with the Scotch Whisky Research Institute (SWRI) and IBM Research UK his team is developing methods to authenticate Scotch whisky and with funding from the Partnership for Clean Competition they are developing sports anti-doping screening tests. He was also part of the large research team that analysed chemical composition of the UK Winchcombe Meteorite, that fell in 2021, the results of which were recently published in Science.

LIST OF THE KEYNOTES

Bioactive compounds from natural resources: from bioprospection to innovative processes and applications

Sónia Santos
CICECO-Aveiro Institute of Materials in University of Aveiro, Aveiro, PORTUGAL

Sónia Santos holds a BSc in Chemical Engineering and a PhD in Chemistry. She is currently Assistant Researcher at CICECO-Aveiro Institute of Materials in University of Aveiro. Her research activity has been focused on the bioprospection of natural components, development of green extraction methodologies and in their upgrading for high value applications (e.g. antimicrobial agents in drug delivery, reducing/stabilizing agents in the green synthesis of nanoparticles, or UV-blockers in active packaging). One of her main research interests has been the development of expedite methodologies to analyze complex matrices or crude extracts by GC-MS and UHPLC-MSn. She published 74 SCI papers, h-index of 26 and +1900 citations (https://orcid.org/0000-0002-6749-7619). S. Santos has been involved in the organization of several Conferences and Workshops and has been also guest-Editor of several special issues in her area.

KN1 Bioactive compounds from natural resources: from bioprospection to innovative processes and applications

Santos SAO[1]

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Natural resources have been widely used since ancient times in folk medicine. The improved knowledge about their effects in the promotion of health and wellbeing has increased the demand for the exploitation of natural products. Lipophilic and phenolic compounds are interesting natural products due to their high biological and chemical diversity. Currently, there is a huge concern to apply the circular economy guidelines in the management of natural resources, whose valorization implies a previous prospective phase towards the detailed knowledge of bioactive compounds composition with potential for industrial applications. In addition, low productivity indexes, high costs and low efficiency of extraction processes are major drawbacks.

This presentation will cover the prospection of polar and lipophilic components in agro-forest residues,[1] food wastes[2] and macroalgae,[3,4] which was performed by constructing a workflow that combined selective extraction steps with advanced instrumental analysis, namely gas chromatography coupled to mass spectrometry (GC-MS) and ultra high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS^n). An overview of sustainable and innovative extraction processes will be provided, considering the most outstanding solutions,[5] and an illustration of the therapeutic potential with selected examples from natural extracts or compounds.[6,7] Finally, future perspectives on their plausible applications for food, nutraceutical or pharmaceutical fields will be presented.

Acknowledgements: This work was developed within the scope of the project CICECO—Aveiro Institute of Materials (UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020), financed by national funds through the FCT/MEC (PIDDAC). FCT is also acknowledged for the research contract under Scientific Employment Stimulus to S. Santos (2021.03348.CEECIND).

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"Catch me if you can!!" – Illicit Substances Detection for Forensic Purposes

André Lobo Castro
Forensic Chemistry and Toxicology Service, National Institute of Legal Medicine and Forensic Sciences - North Delegation, Porto, PORTUGAL

André Lobo Castro has a degree in Biology (University of Aveiro, 1998). Forensic Toxicologist at the National Institute of Legal Medicine and Forensic Sciences since 1999 (starting as a trainee), achieved a M.Sc. in Advanced Biomolecular Methods (University of Aveiro, 2008), and further on, a Ph.D. in Forensic Sciences (University of Porto, 2016).

Allocated to the Forensic Chemistry and Toxicology Service, his work is mainly dedicated to GC-MS(MS), biological samples preparation, LC-MS/MS, analytical validation, according to international forensic guidelines, and results forensic interpretation. Expertise in illicit substances (Drugs of abuse, New Psychoactive Substances). Supervisor of Master's dissertations since 2009.

Is a member of The International Association of Forensic Toxicologists. Published several articles in peer-review journals, considering analytical procedures dedicated to forensic purposes, among other subjects. Is a Reviewer in analytical and forensic science Journals.

KN2 "Catch me if you can!!" – Illicit Substances Detection for Forensic Purposes

Castro AL[1,2]

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The consumption of substances with psychotropic effects is almost as old as human civilization, and is currently a serious public health problem.
Regulatory action by government authorities seeks to monitor and legislate on production, trafficking and consumption by the population. This approach implies a constant updating of information, namely regarding the existence of New Psychoactive Substances, analytical methods for the identification of products and detection procedures in biological samples, in order to assess the clinical effect of the substances on the individual, both at a physical and psychoactive level.
In this presentation, these themes will be addressed, with emphasis on the applied analytical methodologies, the challenges associated with the Medico-Legal interpretation of the results obtained and the transposition to a legislation that needs to be both dynamic and up-to-date.

Organic compounds in primitive extraterrestrial bodies – challenges in sampling and analysis

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Zita Martins is an Astrobiologist, an Associate Professor at Instituto Superior Técnico (IST), Co-Director of the MIT-Portugal Program (MPP), and the Advisor for Higher Education, Science, Innovation and Digital Transition of His Excellency the President of Portugal. Her scientific interests include the detection of signatures of extra-terrestrial life on space missions, and the potential contribution of meteorites and comets to the origin of life on Earth. She graduated in Chemistry at IST in 2002 and obtained a PhD in Astrobiology at the University of Leiden (Netherlands) in 2007. She was an Invited Scientist at NASA Goddard in 2005 and 2006, and an Invited Professor at the University of Nice-Sophia Antipolis (France) in 2012. In 2009 she was awarded by the Royal Society with a University Research Fellowship worth 1 million pounds, having remained at Imperial College London (United Kingdom) from 2007 to 2017.

Zita Martins has been actively participating in space missions. She is Co-Investigator of two projects (OREOcube and EXOcube) of the European Space Agency (ESA) that will be installed on the International Space Station (ISS). She is a Community Scientist of the ARIEL mission (ESA), she is a member of the Hayabusa2 mission (JAXA), and a member of the Comet Interceptor mission (ESA).

She is the Portuguese representative of the Executive Committee of the European Astrobiology Network Association (EANA), member of ESA's Life Sciences Working Group (scientific advisory team), member of ESA's Solar System and Exploration Working Group, and member of the European Space Sciences Committee (ESSC).

She actively does science communication, having given over 100 international media interviews, and was selected by the BBC TV channel as Expert Scientist Women. Her portrait was painted for the Royal Society's exhibition on successful women in science and is now permanently on display at the Royal Society's London headquarters. In 2015 Zita Martins was appointed “Oficial da Ordem Militar de Sant'Iago da Espada” by His Excellency the President of Portugal for exceptional and outstanding merits in science.

KN3 Organic compounds in primitive extraterrestrial bodies – challenges in sampling and analysis

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Extraterrestrial bodies such as comets, asteroids, and their fragments (e.g., meteorites) are known to contain carbonaceous matter. These samples are analyzed *in-situ* through space missions, or on terrestrial analytical laboratories. During the sampling process it is crucial to i) avoid organic contamination from terrestrial sources[1], and ii) to get as much sample as possible, which in most cases is in the order of mg to a few grams. Comets have several extraterrestrial organic molecules[2, 3], including the amino acid glycine, which was detected on comet 81P/Wild2 by NASA's Stardust mission[4]. Samples from the asteroid Ryugu have been analyzed by the Hayabusa2 team and indicate the presence of volatile-rich species[5], while preliminary analysis of the asteroid Bennu, which is expected to return to Earth in 2023 onboard the OSIRIS-Rex sample-return mission, indicates the presence of carbonates and organics matter[6]. Carbonaceous meteorites contain up to 5wt% of organic carbon[7]. More than 70% is a solvent-insoluble kerogen-like polymer (IOM), and the remaining less than 30% are made up of soluble organic compounds[8-10], extracted by solvents of different polarities. Chromatography has a track record of decades in the analysis of extraterrestrial samples[8,11]. However, given the complex nature and distribution of its soluble organic matter, and its low available quantity, it is fundamental to optimize analytical procedures for the separation and detection of extraterrestrial organic matter: (i) low detection and quantification limits, (ii) high enantiomeric resolution, and (iii) minimal co-elution[11].

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Bioanalysis of biomarkers for neurodegenerative diseases by In-tube SPME-LC-MS/MS

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Master in Science (1990) and Doctor in Science (1996) in the Chemistry Institute of São Carlos at the University of São Paulo. In 2003, PhD in Analytical Chemistry (Chemistry Institute of São Carlos of the University of São Paulo). In 2007, international PhD project at the University (Canada) with supervision of the Dr. Janusz Pawliszyn. She is an Associate Professor 3 of the Chemistry Department of the Faculty of Philosophy Sciences and Letters of Ribeirão Preto at the University of São Paulo. She is an International Member of Sample Preparation Task Force & Network - European Chemical Society / DAC. She has about 120 published articles and 7 book chapters and an H 30 index (Web of Science source). Among the current lines of research stand out the development of selective stationary phases for innovative microextraction techniques for coupling with chromatographic systems (LC-MS/MS) or directly to mass spectrometry (MS/MS) for the determination of drugs and biomarkers in biological samples for studies in neurosciences area.

KN4 Bioanalysis of biomarkers for neurodegenerative diseases by In-tube SPME-LC-MS/MS

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Current research into neurological diseases has focused on identifying novel disease biomarkers to improve diagnosis, to provide accurate prognostic information, and to monitor disease progression. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has been the reference technique to determine biomarkers in biological samples, including human plasma, cerebrospinal fluid, and brain tissue. In these complex matrixes, biomarkers coexist with much higher concentrations of exogenous and endogenous compounds (for example, metabolites of the target analyte, proteins, or phospholipids) whose chemical structures resemble the structures of the analytes. Thus, a sample preparation step is mandatory when developing LC-MS/MS methods: this step removes endogenous macromolecules and isolates and pre-concentrates the analytes that are present at trace levels.
Recent trends in biological sample preparation techniques have focused on miniaturized analytical systems, which allow high-throughput performance and online coupling to analytical instruments. These online systems reduce the analysis time and provide better precision and sensitivity than manual offline techniques. In addition, miniaturized systems use smaller volume of organic solvent and biological samples. In this context, in-tube solid-phase microextraction (in-tube SPME) coupled to liquid chromatography is noteworthy.
In this lecture, we will discuss the innovative in-tube SPME-LC-MS/MS methods that employ different types of capillaries (sorbent-packed, porous monolithic rods, and fiber-packed) with selective stationary phases (monoliths, magnetic nanoparticles, restricted access materials, ionic liquids, and hybrid materials) to determine biomarkers in biological samples for neurological studies (Parkinson, Alzheimer, mild cognitive impairment, and Huntington).

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Complete automation for complex online sample preparation approaches using robotic and non-robotic systems

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Fernando Mauro Lanças is the leader of the Chromatography Group and a full Professor at the Institute of Chemistry of the University of São Paulo in São Carlos, Brazil. He started and is Chairman of several meetings, including COLACRO and SIMCRO, and is co-Chairman of WARPA. Prof. Lanças advised ca. 130 Ph.D. and Master Thesis; published ca. 400 peer-reviewed papers, 7 books, and several book chapters. Lanças is the Director of the IIC (International Institute of Chromatography), Editor-in-Chief of Acta Chromatographica, and Associate Editor of J. Chromtogr. Sci. and Guest Editor of several periodicals in his research area.

At the moment, Prof. Lanças's primary research interest is focused on the complete miniaturization of sample preparation-chromatography-mass spectrometry, including new materials, instrumentation, and applications and their entire online automation towards the Unified Chromatography.

KN5 Complete automation for complex online sample preparation approaches using robotic and non-robotic systems

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Sample preparation is usually a mandatory step in most chemical analyses involving complex matrices and a large number of analytes present at low concentration levels, such as in the determination of residue and contaminants in food samples; pharmaceutical compounds in environmental matrices; mycotoxins in beverages such as wine and beer; biomarkers in biological fluids, and so on. This step aims not just to transfer the analytes of interest from the original matrix to a solvent compatible with the analytical method to be used but ideally should also eliminate most contaminants and improve the concentration of the target analytes. Although offline approaches are still widely employed - meaning that the sample prep step has no physical connection with the analytical step - online approaches are receiving substantial attention in the last decade. In our laboratory, a relevant goal is the development of new sorbent materials, extraction devices, equipment, and interfaces aiming to connect online sample preparation with GC-MS(MS) and LC-MS(MS), thus providing a fully automated approach. This presentation reviews two distinct approaches utilized in our research laboratory to achieve this goal: robotic and non-robotic. Several application examples of each approach will be presented and discussed in different application areas, showing the universal character of these approaches to fully automated online sample preparation.

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Future challenges for passive microextraction techniques

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Dr. J.M.F. Nogueira is Associate Professor at the Faculty of Sciences of the University of Lisbon (UL), Lisbon (Portugal). In 1990, he received a degree in Chemistry and a PhD in Analytical Chemistry (UL) in 1995. He worked several months at the Research Institute for Chromatography and University of Gent (Belgium) during his PhD project. Currently, he is researcher at the 'Centro de Química Estrutural' and head of the 'Chemistry for the Environment' group. His main research activity focuses on the development and application of innovative analytical methodologies involving chromatographic, electromigration and hyphenated techniques. He is author and co-author of more than one hundred and sixty SCI articles (h-index: 45) and chapters of international books, as well as supervisor of academic graduation and post-graduation projects and referee in international scientific journals. In 1999, he founded the Chromatography group of the Portuguese Chemical Society being president during several years. He has been chairman and co-chairman of national and international meetings on chromatography, as well as invited to be chairperson and speaker in scientific sessions. In 2017, he was awarded with the "Dr. Janusz Pawliszyn" medal for the outstanding work in the field of Sample Preparation during the IX WARPA (Brazil). In 2020 and 2021, he was part of the World's Top 2% Scientists list in the field of Analytical Chemistry.

KN6 Future challenges for passive microextraction techniques

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Static or passive microextraction techniques have played an important role in the last thirty years as modern and very effective approaches in the field of sample preparation. Some good examples are liquid-based microextraction techniques, namely dispersive liquid-liquid microextraction (DLLME), single drop microextraction (SDME) and hollow fibre liquid phase microextraction (HF-LPME). On the other hand, solid phase microextraction (SPME), stir bar sorptive extraction (SBSE) and, more recently, bar adsorptive microextraction (BAμE)[1], have been the most used solid-based microextraction techniques. In general, the main features of these analytical tools are the use of miniaturized devices, great simplification, easy handling, great reduction or absence of toxic solvents or materials, reduced sample volume, better selectivity and sensitivity for trace analysis, as well as compatibility for interfacing with the common chromatographic and hyphenated systems[2].
Despite the great effectiveness of these technologies, none of them allows "universal application" or "universal usability", since they are neither suitable for certain analyses nor available for the entire community, respectively. On the other hand, if we consider the current state of technological development that we are experiencing, as well as the current environmental requirements, some of the mentioned microextraction techniques have several limitations to be considered effective alternatives for the future. Nowadays, any proposed passive microextraction technique, in addition to being very simple, must be user-friendly, eco-friendly, cost-effective, comprehensive and suitable for the work routine[3,4].
In this contribution, some of the emerging trends in passive microextraction techniques will be discussed, including innovative advances[5-7] and future challenges.

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Sample treatment and bead injection: Quo vadis?

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Marcela Segundo holds a BSc in Microbiology and a PhD in Biotechnology. She is currently the Head of the Analytical Development Group at LAQV/REQUIMTE, Portuguese Government Associate Laboratory for Green Chemistry, Clean technologies and also member of the Executive Board of Faculty of Pharmacy, University of Porto, where she teaches in the Department of Chemical Sciences. Her current research is focused on automation of sample treatment and on development of high-throughput microchemical methods. Her scientific production (ORCID 0000-0003-2938-0214) comprises 14 book chapters, >140 peer-reviewed papers in international journals (h-index 33). In 2016 she received the FIA Award for Science attributed by the Japanese Association for Flow Injection Analysis. Since 2018, she is the Secretary of the Division of Analytical Chemistry of the European Chemical Society (EuChemS).

KN7 Sample treatment and bead injection: *Quo vadis?*

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Flow-based methods have been established over the last few decades to automate (bio)chemical analysis while enhancing the figures of merit regarding accuracy, precision and sample throughput. The bead injection technique was suggested for automation of solid-phase chemistry with the possibility of renewal through microfluidic handling of beads. The beads can be carriers of different (bio)chemical moieties, providing a platform for operation of different chemistries, aiming at analyte preconcentration, in-situ quantification and separative methods.[1]
The present work addresses the state-of-the-art and future developments regarding bead injection towards automatic sample treatment. Online hyphenation to separative techniques will be discussed, namely the issues posed by the compatibility of eluent with conventional HPLC procedures. Additionally, flow programming features will be emphasized, including the reproducible manipulation of sorbents with different shapes and large size distribution through universal handling protocols. Applications concerning food (milk), environmental (water) and biological samples (saliva, plasma) will be overviewed, addressing the problems posed by different matrices. Finally, the advantages of automatic sorbent renewal in solid-phase extraction procedures will be shown, including the reduced volume of eluent required per analysis and the improved sample throughput when compared to conventional batch procedures, featuring the achievement of Green Analytical Chemistry.

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Analytical challenges for the determination of pesticide residues in complex matrices

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KN8 Analytical challenges for the determination of pesticide residues in complex matrices

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The occurrence of pesticide residues is one of the current problems in food production and efforts to minimize these risks must be made. Due to the occurrence of pesticides in food and biological samples, there is a growing demand for new analytical methods that allow fast and efficient sample preparation combined with a reliable determination even in complex matrices. There is a wide variety of pesticides found in food as a result of its permitted use for cultivation or storage, as well as unauthorized pesticides. The number of pesticides used is constantly growing, which implies a great demand for their analysis in foods of animal or plant origin, leading to an ongoing challenge to establish new multiclass methods to analyze a wide range of analytes with high selectivity and sensitivity. Typical difficulties such as complexity and variety of composition of the matrix of foods and biological samples and low concentration of analytes make it difficult to analyze several classes simultaneously.
The QuEChERS procedure, with the possibility of several modifications, is the most widely applied sample preparation procedure for multiresidue analysis of pesticides in foods and biological matrices. The clean-up step based on dispersive solid phase extraction (d-SPE) using a small amount of special sorbents, selected considering the matrix co-extractives, such as proteins, pigments and lipids, allows obtaining suitable extracts for analysis. Precipitation of the interferents using suitable solvents and/or the use of low temperatures is also a quick and efficient strategy. Considering the current situation and the importance of pesticide residue analysis, as well as the limitation of the volume of biological samples, the challenge for analytical chemists is to continue working to improve analytical tools to meet the requirements established by legislation, minimizing costs, time and analysis complexity.

Acknowledgements: We would like to acknowledge the Brazilian agencies CNPq, CAPES and FAPERGS for the financial support and fellowships.

Development and optimization of a targeted GC-MS metabolomics-based approach to study dendritic cells deficient of the subunit A of Succinate Dehydrogenase

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Annalaura Mastrangelo holds First-Class Honours M.Sc. degree in Pharmacy from the University of Modena and Reggio Emilia (Italy) in 2011, International PhD in Medicinal Chemistry and First Class Honours distinction from the University San Pablo CEU (Spain) in 2017. She worked extensively in metabolomics during her PhD in the Metabolomics and Bioanalysis Excellence Centre (CEMBIO) where she mastered the implementation of analytical chemistry methods (chromatography coupled to mass spectrometry, MS) and data science to solve complex biological questions, mainly obesity and its complications. In 2017, she joined Dr. Sancho group at Spanish National Center for Cardiovascular research (CNIC) as a postdoctoral fellow and focused her work on metabolomics-based experiments to investigate the mechanisms underlying the pathophysiology of atherosclerosis and the crosstalk of metabolic pathways and immune response to improve therapy. She is the author of one book chapter and 14 publications in international journals (JCR; among them five as first author and one as last author) in high impact journals (Q1) in the field of metabolomics, analytical chemistry and obesity. Her work has more than 640 cites, H index=12 (google Scholar). She has participated in communication and dissemination activities: 9 oral contributions to international conferences, moderator of the "Healthcare, Pharma & Biotech" group and jury member in the #Nova111StudentList for Healthcare and Bio Science for Nova, collaborated with Fundación Bertelsmann and CNIC in the Xcelence_Escuelas que inspiran project.

KN9 Development and optimization of a targeted GC-MS metabolomics-based approach to study dendritic cells deficient of the subunit A of Succinate Dehydrogenase

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Immune cell activation, proliferation and function are closely linked to, and dependent on, the activation of specific intracellular metabolic pathways. Moreover, several seminal studies with immune cells have shown that targeting these metabolic pathways can greatly influence their functional properties[1]. This has led to the emerging field of Immunometabolism, combining the distinct disciplines of immunology and metabolism. Metabolomics, the discipline that studies the entire set of metabolites in a specific specimen, has been increasingly employed to address the biological questions raised by immunologists[2]. Here, we present a GC-MS metabolomics-based experiment to study dendritic cells (DC) that are deficient in subunit A of Succinate Dehydrogenase (SDHα). SDH, also known as respiratory complex II, is at the crossroads of two essential mitochondrial pathways (the TCA cycle and OXPHOS) and has been suggested as a therapeutic target for cancer, affecting oncogenic metabolites such as succinate, α-ketoglutarate and fumarate[3]. Moreover, SDH inhibiton by itaconate reduces the expression of proinflammatory genes in macrophages[4]. Herein, we describe the development and implementation of a rapid and sensitive method for the sample preparation and data acquisition of five metabolites (i.e. succinate, fumarate, α-ketoglutarate, itaconate and glutamate) in SDHα-deficient DCs supplemented or not with succinate or itaconate. Samples have been analyzed by a GC-MS LECO Pegasus BT and metabolites quantified by the Target Analyte Finding method. The quantification of these metabolites provides a comprehensive readout of the effects of SDHα deficiency in DCs, enabling assessment of its downstream effects on the metabolism and function of these cells.

Acknowledgements: We would like to thank the European Research Council (ERC-2016-Consolidator Grant 725091) and Spanish Ministerio de Ciencia e Innovación (MCIN, PID2019-108157RB/AEI/10.13039/501100011033). GD is supported by a European Molecular Biology Organization Long-term Fellowship (ALTF 379-2019). This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 892965. The CNIC is supported by the Instituto de Salud Carlos III (ISCIII), the MCIN and the Pro CNIC Foundation), and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MICIN/AEI/10.13039/501100011033).

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LIST OF THE SPONSORS LECTURES

S1 Bruker timsTOF: New 4D-applications by Trapped Ion Mobility High Resolution MS

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Ion Mobility Spectrometry (IMS) is a very fast separation technique that studies the movement of ions in the gas phase under the influence of an electric field.
Recently, the development of trapped ion mobility (tims) technology coupled to High Resolution Mass Spectrometry (QTOF) has been a technological revolution for many today`s challenging applications in the analytical chemistry field. The timsTOF technology provides additional separation power for multi-dimensional (4D) characterization and identification of the detected organic compounds with exceptional levels of selectivity and sensitivity not previously achieved.
Since ion mobility separations occur on the milli-second time scale, timsTOF technology can easily be connected to traditional GC or UHPLC chromatographic systems allowing great versatility and resolving power for ultra-trace analyses in complex matrices.
timsTOF technology is currently performing previously difficult to solve analytical problems in both target and non-target workflows. This paper is showing new 4D analytical developments in the fields of food safety, food authenticity and fraud, analysis of unexpected compounds in the environment and analysis of isobaric lipids for lipidomic studies, among others.

S2 Low Adsorption UPLC Systems and Columns to solve challenges in HPLC separations of metal-sensitive analytes

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Since the earliest development of high-performance liquid chromatography (HPLC), stainless steel has been the preferred material of construction for chromatographs and columns. However, it has long been observed that stainless-steel hardware can cause poor peak shapes and low recoveries for certain analytes.

To address these challenges, Waters has developed a family of new technologies, named MaxPeak™ High Performance Surfaces (HPS). When utilized for UPLC™ System and Column hardware, this technology provides a highly effective barrier that mitigates undesired interactions with metal surfaces and provides improved separations of analytes and the best results are obtained when this system is used together with ACQUITY Premier Columns. With the ACQUITY Premier Solution, the need for conditioning with samples or standards is greatly reduced, leading to significant time savings as well as improved precision and accuracy.

S3 Analysis of flavour compounds in milk flavourings by SPME-GC-MS

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Dairy based milk powders offer a healthy alternative to fresh milk whilst also being readily available to incorporate into milk flavoured products during manufacturing. Whilst consumers expect highly soluble and great tasting products, manufacturers need reliable high-quality instrumentation for determining the right chemical composition of their products. Gas chromatography is the most commonly used chromatography technique for analysing food products, especially milk powders, for the identification of aroma compounds. The identification of the aroma compounds is vital as they constitute the taste and smell of all food products.

Solid Phase Micro Extraction (SPME) is a solid phase extraction technique that involves the use of a fibre coated in a polymer or sorbent extracting phase. The fibre is exposed to a sample where sample analytes are absorbed onto the fibre coating. The fibre coating should be chosen to suit the type of analyte in the sample. During injection into the GC inlet, desorption occurs, and the analytes are introduced to the analytical system. The quantity of analyte extracted by the fibre is proportional to its concentration in the sample as long as equilibrium is reached.

The SCION 8500 GC[2] with MS and automated SPME was used to identify flavour compounds commonly found in milk-based products. Both liquid and powered samples containing milk flavourings were analysed with peak identification confirmed via NIST spectra library comparisons. Excellent repeatability was achieved, highlighting the robustness of the SCION SPME autosampler and GC-MS system.

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S4 Two-dimensional Gas Chromatography for food analysis. The ultimate challenge: MOSH&MOAH determination

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In this study comprehensive two-dimensional gas chromatography (GCXGC) in combination with time-of-flight mass spectrometry is shown as a powerful tool for food analysis. LECO´s GC×GC system takes advantage of a dual-stage, quad-jet thermal modulator positioned between the two columns and a secondary oven allows independent temperature control of the second-dimension column, combined with high acquisition rate, full range TOF mass spectra to acquire full information and resolve efficiently and routinary the most complex food samples in order to provide answers to concerns such as quality control, contaminations, frauds or origin.

The ultimate challenge for analytical chemistry is MOSH/MOAH determination. Mineral oil contamination in different consumer products, especially in foods, has become a huge concern in the EU for some years ago. Separation capabilities and comprehensive MS information provided by GC×GC-TOFMS are determining factors to confirm the presence of mineral oils, better understanding the origin of the contamination, and reduce errors in quantitation produce by HPLC-GC-FID preliminary method. This study will also show representative comparison between both analytical methods and which is the state-of-the-art in terms of LECO´s analytical solutions for MOSH/MOAH determination.

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S5 Polar Pesticides Anions in water and food using a UHPLC and Ion Chromatography Hypehenated with Orbitrap HRMSMS or Multiplexing HR-SIM

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The use of polar pesticides has been widely spread over the last years. The control in food and on all water, supply is mandatory in many countries, and of extreme importance for environmental and health purposes. But the routine analysis of Polar pesticides has been a big challenge for the chemists in the last years. The difficulty of having a normal or reversed phase method that performs well is in the base of the problems. Another challenge is matrix diversity, and clean up. Additionally, 3 or four different methods and columns must be used in order to detect a large list of species and metabolites[1]. We have analyzed milk samples for Chlorate and Perchlorate using an Hypercarb column and conditions of analysis and extraction in the European reference method Quppe. The LC was hyphenated to an Orbitrap Exploris 120 in High Resolution Sim Mode. For that, we developed and tested a matrix cleaning method with Dionex matrix removal cartridges, with very good sensitivity and recovery values. Also, superb mass Accuracy under 1ppm was easily and routinely achieved every day, without any instrument calibration in periods of at least 15 days. Orbitrap in SIM HR multiplexing mode, provided a very sensitive and reliable method, with the least matrix interference in milk samples. We have screened some milk samples using a Data Dependent scan with the Polar pesticides referred in the method. As an alternative separation technique, Ion Chromatography provides higher selectivity for polar compounds. Using this approach, and the superb unequivocal identification provided by Orbitrap technology, a setup was done to provide both methods.: Ion Chromatography, coupled with High Resolution or Tandem Mass spectrometry. As most of the species are negatively charged at high pH, is possible to use that property for a good chromatographic separation, in order to achieve selectivity and a good resolution. Separation is improved with a strong basic eluent. As we are interfacing with a mass spectrometer, it is mandatory to suppress the highly ionic signal whilst neutralizing the eluent. This unique feature opens the door for an hyphenated technology such as mass spectrometry. We can use either High Resolution mass spectrometry (featuring the Orbitrap Mass analyzer) or Tandem mass spectrometry (using Triple quads technology) to be sure that the highest sensitivity is achieved. High resolution Mass spectrometry provides another dimension of being unequivocal in its quantitation and identification, representing the best approach for analysis of pesticides and contaminants[2]. The goal of this unique methodology as well, is avoiding any additional sample preparation, or any concentration, for water or food samples. In general, the samples are highly concentrated in several ionic species, and it is quite common to observe some Ion suppression in the Electrospray source. Being capable to achieve a good separation strategy is crucial in order to achieve good limits of detection, and minimize the impact of the sample matrix. The technology involved will be demonstrated, and some chromatograms for chlorate/perchlorate and polar pesticides in milk and other matrixes will be shown.

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S6 Gasin – A importância dos gases BIP na cromatografia

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A GASIN (Grupo Air Products) fornece gases de elevado grau de pureza e equipamentos específicos para cromatografia gasosa, assim como para outras aplicações de semelhante grau de exigência.

Nesta apresentação pretende-se dar a conhecer como se posiciona a GASIN e a Air Products no mercado com particular foco no fornecimento de Hélio; apresentar e realçar as vantagens de utilizar gases com tecnologia BIP (Built-In-purifier) para aplicações tão exigentes como a cromatografia gasosa; destacar os equipamentos mais comuns para um correto controlo e distribuição de gases e por fim a sua associação aos serviços adicionais que a Gasin fornece para aumentar a segurança aos seus clientes no manuseio de gases.

S7 Analytical tools to discover more in complex samples – from high-capacity sorptive extraction to GC×GC

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In recent years, there has been increased demand for non-target workflows across a diverse range of applications, such as identifying novel biomarkers of disease, the detection of food fraud and odour profiling.
Here, we will show how sensitivity and chromatographic performance can be improved using trap-enabled, extraction and enrichment methods. Comprehensive two-dimensional gas chromatography (GCxGC) and BenchTOF2 time-of-flight mass spectrometry then tackles complex samples that are out of reach for traditional GC–MS, while data handling is simplified through automated workflows to spot the differences between samples. We will highlight the use of these powerful techniques to discover more across a range of applications.
Firstly, immersive sorptive extraction coupled with GC×GC—TOF MS will be demonstrated for the comparison of flavour profiles from popular brand soft drinks with those of imitation products.
Next, we will describe the use of direct desorption of various textiles and recycled materials prior to analysis by thermal desorption (TD) and GC×GC–TOF MS to improve identification of malodours and compounds of potential concern. We will demonstrate how to overcome low sensitivity, poor detection of compounds and slow processes when analysing such complex samples.
Finally, the accurate identification and measurement of biomarkers in biological samples - such as breath or saliva - has the potential to provide rapid, minimally-invasive diagnosis of a range of diseases, resulting in the delivery of precision medicine. Here, we will demonstrate the use of TD–GC×GC–TOF MS to uncover hidden changes in breath profiles.

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OC1 How hops or blended hops are related to beer terpenic volatile composition?

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Currently, consumers demand and are attracted to single and distinctive beers. One of the beer characteristics that engage consumers is its aroma characteristics that are essentially associated with its volatile components. Volatiles' origin can result from the metabolism of yeast, from raw materials (including hops), or they can be (bio)transformed during beer production and storage. Indeed, hops play an essential role in the brewing process as they contribute to beer bitterness (resins), and/or also to aroma (essential oils), being these last essentially composed by terpenic compounds. The use of several varieties of hops (one or blended hops, per beer) by brewers had promoted the development of a broad diversity and sensory complexity of beers, and consequently to different beer styles. Despite the brewers' empirical knowledge about the impact of hops on the sensory characteristics of their beers, the chemical information on this impact is still scarce. Thus, the main objective of this work was to disclose the relation between hop or blended hops on the terpenic composition of beer. To achieve this objective, a highly sensitive methodology based on solid phase microextraction (HS-SPME) combined with comprehensive two-dimensional gas chromatography coupled to mass spectrometer with time-of-flight analyzer (GC×GC-ToFMS) was used to study 8 hops varieties and 6 beer styles.
The methodology was implemented to achieve the better chromatographic resolution and separation of the volatile components of hops, using a conventional set of non-polar/polar columns (Equity 5/DB-FFAP). While for beer analysis, the reverse set of columns (polar/non-polar, namely DB-FFAP/Equity-5) provided improved separation power and peak distribution. A total of 102 and 72 mono- and sesquiterpenic compounds were putatively identified, in hops and beer, respectively. Chromatographic data was processed through different statistical methods, namely hierarchical analysis that allowed to obtain the terpenic profiles of hops and beers. β-Myrcene, β-caryophyllene, α-humulene and linalool were the major compounds present in hops. Also, American India Pale Ale was the most complex beer, regarding its terpenic volatile composition. To disclose the relation between hop or blended hops on the terpenic composition of beer, it was performed a reconstruction of the beer volatile composition using only hops data according to their percentage used in each beer style (only common components between the two matrices were used). A similar clustering was achieved between the beers data and the reconstructed beer volatile composition using hops data, which allows to infer their impact on beer terpenic profile. These results clearly show that the terpenic profile of beers is modulated by the terpenic composition of the respective hops (single or blends), which corroborates the brewers' empirical knowledge that sensory perceive the impact of hops on beers.

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OC2 The potential of comprehensive two-dimensional gas chromatography in the disclosure of *Broas* volatile composition

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Broa is a traditional Portuguese maize bread, which has been described as one of the 50 world's best breads [1]. Usually, only a small portion of volatiles, mainly originated from baking, contribute to breads aroma [2]. The volatile composition of breads has been characterized by GC-MS (gas chromatography coupled with mass spectrometry) [2]. However, taking into account the presence of trace levels of key volatiles [2], an analysis with a highly sensitive and high-throughput technique, as comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–ToFMS) may disclose the presence of additional compounds. To the best of our knowledge, GC×GC has never been used to comprehensively characterize the volatile composition of breads.
In this work, GC-MS [3] and GC×GC–ToFMS [4] methodologies were used in the analysis of the volatile compounds of *broas* (n=12) extracted by HS-SPME (headspace solid phase microextraction). Analysis by GC-MS enabled to identify 16 compounds, whereas 128 compounds were identified by GC×GC. These volatiles were mainly furans, furanones, pyrroles, pyrazines and pyranones. Other compounds, as pyrans, pyridines, and sulfur-containing volatiles were detected exclusively by GC×GC. However, the strong odorants sulfur-containing compounds are probably the most relevant to the aroma of broas [5]. GC×GC also allowed the identification of several compounds which have not been described in foods, as some pyrans, pyridines, and sulfur-containing compounds.
This study has highlighted the potentialities of GC×GC in the identification of food character-impact volatiles, and draws attention to the presence of possible relevant food volatiles that currently remain underexplored due to limitations in the separation efficiency.

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OC3 Assessment of the volatile profile of Alentejo varietal wines by HS-GC/TofMS with multivariate chemometric analysis

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The present work aims to characterize the volatile profiles of Alentejo varietal red wines and, as a secondary objective, comparing the most used sample extraction methodologies for volatile wine analysis. To pursue this goal, ten varietal red wines – Alfrocheiro (Alf), Alicante Bouchet (Alic B), Aragonez (Arag), Cabernet Sauvignon (Cab S), Merlot (Merl), Syrah (Syr), Tinta Barroca (TB), Tinta Caiada (TC), Touriga Nacional (TN) and Trincadeira (Trinc) – were produced, under the same conditions, with grapes from Évora University vineyard. The volatile content of the wines was analyzed using a CG from Agillent coupled to a BenchTOF Time-of-Flight Mass Spectrometer from Markes. Before injection, two different sample extraction methodologies were applied to the wines: a liquid-liquid extraction (LLE) and head-space solid-phase microextraction (HS-SPME). Data analysis was performed with SPSS27.0. The qualitative and quantitative models employing principal component analysis (PCA), hierarchical cluster analysis (HCA), and linear discriminant analysis (LDA) were applied. Results illustrated the differences among wine varieties regardless the extraction methodology used.

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OC4 *Thymbra capitata* L. essential oil and hydrodistillation residual water: phytochemical characterization by GC-MS and HPLC-PDA-ESI-MS^n and evaluation of the antioxidant, anti-Inflammatory and wound healing properties

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Thymbra capitata (L.) Cav. is a perennial aromatic plant widely distributed in the Mediterranean region[1]whose aerial parts are widely used in culinary, as well as in traditional medicine for several pathologies, such as diabetes, inflammation, and skin problems[2–4]. In this work, the phytochemical characterization of the essential oil (EO) was carried out by GC-FID and GC-MS, while for the hydrodistillation residual water (HWR) HPLC-PDA-ESI-MS^n was used. In addition, the antioxidant, anti-inflammatory, and wound healing activities were also determined, and the cytotoxicity of both extracts was evaluated. Regarding antioxidant activity, HRW (IC_{50} of 18.86 ± 1.076 µg/ml) was more active than the EO (156.1 ± 1.304 µg/ml). In anti-inflammatory assays the EO displayed better results being able to decrease NO production as well as the protein expression of iNOS and COX-2 at 0.16 µl/ml while the HRW only achieved inhibitory effects at 400 µg/mL. In wound healing assays, both extracts delayed cell migration. At 0.16 µl/ml the EO, weakly delayed the migration of fibroblasts (68 %), while the HRW significantly inhibited cell migration at 400 µg/ml (45 %). In the senescence assay, the EO reverted etoposide-induced senescence, whereas the HRW had no effect. EO is characterized by high amounts of carvacrol, whereas the HRW is predominantly characterized by rosmarinic acid and its isomers. The reported activities might be attributed to the presence of these compounds[5–7]. Our results highlight the industrial interest of *T. capitata* particularly for food and pharmaceutic industries.

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OC5 Anti-glycative effect of phenolic compounds from *Sambucus nigra* L. by trapping methylglyoxal

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Accumulation of advanced glycation end-products (AGEs) are implicated in the development of several pathologies, such as chronic metabolic (e.g. diabetes mellitus) and neurodegenerative diseases (e.g. Alzheimer's and Parkinson's diseases). Where, dicarbonyl compounds are the major precursors of AGEs formation. Among the different dicarbonyl compounds, methylglyoxal (MGO) represent the most cytotoxic and reactive dicarbonyl compound. Bioactive compounds, such as polyphenols are known to display a range of bioactivities as antioxidant, antiradical, metal chelation, therefore, polyphenols may display an important role preventing the formation of AGEs, acting in different stages of glycation, not only by scavenging reactive species, but also, for instance by trapping MGO. *Sambucus nigra* L. commonly named European elderberry is described as a good source of bioactive compounds, mainly phenolic compounds, presenting several bioactivities as the mentioned previously[1].
The aim of this study was to evaluate the anti-glycative potential of phenolic compounds from *S. nigra.* For that, elderberry extracts or phenolic standard were incubated with MGO under physiological conditions, and the formation of MGO-adducts was accessed by chromatography (HPLC-DAD, ESI-MS and UHPLC-MS).
Results showed that among all phenolic compounds present in elderberries, the main anthocyanins, cyanidin-3-glucoside and cyanidin-3-sambubioside, were able to trap MGO efficiently forming mono- and di-adducts. While, quercetin-3-glucoside and quercetin-3-rutinoside reacted slowly, forming MGO-adducts in a lesser extent than monoglycosylated anthocyanins. On the other hand, diglycosylated anthocyanins did not react with MGO, under our experimental conditions. In this way, elderberries present anti-glycative effect by trapping directly MGO.

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OC6 Analysis of volatile compounds of sparkling wines: the effect of using free and immobilized yeasts combined with traditional and Charmat methodologies

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Organoleptic properties of sparkling wines are conferred by the terroir, the grape variety, and technology that influences not only base wine characteristics but the resulting sparkling wine. In the second fermentation, the type of yeast used and the aging over lees are among the most important factors.
The technological process of making sparkling wines comprises two clearly defined stages: the first stage follows (more or less) the same vinification practices typical for white or rosé table wines and allow to obtain the base wine; the second stage is based on the second fermentation with the addition of tirage liqueur, and take place on bottles or in tanks, depending on the production technology, Traditional method or Charmat method.
The aim of this study was to compare the volatile composition of sparkling wine obtained from the same base wine (IPG Bairrada) with different production methods:

A – Traditional method with free yeasts;
B – Traditional method with encapsulated yeasts;
C – Charmat methos with free yeasts.

HS-SPME-GC/MS was used to study the volatiles in sparkling wines.
After normalization, principal component analysis (PCA) obtained from the volatile compounds for each sparkling wine sample showed a clear separation between all sparkling wine samples. The first and second principal components were responsible for 38.11% and 23.85%, respectively, of the system's variance.
Thus, results showed that the production methods and the yeasts used in the winemaking process significantly marked the final sparkling wines, as the characteristics of each sparkling wine were distinct, with sensory analysis notes and diversified volatile compositions.

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OC7 Determination of selected carbonyl compounds in Port wines by reductive amination of aldehyde 2,4-dinitrophenylhydrazones using cyanoborohydride

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Carbonyl compounds are present in wines due to the microorganism metabolism during alcoholic and malolactic fermentation, oxidation of wine compounds during the winemaking process, and oxidation and other chemical reactions through the ageing process in wood barrels. Consequently, their concentration in different wines is highly dependent on winemaking practices and storage conditions[1]. These compounds are mainly analysed by HPLC with ultraviolet detection after reaction with DNPH, yielding carbonyl-hydrazones[1,2]. However, the presence of geometrical isomers whose relative abundance is dependent on the reaction condition introduces analytical errors and increases the complexity of the chromatograms. One way to solve this problem is to reduce the C═N double bond to the C—N single bond and eliminate the geometrical isomers[3]. In this work, a simple, fast, and robust method for determining pyruvic acid, acetaldehyde, and α-ketobutyric acid in Port wines by reductive amination of aldehyde 2,4-dinitrophenylhydrazones using cyanoborohydride followed by HPLC-DAD was developed, optimised and validated. The method showed good performance in several parameters such as linearity (0.025-200 mg/L), detection limits (0.0043-0.0097 mg/L), quantification limits (0.014-0.032 mg/L), precision (1.1-4.2%) and accuracy (99.02%). The method was applied to White, Tawny, and Ruby Port wines. The quantification of these carbonyl compounds simultaneously was achieved in Port wines for the first time. Pyruvic acid is present in higher concentrations in Ruby Port wines and decreases with age in White and Tawny Port wines. Ruby Port wines have lower acetaldehyde levels, and Tawny Port wines have higher amounts α-ketobutyric acid.

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OC8 Gas chromatography and chemical sensors as reliable tools for the assessment of adulterations in coffee

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Coffee is an economically important commodity worldwide, being its popularity greatly linked to its pleasant aroma and flavour. However, coffee is frequently adulterated for economic gains by adding low-cost raw materials, such as coffee husks[1]. Thereby, there is a need to adopt fast and reliable methodologies for the detection of coffee adulterations to ensure coffee quality. This work aims to evaluate the feasibility of using headspace solid-phase microextraction coupled to gas chromatography/mass spectrometry (HS-SPME/GC-MS) and chemical sensors (electronic tongue) for the detection of coffee adulteration promoted by coffee husks. Commercial medium roast Colombia coffee was admixed with coffee husks at different concentrations (2% to 20%, w/w). A total of 72 volatile compounds were determined in all samples. From these, β-damascenone and linalool oxide, both related to the secondary metabolism of the coffee plant, were detected only in coffee husks and adulterated samples, suggesting that these compounds can be used as adulterant markers in coffee brews. Besides, both volatile composition and electronic tongue responses were used for constructing calibration models for the prediction of the coffee husk concentrations added to coffee. Calibration models were calculated using Partial Least Square regression with leave-one-out validation. The results confirmed the feasibility of HS-SPME/GC-MS for adulterant detection down to 2% w/w of coffee husks added, while the chemical sensors allowed its detection down to 5% w/w. This study showed that HS-SPME/GC-MS and chemical sensors can be used as simple and sensitive tools that can be easily applied at industrial level to detect adulterations in coffee brews.

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OC9 Liquid and gas chromatography as a tool for the characterization of *Fucus vesiculosus*-rich extracts as potential food ingredients

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Brown macroalgae are rich sources of health-promoting compounds, which makes them attractive for development of added-value functional foods[1]. Therefore, this work aims to develop a holistic strategy to obtain economic-affordable extracts rich in target compounds from the brown macroalgae *Fucus vesiculosus*, to be applied as added-value ingredients in functional foods. To achieve this, two sequential extraction approaches were developed, differing mostly on the solvent used in the first step: water (1st approach) or food-grade ethanol (2nd approach). The resulting macroalgae residues from this step were extracted separately with 70% EtOH, to recover phenolics and pigments, followed by hot water extraction with $CaCl_2$, to recover polysaccharides. The phenolic compounds were quantified by DMBA assay, pigments were elucidated by UHPLC-DAD-ESI-MS analysis, and polysaccharides were characterized in neutral sugars by GC-FID and uronic acids[2-4].
The results revealed that the use of water at first allowed the recovery of water-soluble phlorotannins (0.2%) and branched-laminarans (1.1%), while sequential 70% EtOH extraction yielded the highest fucoxanthin recovery (1.3%). Contrarily, the use of food-grade ethanol at first yielded phlorotannins-richer extracts (0.4%). The sequential hot water extraction in the first approach resulted in fucoidans-purer fractions, whereas fucoidans were co-extracted with laminarans when using the second approach. The amount of recovered calcium-alginates accounted for 2-4% and M/G ratios were assessed by ionic chromatography. Overall, this work allowed the development of a sustainable extraction strategy to produce macroalgae food-grade extracts rich in specific target compounds with bioactive potential to be used in the formulation of new functional foods.

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OC10 Development of statin-like and ergosterol enriched extracts from mushroom bio residues

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Although cholesterol is essential for the functioning of the human body, its excess is responsible for causing atherosclerosis, along with several other diseases that affect the bones, liver, hormones, and immune system[1]. As a natural solution, mushrooms are being studied for their ability to reduce cholesterol due to their richness in molecules capable of reducing both cholesterol absorption and synthesis[2,3]. Therefore, the present work investigated two mushrooms species (*Agaricus bisporus* L. and *Pleurotus ostreatus* L.), aiming at extracting the highest contents of statins and ergosterol. Ultrasound assisted extraction (UAE) optimization was performed followed by quantification of ergosterol and statins of the extracts by HPLC-UV[4,5], as well as the effect of the temperature on both extractions (with and without iced bath), since statins are described as sensitive to high temperatures. For *A. bisporus*, there was no significant differences in the concentration of ergosterol and pravastatin when applying ice bath during the UAE. For *P. ostreatus*, the ice bath resulted in a 6% decrease in the ergosterol concentration and a 35% increase in pravastatin content, suggesting that the best extraction method to obtain an extract rich in hypocholesterolemic compounds is a cold extraction. For optimization, the *P. ostreatus* extract revealed the highest amount of pravastatin and ergosterol (63.85 and 23.95 mg/g of extract, respectively), in which the best extraction conditions were 400 W of ultrasonic power, 4 minutes and 44 seconds of extraction time, and 15.2 g/L of solid:liquid ratio, using methanol as solvent, besides using an ultrasound extraction with ice bath.

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OC11 Headspace-solid phase microextraction coupled with gas-chromatography as a useful tool to detect volatile compounds in a pulmonary arterial hypertension pre-clinical model

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Pulmonary arterial hypertension (PAH) is a rare, life-threatening pulmonary vascular disorder with a low survival rate[1], when left untreated, mainly due to right heart failure. PAH is characterized by excessive vasoconstriction and vascular remodelling of the small pulmonary arteries[2], which lead to increased lung vascular resistance[3], culminating with right heart failure. Current therapies prevent the triad of events that lead to PAH[4], but fail to target right heart failure. In this scenario, new therapeutic strategies that reduce pulmonary vascular remodelling and, concomitantly, improve right ventricle function would contribute to mitigate PAH's outcomes.
Herein we demonstrate the protective effect of 1,8-cineole, a monoterpene widely found in aromatic plants, on the monocrotaline-induced PAH pre-clinical model. The compound applied topically was able to enter the blood stream and reach both relevant organs, the lungs and the heart, as shown by headspace-solid phase microextraction followed by gas chromatography coupled with mass spectrometry (HS-SPME-GC/MS).
Following 3 weeks of daily treatment, the compound decreased right ventricle hypertrophy, improved heart function, and prevented pulmonary vascular remodelling associated with this disease.
Overall, our work reports for the first time the use of HS-SPME-GC/MS to detect volatile compounds in biological matrices and paves the way for the development of amenable effective therapeutic approaches for PAH.

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OC12 Chromatographic-mass spectrometry methods for risk evaluation of anthropogenic and natural contaminants in raw milk

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Raw milk consumption has been increasing in Europe, especially between health-conscious people, due to its rich composition in micro and macro-nutrients, which can be diminished by industrial processing. These consumption patterns can lead to eminent hazards on human health due to milk contamination through consumption of contaminated feed (mycotoxins) and/or due to the use of veterinary drugs for promotion of growth and treatment of livestock (antibiotics). It is crucial to perform integrated assessments to fully understand the health risks associated to the high intake of this food product, and, consequently, to establish efforts aiming at the development of sensitive and robust multi-analyte methods.

This study was focused on the validation of analytical methodologies by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-QTrap-MS/MS) and high-resolution Time-of-Flight mass spectrometry (UHPLC-ToF-MS) for determination of 23 regulated, non-regulated and emerging mycotoxins, and 43 antibiotic residues from 7 different families (tetracyclines, sulphonamides, quinolones, penicillin, macrolides, cephalosporins and trimethoprim)[1], respectively, in raw milk. Blank samples fortified with standards of target compounds were used for validation of parameters including linearity, limits of detection (LoD) and quantification (LoQ), repeatability, reproducibility, and recovery. Performance criteria for regulated mycotoxins were evaluated according to specific requirements for confirmatory methods stated in Commission Regulation nº 401/2006[2]. For non-regulated and emerging mycotoxins, and antibiotics, method validation guidelines were followed in accordance with ICH guidelines and CIR 808/2021[3,4]. For application purposes, a qualitative characterization of contamination profiles in raw milk samples from Portuguese dairy farms was performed.

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OC13 Treatment of wastewater effluents using nanofiltration and low pressure UV treatment to produce high quality water that can be reused for irrigation for food production

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Agriculture irrigation accounts for approximately 70% of all water withdrawals. Climate change and the increase in human population will lead to a higher food and water demand. Hence, it is crucial to explore new water sources for agriculture irrigation, such as the reuse of wastewater effluents. Prior to irrigation with wastewater effluents, it is important to evaluate the potential uptake of contaminants by food crops.

In this study, different water sources (wastewater effluents, wastewater effluent after nanofiltration treatment and tap water) were used for the irrigation of raspberries. Several antibiotics (ciprofloxacin, levofloxacin, ampicillin, ertapenem and meropenem) as well as total coliforms and *E. coli* resistant to different antibiotics (ciprofloxacin, levofloxacin, meropenem, streptomycin and ampicillin) were quantified in the different irrigation waters over a 5 month irrigation period.

Of all the target antibiotics, only ciprofloxacin, levofloxacin, and meropenem were detected after solid phase extraction and liquid chromatography-tandem mass spectrometry at different concentrations in the different irrigation waters. The discharged wastewater effluent had higher levels of ciprofloxacin and levofloxacin, ranging from 174 to 1335ng/L.

The combination of UV photolysis with membrane filtration was tested to further treat the permeate and retentate samples from the nanofiltration unit. When ciprofloxacin was present in the permeate samples, after exposure to UV photolysis using a low pressure mercury lamp the antibiotic was not detected. In the highly concentrated retentate samples, 57% of ciprofloxacin and 31% of levofloxacin were degraded by UV photolysis using an extremely low UV fluence. If a higher UV fluence was used, higher degradation levels would be achieved. These low UV fluences were able to successfully inactivate the antibiotic resistant bacteria in the retentate samples (log reductions higher than 4).

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OC14 Development of a solid phase extraction methodology for pharmaceuticals quantification by HPLC using waste-based sorbents.

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In recent years, the level of contamination of aquatic ecosystems with sulfamethoxazole (SMX)[1] and carbamazepine (CBZ)[2], which are among the most persistent aquatic organic micro-contaminants, has increased significantly. The low levels at which these contaminants occur together with the complexity of the samples imply the need of sample preconcentration strategies prior to the quantification methodologies[3,4]. Solid phase extraction (SPE) is more frequently used due to the vast diversity of sorbents available, however, its use in large environmental screenings brings a significant increase in the cost of the analytical process, generally associated to the cost of the sorbents[5]. Thus, the preparation of alternative sorbents from wastes has received growing attention[6]. In this regard, two sorbents, derived from waste materials (spent brewery grains and primary papermill sludge) were produced by chemical activation, followed by conventional pyrolysis, and subsequently physically and chemically characterized. These sorbents were used in SPE cartridges and a preconcentration methodology for SMX and CBZ in water samples was optimized and high-performance liquid chromatography was subsequently used for their quantification. Several parameters of the SPE procedures were considered in the optimization of the process for each pharmaceutical. When using the optimized conditions for the determination of pharmaceuticals in real water samples, the recovery efficiency in river water was maintained for both pharmaceuticals for a range of concentrations, however for wastewater it decreased significantly. It was concluded that the developed method was accurate and repeatable, being suitable to be applied in the determination of SMX and CBZ in surface waters.

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OC15 Removal of estrogens from water using activated carbon from olive stone

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Micropollutants are substances that are continuously released to environments and can present adverse effects to the environment, even when present at very low concentrations (trace levels). Among these compounds are the estrogens pharmaceutical drugs, since traditional sewage and drinking water treatment plants are not able to remove or degrade them[1,2]. Thus, new and more efficient treatments are required, such advanced oxidation processes or adsorption.
Activated carbons (ACs) are known as low-cost carbonaceous materials used for removal of pollutants using adsorption processes[3,4]. This work aims to produce ACs from olive stone and to evaluate the simultaneous removal of four different estrogens by adsorption with the produced materials.
From the olive stone by product generated in the olive oil extraction, five different materials were produced, namely (i) powdered olive stone, (ii) physical activated at 800°C (iii) carbonized at 500°C, (iv) chemical activated using phosphoric acid and (v) chemical activated with sodium hydroxide. The carbonization yield was calculated and the pH at point of zero charge (pH_{PZC}) of the carbonaceous materials determined. The simultaneous quantification of estriol, estrone, 17β-estradiol and 17α-ethinylestradiol in aqueous solution was performed by high performance liquid chromatography with diode array detector (HPLC-DAD). The highest carbonization yield (57.5%) was observed using acid activation. The adsorbents production method also influences the pH_{PZC} of the adsorbents, being more expressive by the acid activation with the lowest pH_{PZC} (3.84). The olive stone raw-material shows an important potential to be used on the production of activated carbons with high carbonization yields.

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OC16 Bioaccessibility determination of omega-3 and conjugated linolenic acid using an *in vitro* standardized digestion model (INFOGEST) by GC-FID

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Omega-3 fatty acids such as EPA and DHA are essential fatty acids with relevant health benefits. Conjugated linoleic and linolenic acid also possess important health effects: anti-carcinogenic effect, anti-inflammatory properties, and body weight reduction1,2. To achieve therapeutical doses, high amounts of these fatty acids' food sources must be consumed. Thus, the intake of enriched oils with high concentration of these fatty acids are often used. But several factors influence the bioavailability of the bioactive fatty acids. Here, by using the INFOGEST static in vitro protocol of gastrointestinal tract digestion3 and through a gas chromatograph HP6890A (Hewlett-Packard, Avondaale, PA, USA) equipped with a flame-ionization detector (GLC-FID)4, we studied the bioaccessibility of these bioactive fatty acids in different matrixes: Pomegranate and Fish oil and Omega-3 and CLNA enriched capsules. There is a strong degradation effect of the general lipid fraction in the intestine. When focusing specifically on the relevant bioactive FAs, EPA and DHA (omega-3) and PUA (CLNA), comparing the oil and capsule matrixes we found that higher initial concentrations of these FAs seem to be related with lower recovery indexes after digestion. We complete the bioaccessibility studies using 3.5 kDa dialysis membranes and verified that the bioaccessibility indexes were very low or null for most of the major bioactive fatty acids of all the samples. These results indicated that most fatty acids were retained in the non-bioaccessible fraction, which could be interesting for further studies regarding their effects in gut health modulation.

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OC17 Chromatography for the analysis and purification of phenolic compounds

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Phenolic compounds are widespread in nature being present in many foodstuffs, flowers, and beverages.[1, 2] Several health benefits have been associated with the high consumption of these compounds, mainly due to their anti-oxidant, anti-mutagen, chelators of catalytic metals, and free radical scavengers properties.[3] The great abundance of these compounds in our diet combined with their role in the prevention of several health diseases turned these compounds into a target for nutritionists, and food scientists.

Moreover, it is known that dissimilar phenolic compound compositions are observed in different vegetal sources and this chemical composition varies with factors such as the vegetal source, the geographic origin, the soil composition, the maturation, the food processing, etc. Due to the apolar nature of these compounds, reversed-phase liquid chromatography has been widely used to characterize phenolic compounds in different vegetal sources, and to follow the formation of new compounds during phenolic compounds functionalization. This technique can also be used to isolate new compounds or to obtain pure standards.

Our research group has been using Reversed-Phase High-Performance Chromatography coupled with Diode Array Detector and Mass Spectrometry to study the phenolic composition, especially anthocyanins and anthocyanin-derived compounds present in grapes, wines, and respective byproducts.[4, 5] The use of chromatography to isolate new anthocyanin-derived pigments that were found to occur in red wines during aging is also a target of our research.[6]

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OC18 Modified capillary column for comprehensive two-dimensional liquid chromatographic separation of complex environmental matrices

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Comprehensive multidimensional liquid chromatography is a very common technique in proteomics and polymer research, however, its use for separation and analysis of environmental and natural products samples is still very limited. The main advantage of multidimensional liquid chromatography is the increased peak capacity allowing the separation of chromatographic peaks that would coelute in a regular 1D-LC run. However, the extra separation dimensions pose additional complexity that leads to new challenges, including the selection of stationary phases, orthogonality of separation modes, compatibility of mobile phases, and data analysis[1]. Furthermore, the flow of the mobile phase in the first dimension must be significantly lower than the subsequent dimensions, which leads to chromatographic runs of several hours to even days. To address this issue, a fused silica capillary column modified with a magnetic hybrid material composed of Fe_3O_4-CTAB micelles supported on SiO_2[2] was used as first dimension separation column alongside with a size-exclusion chromatographic column in the second dimension of a 2D-LC system.
The developed separation method was applied for the separation of complex environmental matrices such as the water-soluble fraction of particulate aerosols. The results show a remarkable increase of the separation capacity when compared to a 1D-LC system. Furthermore, the use of the modified capillary column significantly decreased the total time of analysis, the solvent consumption, and solvent compatibility issues between both dimensions.

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OC19 Unraveling the metabolites of *N*-ethylpentylone in mice serum and urine

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Synthetic cathinones (SC) have increasingly proliferated in the illicit market [1,2]. SC show psychostimulatory effects that are similar to methamphetamine and cocaine, being responsible for many intoxications and overdose deaths worldwide [1,2]. *N*-Ethylpentylone (NEP) is a popular emergent cathinone [1,2], yet little information is available about its metabolism. A higher knowledge of NEP metabolism is of major importance, in order to find out potential new markers to estimate drug consumption and confirmation of drug use.

The aim of this study is to identify possible metabolites of NEP in mice serum and urine, using ultra-high performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS). NEP and metabolites derived from its *N*-deethylation, β-keto reduction, demethylenation, and *N*-deethylation and reduction were synthesized in-house and used as standards. Urine and serum samples of mice were collected 1 h and 24 h post-dose. MS full scan mode was used for a preliminary screening aiming at searching for precursor ions corresponding to parent drugs and expected metabolites. Further MS/MS analyses were performed, and the fragmentation mechanisms were proposed. Identifications were confirmed by high resolution mass spectrometry (UHPLC coupled to an Exactive Orbitrap).

Results showed that, besides NEP, several phase I and phase II metabolites were present in urine and serum, including those derived from demethylenation, β-keto reduction, *N*-dealkylation, glucuronic acid conjugation, and others resulting from combination of various reactions. Some sulfoconjugates were also identified in both 1 h and 24 h urine samples. A further optimization of the UHPLC method allowed the resolution of several isomers of the identified metabolites. This work contributes to a better understanding of NEP metabolism. The proposed UHPLC-MS/MS method allows the identification of several NEP metabolites, which can be potential new markers to estimate drug consumption.

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OC20 Development of a GDME-HPLC-DAD-MS/MS methodology for the extraction and identification of volatile carbonyl compounds in wood-based panels

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Wood-based panels (WBPs) is a general term used for board products made with fibres, particles, or veneers, which includes particleboards, medium-density fibreboards, oriented stand boards, and others, that share similar characteristics: an adhesive is combined with particles or fibres to create a wood-adhesive matrix that, by means of heat and/or pressure in a press, bonds the particles to form a solid panel. The emission of volatile organic compounds (VOCs) from WBPs and their influence on the quality of the indoor air is a topic of interest among different European agencies, due to the increasing periods of time spent indoors and the possible impacts of these compounds on human health[1].

Gas-diffusion microextraction (GDME)[2,3] is a technique that consists on the extraction of volatile analytes through a permeable membrane to an acceptor solution (containing a derivatization reagent). GDME is a faster, simpler, and cheaper alternative method for the determination of volatiles, when compared to commonly applied methods, as it allows for the simultaneous isolation, concentration, and derivatization of analytes.

In this work, GDME was used for the extraction of carbonyl compounds directly from WBPs without any sample pre-treatment. Different extraction parameters were studied and optimized, such as the influence of the temperature, time, sample mass, volume of acceptor solution, among others. The choice of derivatization reagent was also evaluated, between 2,4-dinitrophenylhydrazine and 4-hydrazinobenzoic acid. HPLC-DAD-MS/MS studies were performed to identify the volatile carbonyl compounds extracted from particleboards and medium-density fibreboards with different characteristics and production processes.

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OC21 A fast analytical approach based on µSPEed/UHPLC-PDA for the simultaneous determination of pesticides in wastewaters

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Excessive use of pesticides constitutes a major health problem because many of them are stable enough to accumulate in the environment, contaminating soils and water and eventually being amplified through the food chain, causing diverse disruptive events.[1]
In this work, a 5-min analytical approach using an innovative microextraction procedure, µSPEed,[2] operated by the digital syringe digiVol®, was coupled with a 7.5-min chromatographic separation for the simultaneous analysis of the pesticides paraquat, thiabendazole, asulam, picloran, ametryn, atracine, linuron, and cymoxalil. Following the selection of the best µSPEed extraction conditions (C18 sorbent, minor solvent, and sample volumes - 2×250µL methanol activation, water equilibration and sample loading, and 2×50µL methanol elution), which take less than 5 min to complete, chromatographic analysis of the selected pesticides was thoroughly optimized. Overall, an acidified acetonitrile gradient was used to separate the 8 pesticides in a 1.8-µm ACQUITY UPLC HSS column, at 40ºC, followed by PDA detection. The optimized methodology was validated, and, despite its simplicity and speed, good analytical performance was obtained in terms of linear range, specificity, LODs and LOQs, recovery and matrix effect. Finally the proposed methodology was applied to the analysis of wastewater samples, exhibiting a great potential for application to other matrices.

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OC22 Monitoring of bladder cancer patients through the urinary proteome

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Biomarker discovery as a tool for diagnostic and prognostic of diseases has proven to have many shortcomings. The vast majority of recently discovered biomarkers for disease have very limited diagnostic and prognostic value. Many of these biomarkers stem from physiological conditions brought on by the disease, but cannot be directly linked to the disease itself, let alone prognostic and treatment response. Another contributing factor to these shortcomings is patient phenotype and the role it plays in biomarker response. However, modern mass spectrometry has opened up a world of new possibilities to use the evolution of the patient's proteome to evaluate the disease progression. High resolution mass spectrometry allows for the monitoring of the levels of thousands of proteins, instead of just the few biomarkers, and can provide insight into the roles proteins play in the biochemical pathways they are involved in. Thus, it becomes easy to evaluate which biochemical pathways the disease is affecting, as well as treatment response, providing useful insight into therapy adjustment. This concept was applied to a pool of patients with different stages of bladder cancer, ranging from Ta, T1, and T2+, and it was shown that through this approach it is possible to both provide an accurate diagnose of disease stage, as while also providing valuable insights into patient outcome.

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OC23 Bioanalytical method using DLLME and LC-MS/MS to determine doxycycline residues in fish

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Doxycycline (DOX) is a broad-spectrum antimicrobial with well-recognized efficacy for the treatment of bacterial diseases in fish. DOX is not yet regulated in Brazil for aquaculture, although it is already approved in other countries[1,2]. To be considered for regulation, studies of drug depletion must be executed in target animal species which demands for bioanalytical methods suitable to determine drug residues accurately at concentration levels below the Maximum Residue Limit (MRL: 100 µg kg^{-1}) in fish meat (muscle plus skin in natural proportion) [3,4]. Therefore, the method must be able to determine drug residues as below as possible the MRL in such highly complex samples[5]. Although the implementation of liquid chromatography associated with mass spectrometry in tandem (LC-MS/MS) has allowed the advances in analytical challenges, the sample preparation remains as the critical step specially concerning demands requiring high detectability in complex samples. For so, we developed a bioanalytical method starting by DOX extraction from fish meat with ethyl acetate followed by the Dispersive Liquid-Liquid Microextraction (DLLME) with innovative modifications by using methanol as dispersing solvent and water as the extracting solvent. Hexane was employed as polarity modifier. The LC-MS/MS was configured with electrospray interface operating in positive mode, and mobile phase composed by water and methanol (35:65 v/v) containing 0.5% formic acid. The method presented adequate selectivity, linearity (r>0.99, homoscedastic), high detectability (LOQ: 5 µg kg^{-1}), precision (CV<8%) and recovery (>96% and <105%).

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OC24 Encapsulation efficiency measurements on polymeric and lipid drug-delivery nanocarriers: are we there yet?

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Encapsulation efficiency (EE) belongs to the set of quality attributes that must be mandatorily and accurately characterized during the development of any drug-delivery system for clinical aims.[1] EE refers to the amount of drug associated with NPs (relative to the quantity added initially), being critical for *in vivo* therapeutic efficacy and toxicity. Nevertheless, accurate EE measurements remain challenging, particularly for organic NPs as those made of polymers and lipids, due to their susceptibility to suffer alteration under certain pre-treatment or analysis conditions.[2-4] For instance, leakage of organic NPs drug-cargo while separating NPs from free (*i.e.* unloaded) molecules for EE determinations has been reported.[3,4] Such hurdles have raised awareness on the need for innovative strategies compatible with the maintenance of NPs integrity for these separations, along with the importance of validating EE results by complementary protocols.

In this talk, the different separative strategies developed in our research group for EE measurements in polymeric NPs (poly-*D,L*-lactide-co-glycolide – PLGA, and polyethylene glycol-PLGA – PEG-PLGA) and in nanostructured lipid carriers will be addressed. The importance of adapting chromatographic conditions (*e.g.* stationary phase chemistry) depending on the nanoformulation under study (*e.g.* PLGA *vs.* PEG-PLGA) and on the goal of the analysis (*e.g.* total *vs.* loaded drug measurements) will be addressed. Moreover, the impact of the NP material (PLGA, lipid NPs) and of the pharmaceutical drug (*e.g.* methotrexate, diclofenac) on the conditions used for EE measurements using ultrafiltration as separative strategy and HPLC for quantification will be discussed.[3]

Finally, a size exclusion-based strategy for the online separation of NPs from free molecules (rhodamine B) that allows simultaneous measurements of NPs EE and quantity under HPLC will be also covered.

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OC25 Semi-preparative enantioseparation of cathinone derivatives on amylose-derived LC columns and binding studies to human serum albumin by HPALC

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Synthetic cathinones are widely abused substances due to their stimulant and hallucinogenic effects representing a public health threat since they are easily accessible online and are sold as a legal alternative to illicit drugs. All are chiral meaning that each enantiomer may present different toxicodynamic and toxicokinetic effects.[1] Hence, enantioselectivity studies are pivotal for this class of drugs. In addition, the interaction between human serum albumin (HSA), the most abundant plasma protein, and drugs has been widely explored over the past years and, nowadays, increasing attention is paid to innovative methods, such as high-performance affinity liquid chromatography (HPALC).[2]
Herein, the binding affinity to HSA of a series of synthetic cathinones was investigated by zonal elution HPALC analysis and the drug bound percentages were calculated. For some cathinones the binding to HSA was enantioselective. In addition, information on the HSA binding site was obtained, for selected synthetic cathinones, by zonal displacement chromatography, using the competitors with known specific binding sites on HSA. Competition was observed between the tested drugs and both competitors consistent with an allosteric competition with a non-cooperative binding mechanism.
To better understand the chiral recognition mechanisms and considering that for most of the synthetic cathinones the stereochemistry is not known, enantioresolution at a multi-milligram scale was performed by liquid chromatography (LC) using semi-preparative amylose-derived columns and subsequent determination of the absolute configuration of the enantiomers by electronic circular dichroism (ECD) spectroscopy, with the aid of theoretical calculations.[3] Docking studies were performed and the results were in good accordance with chromatographic experimental data.

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OC26 Anticancer drugs in the aquatic environment: where should we act and is membrane filtration a solution to this problem?

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Anticancer drugs have been detected in hospital effluents, sewage effluents and river water samples. Due to the increasing number of cancer patients, an increase in the consumption of anticancer drugs is expected and therefore the development of effective treatment options is important to avoid the discharge of these drugs in the aquatic environment. To define the most relevant target drugs to monitor, the environmental concentrations in surface waters of 123 anticancer drugs used in Portugal, Belgium and India were predicted[1]. Based on the predictions, an analytical method that comprises solid phase extraction and liquid chromatography with tandem mass spectrometry was optimized to detect the compounds with the highest predicted environmental concentrations in wastewater effluents. An occurrence study was then performed by collecting several grab samples throughout a year and comparing the results obtained in grab samples with the less time-consuming passive samplers[2].

The viability of nanofiltration for treatment of these compounds was addressed at laboratory scale using different matrices: laboratory grade water, synthetic urine, and real secondary effluent. Experimental results showed that the Desal 5DK membrane is more effective than the NF270 membrane for the rejection of these compounds, showing no significant matrix influence on the rejection results[3].

A pilot scale nanofiltration unit was finally installed in a wastewater treatment facility to confirm the laboratory scale findings and propose the operating conditions that would maximize the rejection of the target anticancer drugs and minimize fouling[4].

The anticancer compounds tested in this work have very different structures and physicochemical properties and thus the high effectiveness reported for nanofiltration at pilot scale is a good indication of what can be expected to a multitude of other pollutants. The implementation of nanofiltration in wastewater treatment plants may thus contribute to the protection of the aquatic environment.

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OC27 Profiling the phlorotannin composition from *Laminaria digitata* before and after gastrointestinal digestion

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Phlorotannins are phenolic compounds made of several units of phloroglucinol that are exclusive from brown macroalgae and have been recognized for their promising bioactive properties[1]. However, the bioactive properties described for these compounds have usually been described on pure compounds and/or phlorotannin-rich extracts without considering the possible alterations that they may incur during their passage of the gastrointestinal tract. The goal of this study was to investigate whether the gastrointestinal digestion might influence the phlorotannin profile of *Laminaria digitata* 70% acetone extracts and how these alterations may affect the extract's anti-inflammatory properties. After submitting the samples to the simulated digestion protocol (infogest), the samples were analyzed via UHPLC-ESI-MS/MS revealing interesting differences between the phlorotannin profiles of non-digested versus digested extracts, especially when looking at the fuhalol-type compounds which were completely absent in the digested extract. These results were in agreement with the total phlorotannin content (quantified via DMBA assay) and antioxidant activity (measured via the scavenging activity against $NO^{\bullet}$ and $O_2^{\bullet -}$), which were lower on the digested samples compared with the non-digested ones. Nevertheless, when non-digested vs digested extracts were tested on LPS-stimulated Raw 264.7 macrophages, both showed strong inhibitory effect on the cellular $NO^{\bullet}$ production, suggesting that even though the phlorotannins' concentration is affected by digestion, the possible products that are being formed either by degradation or by interaction with other components of the digestive medium, may exert their effects through the modulation of the intracellular signaling mechanisms. Overall, this study contributes to disclose the effects of gastrointestinal digestion in the phlorotannin profile of *L. digitata* extract, as well as to understand that, although the digestive process may affect total content of phlorotannins, it does not necessarily translate into loss of bioactivity, in particular the anti-inflammatory activity.

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OC28 Regimes de fertilização por medida como estratégias para o incremento da composição fenólica: estudo de caso em *Cichorium spinosum* L.

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A *Cichorium spinosum* L. é uma halófita perene da bacia do Mediterrâneo, consumida pelas suas folhas verdes.[1] É normalmente colhida à mão na natureza, mas o cultivo controlado permite a sua recolha várias vezes por ano, recorrendo ao uso de fertilizantes químicos.[2,3] Escusado será dizer, que a mudança para práticas agrícolas inovadoras e sustentáveis é da maior importância, num mundo em plena crise climática, degradação de terra e, principalmente, seca extrema, permitindo a produção de culturas promissoras com baixo consumo energético, pegada sustentável e ricas em compostos de alto valor agregado. No presente trabalho, o perfil individual em compostos fenólicos foi obtido por HPLC-DAD/ESI-MSn nos extratos aquosos e hidroetanólicos de plantas de *C. spinosum* cultivadas em vaso, não fertilizadas e fertilizadas com diferentes concentrações (mg/mL) de uma solução nutritivas N:P:K. Em ambos extratos, foram identificados sete compostos fenólicos, sendo o ácido *p*-cumaroilquínico e derivados de isorhamnetina *O*-glicosilados os mais abundantes (entre 33% e 44% dos compostos fenólicos totais). Os resultados obtidos mais relevantes foram a constatação de que em concentrações mais elevadas de N:P:K (C3:3:3 mg/mL) obtiveram-se maiores teores de ácidos fenólicos; enquanto teores mais elevados de flavonóides foram obtidos em concentrações mais moderadas (C 2:1:1 e C2:2:2 mg/mL). Os regimes de fertilização por medida, ou seja, desenhados de acordo com os compostos pretendidos, podem, portanto, ser usados para implementar uma estratégia de produção de plantas inovadoras para obter produtos finais de alta qualidade e de grande valor de mercado.

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OC29 HPAEC-PAD as a tool towards the characterization and design of novel carbohydrate-based sweeteners

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Fructooligosaccharides (FOS) are sweet tasting carbohydrates useful for sugar reduction in foods.[1] These oligosaccharides can be obtained by inulin depolymerization that, when taken to its limit, leads to the loss of the low caloric and glycemic features of FOS-rich food ingredients.[1,2] However, by taking advantage of chromatographic techniques, it is possible to monitor the amount and type of FOS resulting from inulin depolymerization[3]. Having this into account, this work aimed to take advantage of high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) to predict the sweetness of FOS-rich-syrups produced from yacon inulin depolymerization under different citric acid concentrations.
HPAEC-PAD discriminated all sweet tasting sugars in yacon: free sugars, FOS, and inulin, being the latter the most abundant (311 g/$kg_{dry\ matter}$). Following inulin acid depolymerization, with 0.5% (w/v) of citric acid, the inulin/FOS proportion decreased from 1.8:1 to 0.5:1. HPAEC-PAD of the hydrolysate also showed the formation of new FOS structures, of kesto and inulo-type, with degree of polymerization from 2 to 9. Accounting these changes, the syrups sweetening power was estimated to reach up 0.6 when compared to sucrose (1.0). In this context, HPAEC-PAD represents a valuable tool to design the production and predict the sweetening properties of FOS-rich syrups for their use as carbohydrate-based sweeteners.

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OC30 Digestion of a phenolic-rich extract from extra virgin olive oil using a dynamic *in vitro* gastrointestinal model: exploring the metabolomic profile

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In Mediterranean diet, virgin olive oil (VOO) is a source of fatty acids, and minor compounds (nearly 2% of the total weight) that include phenolic compounds (phenolic acids, phenolic alcohols (e.g., hydroxytyrosol, OHTyr), flavonoids, lignans, and secoiridoids). VOO has been widely associated to the prevention of several chronic diseases like cardiovascular diseases[1,2]. Despite of the claimed health effect, few studies have been dedicated to the gastrointestinal digestion impact on the VOO metabolite composition[1]. In the present study a Simulator of Human Intestinal Microbial Ecosystem (SHIME®) was used as a dynamic *in vitro* digestion model to obtain, starting with an ethanolic phenolic-rich VOO extract, different fractions of the digestion process (gastric, intestinal, and colonic) at different time points. The VOO extract, as well as the digested fractions (stomach, ST after 2h; intestinal, SI after 1.5h; colon after 6h and after 48h), and the corresponding blank samples, were analysed by a Q Exactive™ Orbitrap Focus (Thermo Scientific®) for the untargeted metabolomic analysis. The relative peak areas of the filtered compounds were compared by multivariate analysis (principal component analysis and heatmap representation). The data showed a huge diversity of metabolites that distinguish VOO from the digested fractions. If in VOO, the main metabolites were phenolic compounds, in the ST2h, SI1.5h and in colon, the furans, the lipid and lipid-like molecules and the dicarboxylic acids, were, respectively, the most abundant metabolites. Future analysis using commercial standards should be conducted and *in vitro* bioactivity studies in colon cells should be performed.

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OC31 Brewer's spent yeast gluco-oligosaccharide profiling by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)

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Brewer's spent yeast (BSY) represents a great source of bioactive ingredients, such as the cell wall polysaccharides (β-glucans, glycogen mannoproteins)[1]. In particular, BSY β-glucans have been highly exploited due to its health benefits as well as effective stabilizers, emulsifiers and potential fat replacers. Comparatively to commercial glucans, BSY β-glucans exhibit higher apparent viscosity, water holding capacity and increased emulsion stabilizing capacities[2]. These BSY glucans functionality and potential applications are closely related with their structural features.
In this work, it was studied the structural details of BSY *S. pastorianus* cell wall glucans. As BSY glucans are homopolymers of glucose, one of the major challenges of their structural analysis is the sequencing of glycosidic linkages as well the distinction of anomeric configuration. Thus, BSY *S. pastorianus* glucan details were identified upon enzymatic release of oligosaccharides followed by their analysis by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). BSY glucans were hydrolysed with a zymolyase (endo-1,3-β-D-glucanase), lichenase (endo-1,3:1,4-β-D-glucanase), amylase (1,4-α-D-glucanase) and a cellulase (1,4-β-D-glucanase). Through HPAEC-PAD, it was identified gentiotriose, maltotriose, cellotetraose and maltotetraose oligosaccharides, confirming the covalent linkage of (β1®3), (β1®6) and (α1®4)-linked glucans, but also with (β1®4)-glucan motifs. HPAEC-PAD also allowed the confirmation that lichenase released mainly (α1®4)-Glc oligosaccharides, suggesting that (β1→4)-Glc function as key connector between (β1→3)-glucans and glycogen as –(β1→3)-Glc-(β1→4)-Glc-(α1→4)-Glc motifs, on yeast cell wall. HPAEC-PAD is a powerful technique to oligosaccharides separation and identification, which allowed a step forward into BSY glucans structural characterization.

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OC32 Use of *shotgun* proteomics for the assessment of the chemical composition of biscuits melanoidins

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Melanoidins are the final product of Maillard reaction (MR), a non-enzymatic browning reaction that occurs between the carbonyl group of reducing sugars and the amine group of amino acids and proteins, at high temperatures. Melanoidins are responsible for the sensory properties of foods, such as taste, colour and aroma, occurring during the thermal process of a wide variety of foods.[1,2] It was estimated that melanoidins have a huge presence in the European diet, especially because of bread and coffee. Biscuits are also widely consumed, especially by the younger population, and therefore are amongst the main sources of melanoidins.[2] Thus, the study of the chemical structure and the biological activities of those compounds are mandatory to understand the impact of those foods on the consumer's health. Although the chemical structure of melanoidins is not fully completely stablished, it is hypothesised that melanoidins from wheat-based products are supposedly generated by coloured Mailllard reaction products (MRP's) cross-linked with gluten proteins, while other low molecular weight (LMW) MRP's are entangled in the gluten network[2,3].

In this work, we developed a new method to extract the melanoidins from samples of biscuits, using successive enzymatic digestions and solvents, and a method to identify the proteins involved in the melanoidin formation, by *shotgun* proteomics (high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). We were able to identify peptides derived from gluten proteins, such as gliadins and glutenins, as well as peptides from soluble proteins present on wheat flour, showing their involvement in melanoidin formation.

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OC33 Chromatographic approaches to study pine nut skin: exploitation of its composition and bioactivities

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Pine nut skin (PNS) is an unexploited and uncharacterized by-product recovered during pine nut processing. The exploitation of by-products as sources of valuable compounds agrees with the current demand for the reduction of waste, and a transition to more sustainable production and consumption[1]. Therefore, PNS characterization and bioactive potentialities were assessed.

The utilization of several chromatographic techniques allowed the characterization of PNS phenolic compounds (HPLC-DAD-UV and HPLC-DAD-ESI-MS^n), and the carbohydrates quantification and structural characterization, after specific derivatization (GC-FID and GC-MS). PNS subcritical water extraction using microwave was optimized and the obtained extracts, separated into low-molecular-weight (rich in phenolic compounds) and high-molecular-weight (rich in carbohydrates), were evaluated regarding their digestibility and prebiotic activity. The prebiotic potential was assessed by quantifying the short-chain fatty acids (HPLC-UV) produced after the *in vitro* faecal fermentation.

HPLC-DAD-ESI-MS^n allowed to identify PNS phenolic compounds, namely protocatechuic, *p*-coumaric, and caffeic acids, while HPLC-DAD-UV enabled the monomers identification of proanthocyanidins ((epi)catechins) and hydrolysable tannins (protocatechuic acid), after acid methanolysis. GC techniques allowed to disclose the polysaccharides structures (xyloglucans and pectic polysaccharides) and their degradation by microbiota. The fermentation of both extracts rich in phenolic compounds and rich in polysaccharides resulted in an increased production of acetic, propionic, and butyric acids when compared to the commercial prebiotic inulin, proposing these PNS extracts as prebiotic agents.

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OC34 Fig (*Ficus carica* L.) bioresidues: A chromatographic study of five varieties for its valorization

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Fig (*Ficus carica* L.) is a fruit highly recognized in history and it's been used for centuries as food, thanks to its fascinating nutritional profile and, in folk medicine due to its recognize therapeutic properties, mainly due to the presence of bioactive molecules both in its fruits and leaves[1]. Due to the perishability of the fruit and particularly the disposal of leaves in land fields, huge amounts of bioresidues are produced in its chain production. Greater sustainability in the process is therefore required, which is why the current study was designed to identify and quantify, resorting to different chromatographic techniques, various bioactive substances (organic acids, phenolic compounds, tocopherols, and fatty acids) from the produced bioresidues (leaves, peels, pulp and hole fruit) of five different fig varieties (Dauphinie, Marseille, Boujassote Noire, Longue d'Aout and Pasteliere), aiming to valorise the bioresidues as alternative sources of bioactive compounds for industrial applications. According to the obtained results, Marseille leaves stood out in organic acids composition (139.62±0.43 g/100g dw). Regarding, the polyphenolic composition, it was possible to tentatively identify 12 phenolic compounds in leaves of the Longue d'Aout variety (42.44 ± 0.27 mg/ g dw), being this variety the most interesting in terms of fatty acids, displaying the higher amount of unsaturated fatty acids (70.72%), with C18:3n3 (alphalinolenic acid) as the major molecule present. The Dauphine peel variety revealed the highest total tocopherol content (16.65±0.03 mg/100 g dw), with the prevalence of α-tocopherol (7.49±0.01 mg/100 g dw) and γ-tocopheral (6.53±0.02mg/100 g dw), with lower significant values of δ- and β-tocopherol.

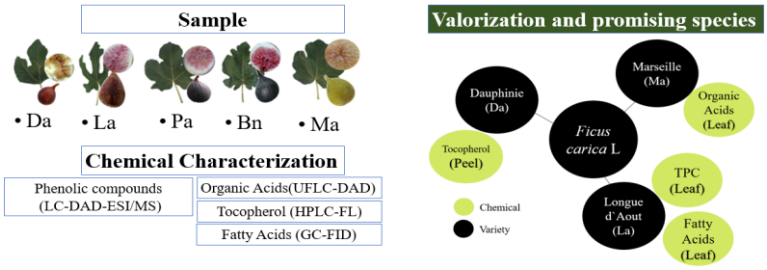

Figure (1): Varieties analysed with emphasis on those with the highest amounts of Organic Acids, Phenolic Compounds, Tocopherol and Fatty Acids

The preferred source will vary depending on the target bioactive compound, as shown in **Figure 1**. The current study, however, gave important insights into the chemical composition of the bio-waste generated during the production of figs, validating these underutilised resources as promising natural alternative sources of bioactive compounds for usage in the food, cosmetics, and pharmaceutical industries.

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OC35 Impact of growth medium salinity on galactoxylan exopolysaccharides of *Porphyridium purpureum*

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Porphyridium purpureum is a red saline microalga that can excrete high levels of sulfated polysaccharides (sEPS) into growth medium. Moreover, *P. purpureum* changes their growth rate and sEPS production and composition in response to environmental variations[1,2]. Thus, the impact of growth medium salinity (18, 32, and 50 g/L NaCl) on the sEPS production yield and chemical structure were evaluated. The excretion yield was higher for the culture with 32 g/L NaCl (90 mg/L), reflecting the effect of salinity in sEPS production[3]. Neutral sugars, determined as alditol acetated by gas chromatography with flame ionization detection (GC-FID), were mostly xylose (45-49 %mol), galactose (19-22 %mol), and glucose (15-17 %mol). The extracellular polysaccharides contain also sulfate esters (8-9%) and uronic acids (17-18 %mol). These sEPS composition was maintained for the different growth media salinities. Thus, to disclosure differences in sEPS structures, glycosidic-substitution analysis of the partially methylated alditol acetates was performed using a gas chromatography coupled to quadrupole mass spectrometry (GC-qMS) to EPS before and after desulfation. The polysaccharides obtained from the 3 cultures were mainly composed by t-Xyl, t-Xyl4S, 3-Xyl, 4-Xyl, t-Glc, 3-Glc6S, t-Gal, and 2,3,4-Gal. Moreover, it was possible to observe that the growth medium salinity slightly changed the sulfation pattern of the glucuronoglucogalactoxylan, since sEPS produced from *P. purpureum* grown at lower salinity tend to be more sulfated in *O*-3 position of xylose and *O*-6 position of glucose. This work highlighted the relevance of gas chromatography on the structural characterization of sulfated polysaccharides from microalgae.

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OC36 How do extraction methodologies influence the biological properties of pomegranate leaves?

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One of the oldest known plants is the pomegranate tree (*Punica granutum* L.), native to the Mediterranean region. This plant's edible and non-edible portions have been shown to provide several health advantages. It has long been recognized as a source of bioactive chemicals, such as phenolic acids, flavonoids, tannins, alkaloids and amino acids. Large amounts of industrial waste are generated during the industrial processing of pomegranates, and 40 to 50% of the whole fruit is typically discarded [1]. In this context, the present work was conducted to determine the chemical composition and bioactive properties of pomegranate leaves to support their potential utilisation as functional ingredients. Three extraction methodologies were evaluated: maceration, microwave and ultrasound-assisted extractions. The chemical composition of the different extracts was determined using HPLC-DAD-ESI/MS. The antioxidant potential was assessed using two cell-based assays: TBARS and CAA. Sulforhodamine B colourimetric assay was used to assess the antiproliferative capacity using several tumour cell lines and the primary culture of non-tumour cells (PLP2). The anti-inflammatory activity was measured through the extract's capacity to inhibit nitric oxide production. Finally, antimicrobial activity was evaluated using the microdilution method. The results showed that the main phenolic compounds found in the three extracts were gallic and caffeic acid derivatives, and flavonoids such as luteolin, apigenin, quercetin, and kaempferol derivatives. All the extracts exhibited capacity to inhibit tumor cell lines proliferation (GI_{50} of 19 - 76 µg/mL). Gastric adenocarcinoma (AGS) showed a higher sensitivity to all three leaf extraction methodologies. All three extracts presented lower IC_{50} values in the TBARS antioxidant assay (0.83 - 1.70 µg/mL) than the positive control Trolox (IC_{50} = 9.1 ± 0.3 µg/mL). The extracts presented a broad-spectrum antimicrobial inhibition with. *K. pneumoniae* showing the highest susceptibility to the extracts, (MIC values 0.6 mg/mL). These results suggest that pomegranate leaves can be sustainably exploited as a source of health-promoting biomolecules, to be used as functional ingredients in some biobased applications.

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OC37 Establishment of the volatile fingerprint of the PDO pear "Pera Rocha do Oeste" by HS-SPME/GC×GC-ToFMS

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"Pera Rocha do Oeste" (*Pyrus communis* L. cv. Rocha) is an important Portuguese Protected Designation of Origin (PDO) product, due to its characteristics, namely its aroma. The volatile organic compounds (VOCs) of the fruit aroma are formed through metabolic pathways and vary according to the cultivar[1]. To characterize the volatile fingerprint of PDO "Rocha" pear, it is important to select only the varietal compounds, which not vary according to the orchards, the storage conditions, and the harvest year[2,3]. In this study, a method based on HS-SPME/GC×GC-ToFMS[1,4] was used to analyze the VOCs of "Rocha" pears. PDO pears from different orchards stored in normal atmosphere (NA) (-0.5°C, 21.0% O_2, 0.5% CO_2, for 2 months), harvested in 2018 (TSS=11.1±0.9) and 2019 (TSS=11.4±0.7) and stored in controlled atmosphere (CA) (-0.5°C, 0.7% O_2, 0.5% CO_2, for 6 months) harvested in 2018 (TSS=12.0±1.0) were used. Also, non-PDO "Rocha" pears from Alentejo harvested in 2019 (TSS=12.1±0.4) stored in NA were used. From 130 compounds identified, 14 were selected (2 alcohols, 11 esters, and 1 terpene) that were constant between PDO pears from different orchards, stored in different atmosphere conditions and harvested in two consecutive years. A PCA using these VOCs allowed to discriminate PDO pears from those of a non-PDO region. The alcohols and esters have origin on amino acids metabolism and oleic and linoleic acids oxidative pathways during ripening, and the terpene from mevalonate pathway[1-3]. These 14 VOCs can be considered as varietal markers of the PDO "Pera Rocha do Oeste".

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FC1 Multi-detection and quantification of pharmaceuticals residues in seafood by a liquid-chromatography tandem mass spectrometry method

Cunha SC,[1] Mello F,[1] Fernandes JO[1]

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The presence of pharmaceutical residues in food has recently received much attention due to the growing concerns on consumers safety. A sensitive and accurate quantification of these analytes can be achieved by liquid-chromatography coupled to tandem mass spectrometry (LC-MS/MS). In this study, for detection and quantification of 11 pharmaceutical residues in seafood matrix including lean fish, fat fish and mussels, a LC-MS/MS method was developed following a QuEChERS procedure. The use of acetonitrile added with 10% formic acid and NH_4Cl in QuEChERS procedure allied with the high sensitivity of LC-MS/MS allowed a very accurate and sensitive method, able to be used on complex seafood matrices. Limits of detection (LOD) of chloramphenicol, furosemide, carbamazepine, 4'-hydroxydiclofenac, ketoprofen, naproxen, bezafibrate, diclofenac, ibuprofen, gemfibrozil, and simvastatin, ranged from 0.002 to 2.16 $ng.g^{-1}$ ww (6.06 $ng.g^{-1}$ dw) while limits of quantification from 0.01 $ng.g^{-1}$ to 7.14 $ng.g^{-1}$ ww (20 $ng.g^{-1}$ dw) were obtained[1]. Experiments using spiked samples at 3 levels of concentration in the three different matrices showed recoveries ranging from 72 to 128%. Linearity determined from matrix-matched curves in the range from 1.75–17.5 $ng.g^{-1}$ ww for fatty fish, 2–20 $ng.g^{-1}$ ww for lean fish, and 3.5–35.75 $ng.g^{-1}$ ww for mussels, provided good coefficient of correlation r>0.99.

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FC2 Multi-detection of pharmaceuticals in environment matrices by UHPLC-ToF-MS

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The constant release of veterinary and human pharmaceutical active compounds to the environment, through wastewater treatment plants, runoffs from intensive animal production, use of uncontrolled manure, can result in the presence of those contaminants in the ecosystems. These emerging pollutants are considered a global concern due to the undesirable negative effects that can cause to human, animal and environment health. The development and spread of antimicrobial resistant bacteria strains is one of the examples that can be correlated with the excess use of antibiotics. Environmental matrices, such as water, algae and sediments can work as important contaminant bioindicators and for that, should be included as matrices of choice to evaluate the levels and sources of anthropogenic contaminations.
Multi-detection and multi-class methods, based on ultra high-performance liquid chromatography coupled with high resolution mass spectrometry detector, time-of-flight, has been developed and validated to access the presence of pharmaceutical compounds in water, algae and sediments. Those analytical tools are able to detect and quantify more than 60 compounds from diverse family drugs, including antibiotics, anti-inflammatory, psychiatric, antidepressants and anticonvulsants drugs. Parameters of validation, including limit of detection (LoD), limit of quantification (LoQ), precision, recovery, linearity, selectivity and specificity, were evaluated to prove the applicability of the methods. For the matter of environmental contaminations, keeping a digital print gives the possibility of reevaluating results in the future in order to search untargeted compounds at the present time.

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FC3 Advances in the extraction of antibiotics from hen eggs

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Antibiotics are widely applied in laying hens to avoid production losses, as environmental conditions favor the rapid spread of diseases among animals. Residues of these drugs can be present in eggs and, when consumed frequently, can be a source of antimicrobial resistance (AMR), which is classified as a serious threat to health, development and world food security[1]. To ensure the quality and safety of the food, the antibiotics present must be quantified, however, biological matrices such as the egg are very complex, and extraction is a critical step for the quantification efficiency. Therefore, this study aims to share the advances made in sample preparation through the development of 12 methods of antibiotics extraction in the egg matrix varying the parameters: egg mass per sample, liquid-liquid extraction, solid phase extraction, and degreasing. Based on the maximum residue limits (MRL) of pharmacological substances in animal-origin foods and on the directives of analytical methods established by the European Commission[2,3], the blank samples were fortified with 64 antibiotics at the MRL level concentrations and analyzed in the equipment of liquid chromatography coupled to a mass detector, namely the UHPLC-TOF-MS. The number of compounds detected and their recovery rates were evaluated in order to elect the best method to, in the near future, validate and use it as a food safety tool that contributes to the containment of AMRs.

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FC4 Development and validation of a method for sugar analysis by HPLC-ELSD

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Sugars play an important role in beer, as they are essential substances for its production process. Some of the most appropriate aspects of this process for monitoring sugars are the production of wort and its use during fermentation [1]. Additionally, sugars are directly associated, for example, with the flavor and body/mouthfeel of beer [2].
In this work, a high-performance liquid chromatography (HPLC) method with evaporative light scattering detection (ELSD) was implemented and validated for the quantification of the fermentable sugars fructose, glucose, sucrose, maltose and maltotriose, in several matrices of the brewing process. Several parameters were optimized for both systems (HPLC and ELSD), in order to improve the analytical response. The stability of some of the matrices and variation in sample preparation were also evaluated.
Precision was estimated, demonstrating that the method exhibits good repeatability, with relative standard deviation (RSD) values between 0.6 and 1.9%. Regarding intermediate precision, RSD values are between 8.8 and 30.4%, only being verified as acceptable for higher concentrations, since for the lowest RSD values exceed 10%.
The trueness of the method was evaluated and accepted by performing proficiency tests and recovery tests on some of the matrices. Recovery percentages between 88 and 116% were established.
The validated method was applied to determine sugars in different matrices, aiming at the evaluation of a wort/beer fermentation profile and the evaluation of the variability between different productions and their corresponding batches of malt.

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FC5 Comparison of microwave assisted and conventional solid-liquid techniques for the chlorogenic acids extraction from silverskin: an analysis by HPLC-DAD

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In recent years, interest in naturally occurring chlorogenic acids (CGA) has increased due to their bioactive characteristics (antimicrobial and antioxidant).[1] By-products rich in CGA, such as silverskin, can be found in the coffee industry. Aqueous microwave-assisted extraction (MAE) may be a more efficient way to obtain bioactive compounds than conventional extraction by organic solvents since it is more sustainable and decreases process time.[2]
In this work, two techniques for the extraction of CGA from silverskin were compared: MAE (aqueous) and solid-liquid extraction (SLE) (aqueous and hydroethanolic). Initially, the influence of temperature on the MAE of CGA from silverskin was studied. The MAE extract with the highest CGA yield (namely, 5-CQA) was compared to the SLE technique previously optimized for the extraction of these compounds. CGA were analyzed by RP-HPLC-DAD and monitored at 320 nm.[3]
The results showed that 5-CQA is the most abundant CGA in silverskin (0.34, 0.41, and 0.57 mg/g dry sample, for aqueous SLE, MAE [80 °C] and hydroethanolic SLE, respectively). Temperatures higher than 80 °C resulted in a lower extraction yield for this compound and higher for 3- and 4-CQA, which may be due to the isomerization of 5-CQA when subjected to temperatures of 100-200 °C.[1] Except for 5-FQA, all CGA were significantly higher in MAE compared to aqueous SLE. This means that MAE enhances the extraction of CGA from silverskin.
MAE can be a viable alternative to conventional techniques that use organic solvents, producing CGA-rich extracts that can be used in the production of functional foods.

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FC6 Comprehensive two-dimensional gas chromatography as a tool to unveil the volatile profile of grape spirits used in Port wine fortification

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Port wine is a fortified wine exclusively produced in the Douro Appellation (Portugal) under very specific conditions resulting from natural and human factors. Its intrinsic aroma characteristics are modulated upon a network of factors, such as the *terroir*, varieties and winemaking procedures that include a wide set of steps, namely the fortification with grape spirit (*ca*. 77% v/v ethanol). The grape spirit comprises roughly one fifth of the total volume of this fortified wine, thus it is a potential contributor to the global quality of this beverage, including the aroma notes. Nonetheless, the information about the influence of the grape spirit on the final aroma of Port wine, as well as the grape spirit volatile composition, are extremely limited. This work intends to perform an in-depth mapping of grape spirits volatile organic compounds and to generate new data that may contribute for molecules' identification. To fulfill this goal, the experimental parameters of a methodology that combines the two-dimensional gas chromatography-mass spectrometry with time-of-flight analyser (GC×GC-ToFMS) with a solvent-free solid phase microextraction technique (SPME) were optimized. The SPME experimental parameters (fiber coating, extraction temperature, and time, sample volume and dilution conditions) were selected. Also, different column sets (first × second dimensions) were tested to obtain the best chromatographic resolution and peak capacity. Firstly, the GC×GC-ToFMS experimental parameters were implemented using a reversed phase column set (polar ^{1}D × nonpolar ^{2}D) that presented advantages compared to the conventional column set (nonpolar ^{1}D × polar ^{2}D) regarding the analytes´ separation. Secondly, the SPME conditions that promoted the highest extraction efficiency were: 2.0 mL of spirit (diluted at 10% v/v ethanol) were extract with 50/30 µm DVB/CAR/PDMS StableFlex™ (1 cm), at 40 ˚C, using 10 min of pre-equilibrium followed by 30 min of extraction. To test the applicability of this methodology, a set of grape spirits were analysed, which allowed the detection of hundreds of volatiles. This study adds further insights unveiling the complex nature of the grape spirits chemical volatile data, through the identification of compounds not yet determined in these matrices. These novel data may be useful in the production of Port wines that promote novel sensorial experiences, respecting tradition and quality of this highly recognized fortified wine.

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FC7 Phenolic profile and bioactive potential of cardoon (*Cynara cardunculus* L.) inflorescences: selection of the best genotype for food application

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Cynara cardunculus L. (syn. cardoon) belongs to the Asteraceae family and is native to the Mediterranean basin. This species is widely used for milk coagulation of PDO cheeses and consumed due to its rich nutritional value and related medicinal properties (e.g. choleretic, cardiotonic, antidiabetic, liver disease treatment). In the last decade, the interest in this crop increases due to its multifaceted industrial applications. It has been explored for animal feed and lightwood panels, biodiesel, paper pulp, and food oil production. Their production countries estimate a generation of approximatly 15 to 30 t/ha of dry biomass. The adequate exploitation and characterization of the species are essential for circular economic stimulation and environmental impact reduction. The hydroethanolic extracts of four cardoon genotypes and their vegetable tissues (stigma, corolla, bracts, pappus, receptacle) were studied. The polyphenolic profile was analyzed by HPLC-DAD-ESI/MS. The antioxidant activity was evaluated TBARS and OxHLIA cell-based assays.The anti-inflammatory activity was assessed through the extracts' capacity to inhibit the pro-inflammatory mediator nitric oxide. Finally, the cytotoxic potential was evaluated against four tumor and a non-tumor cell line (PLP2) using the sulforhodamine B assay. Fourteen phenolic compounds were tentatively identified. The corolla presented the higher variety and bracts the higher content of phenolic compounds. In terms of bioactivity, none of the tested extracts exhibit anti-inflammatory potential. Genotype F1-1-1 exhibits a higher cytotoxic potential. All the four genotypes and vegetable tissues tested exhibited antioxidant capacity, especially corolla of F4-1-4 genotype for TBARS assay (IC_{50} = 38 µg/mL) and its receptacle for OxHLIA (IC_{50} = 71 µg/mL). In conclusion, this study showed that the phenolic composition and biological activities of cardoon are influenced by both genotype and plant tissue. Further studies are needed to determine the genetic information for obtaining the highest bioactivity.

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FC8 Salt pan brine water glycans: complementary characterization by GC-FID and HPAEC-PAD

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Salt pans are man-made systems where seawater gives rise to sea salt due to its evaporation by wind and sunlight. Seawater is a source of highly heterogeneous sulfated glycans that are excreted by marine organisms, being accumulated in salt pan brine water. These glycans have been shown to improve the immune system[1]. Therefore, to establish the structural features responsible for biological activity[2-4], glycans recovered by dialysis (cut-off 12 kDa) in salt pan brine waters were characterized by colorimetric and chromatographic techniques. These glycans were acid hydrolyzed and analyzed by gas chromatography with flame ionization detector (GC-FID) after derivatization to determine neutral sugars[1], by the m-phenylphenol colorimetric method to obtain the total content of uronic acids[1], by high-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) to identify and quantify the uronic acids[5], and by $BaCl_2$ turbidimetric method to access sulphate esters content[1]. The main neutral sugar of salt pan brine waters was galactose (13 mol%), followed by small amounts (1-6 mol%) of glucose, mannose, xylose, fucose, rhamnose, arabinose, and ribose. HPAEC-PAD analysis allowed to reveal that the uronic acids, quantified by colorimetry, were galacturonic acid (13 mol%) and glucuronic acid (10 mol%). The content of uronic acids (23 mol%) and sulfates (45 mol%) showed that salt pan brine water glycans are highly charged carbohydrates. These results show how GC-FID and HPAEC-PAD techniques are complementary to analyze both neutral sugars and uronic acids, respectively, and contribute to the establishment of structure-function relationships.

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FC9 Perfil lipídico de óleos de subprodutos de peixe obtidos por extração assistida por micro-ondas e Soxhlet

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A indústria pesqueira produz anualmente grandes quantidades de subprodutos de pescado, atualmente pouco valorizados pelo setor agroalimentar. No entanto, a exploração destes recursos naturais tem vindo a ser promovida pelos teores lipídicos geralmente elevados e pelo surgimento de técnicas de extração mais sustentáveis, no entanto ainda pouco exploradas para obtenção de frações lipídicas de subprodutos de peixe. Portanto, este trabalho teve como objetivo obter óleos de subprodutos de peixe por extração assistida por micro-ondas (EAM) e extração Soxhlet (ES) e caracterizar o seu perfil lipídico. Os subprodutos de peixe de categoria 3, fornecidos e considerados standard pela indústria transformadora (Grupo ETSA), foram liofilizados e submetidos às seguintes condições de extração: 17 min de irradiação a 750 W, numa razão sólido/líquido de 70 g/L, para EAM e 6 h de extração a 80 ºC, numa razão de 20 g/L, para ES; hexano foi o solvente utilizado[1]. O rendimento de óleo foi determinado gravimetricamente e o perfil de ácidos gordos foi analisado por cromatografia gasosa com deteção de ionização de chama (GC-FID), após um processo de derivatização realizado para obtenção de ésteres metílicos de ácidos gordos. A EAM permitiu obter um rendimento de óleo semelhante ao da ES (cerca de 18 g/100 g dw). O perfil lipídico foi constituído maioritariamente por ácidos gordos insaturados, devido aos teores elevados dos ácidos oleico, docosahexaenóico (DHA) e linoleico, os quais não foram afetados significativamente ($p < 0,05$) pelos métodos de extração. Estes óleos serão de interesse para formulação de alimentos para animais.

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FC10 GC×GC-ToFMS analysis of passion fruit volatile profile: a step to unveiling consumers aroma perception

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Passion fruit has an average global production of 1.5 million tonnes and is mainly directed to juice production or consumption in fresh[1]. Its purple variety (*Passiflora edulis* f. *edulis*) is considered more pleasant for raw fresh consumption due to its higher sweetness and lower acidity[2].
Flavour perception of fresh fruits is considered among the most relevant factors that influence the purchase decision and such perception is deeply influenced by aroma, which is determined by the emitted volatile organic compounds (VOCs). Headspace solid-phase microextraction (HS-SPME) combined with comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-ToFMS) is known as a powerful tool to unravel complex mixtures of VOCs and semi-volatile organic compounds mainly due to its improved resolution and sensitivity[4].
The objective of this study is to perform an in-depth characterization of purple passion fruit juice VOCs profile and identify key markers that influence the consumer aroma perception at the moment of purchase and consumption. The VOCs released from whole fruit, halved fruits and juice were investigated by HS-SPME/GC×GC-ToFMS which allowed the detection of hundreds of chromatographic peaks, among which a set of compounds that have high impact on consumers aroma perception were identified. Brix°, pH, total titratable acidity, total phenolic compound (TPC) and antioxidant activity were also quantified and chemometric tools were used to combine all domains of information to correlate VOCs profile and physicochemical parameters.

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LIST OF THE POSTERS

P01 Development and validation of a UHPLC-UV method for MDPV quantification to evaluate enantioselectivity on their absorption using the Caco-2 cell line

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Synthetic cathinones, such as 3,4-methylenedioxypyrovalerone (MDPV), are psychoactive substances well absorbed by the oral mucosa[1]. However, the level at which these compounds and their enantiomers cross the intestinal barrier has only been determined for methylone and pentedrone[2]. Actually, literature on enantioselectivity studies with synthetic cathinones is still scarce[3]. Thus, the present study aimed to analyze the potential enantioselectivity of the enantiomers of MDPV in the passage across the Caco-2 monolayer, a widely used *in vitro* model for intestinal permeability studies. The Caco-2 monolayer was exposed to 300 µM of each enantiomer of MDPV in both apical (AP) to basolateral (BL) and BL to AP directions and samples were collected for 5 hours. To detect and quantify MDPV in the transport buffer of the study, HBSS(+/+), an UHPLC-UV method was developed and validated. The parameters studied were specificity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). Additionally, evaluation of samples' stability was also performed.
High selectivity for MDPV and good linearity were observed in the tested concentration range (0.5-500 µM) with correlation coefficients always higher than 0.999. An accuracy ranging between 102 and 109%, inter-day and intra-day precisions with coefficients of variation below 15%, LOD and LOQ of 0.063 µM and 0.19 µM, respectively, were also observed. Samples were stable for 6 weeks of storage in different temperatures (room temperature, 4 °C, -20 °C and -80 °C). No statistically significant differences were found between the enantiomers in the passage across the Caco-2 monolayer (no enantioselectivity).

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P02 Nicotine-like insecticides a new environmental concern

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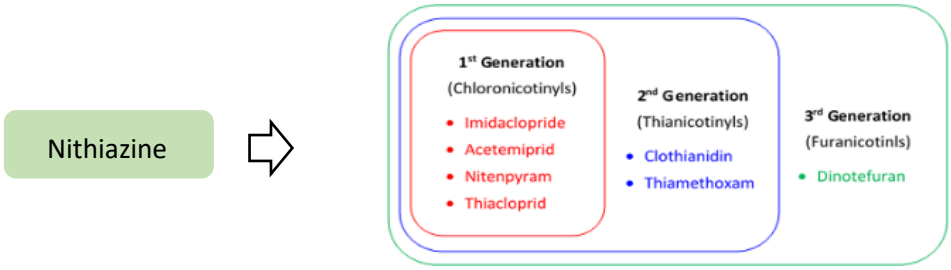
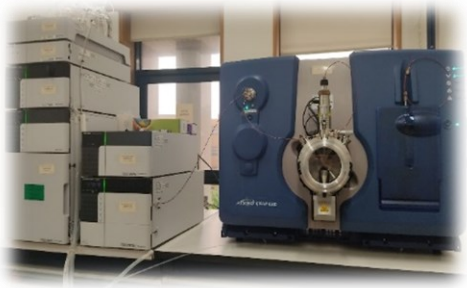
Neonicotinoids are a class of insecticides with nicotine-like molecular structure that have been used widely in the last two decades.
Nithiazine was the first neonicotinoid developed by Shell (USA) in the 1970s, it is selectively toxic to insects but its field application is limited due to low photostability, nevertheless it was the lead compound in syntheses of the first commercially successful neonicotinoid, imidacloprid.



Neonicotinoids are systemic pesticides that act in different parts of the plants. They are much more toxic to invertebrates, than they are to mammals. Recent studies suggest some relation to the Colony Collapse Disorder of bees, with effects on the central nervous system of these insects, leading to eventual paralysis and death. So in recent years, the EU severely restricted the outdoor use of Clothianidin, Imidacloprid and Thiamethoxam due to the identified risks to bees.
An efficient LC-MS/MS method was developed to measure seven neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam) in water samples in order to evaluate the Portuguese river contamination.



Neonicotinoid Analysis	
Method of analysis	Direct injection/SPE – UPLC-MS/MS
Limit of Quantification	50 ng/L (0,1 ng/L with SPE)
Quantification range	50 – 2000 ng/L (0,1 – 100 ng/L with SPE)
Calibration curves	R ≥ 0,99
Acceptable recovery (n=5)	80 – 120 % (SD < 20%)
Method uncertainty	< ± 35 %

Acknowledgements: We want to express our gratitude to the other members of the APA, IP especially the LRA partners.

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P03 Fatty acid profile of subcutaneous and visceral adipose tissue from north Portuguese obese women

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Fatty acids (FA) are crucial autacoids molecules in the synthesis of bioactive lipids. The interest on FA has increase as these can prevent and treat numerous metabolic conditions. However, at high levels FA are associated to disorders as diabetes, obesity, amongst others[1]. Usually, FA analysis is conducted in plasma, representative of a recent diet. Although, for a long-time vision of FA profile, adipose tissue (AT) is a more assertive matrix. FA are storage in AT and are released to plasma during fasting[2]. AT can be divided in subcutaneous AT (scAT) and visceral AT (vAT), located either close to the skin or around an internal organ, respectively[3]. These AT behave distinctly, scAT take FA from blood and develops by fat cell size increase while vAT releases FA to blood or organs and it grows by number of fat cells. vAT is also more innervate, vascularized and active. In obese individuals AT acts differently than in nonobese individuals, which can cause metabolism alterations[4].
The scAT and vAT FA profile for 188 obese women from North Portugal was assessed. AT lipids were extracted by ultrasound-assisted extraction with *n*-hexane[5] and FA methyl esters were obtained by base-catalysed derivatization. FA identification and quantification was carried out using gas chromatography with flame ionization detector[1].
In all samples analysed for both tissues were detected the FAs C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C14:1, C16:1, C18:1cis, C20:1, omega-3 C18:3, and omegas-6 C18:2cis C20:2, C20:3, C20:4 and C22:6. Significantly differences on FA profile between scAT and vAT were obtained.

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Funding: The authors are thankful to Fundação para a Ciência e a Tecnologia (FCT)/Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) for the support of this work through projects UIDB/50006/2020, UIDP/50006/2020, and LA/P/0008/2020.

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P04 Polycyclic aromatic hydrocarbons analysis in human adipose tissue by HPLC – analyte loss evaluation during extraction and sample preparation

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Polycyclic Aromatic Hydrocarbons (PAHs) are endocrine disruptors released into the environment by incomplete combustion and pyrolysis processes. Either through ingestion, inhalation or dermal contact, humans are chronically exposed to these lipophilic compounds. After adipose tissue (AT) accumulation, PAHs can lead to diabetes, oxidative stress, inflammation, cancer, infertility, and others. However, analytical methods for PAHs assessment in AT are scarce[1].

The extraction and quantification of 18 PAHs in AT were studied using an ultrasound-assisted extraction (UAE) and High-Performance Liquid Chromatography (HPLC). An important issue in PAHs analysis is the analyte loss during the analytical method either by the retention (using clean-up step and syringe filter), by the adsorption of heavier PAHs (to glass surface), or/and by the evaporation of volatile PAHs (using nitrogen)[2]. Thus, several steps were assessed, namely: UAE, concentration factor, clean-up, and filtration of extract before HPLC.

The methodology achieved provided a simple, faster, reliable, and eco-friendly extraction of PAHs: 0.4 g of AT was homogenized with 6 mL of *n*-hexane in an ultrasonic processor[3], and quantification was carried out in a Shimadzu LC system with photodiode array and fluorescence detectors inline. PAHs were separated using a CC 150/4 Nucleosil 100-5 C18 PAH column[4]. The method was validated with linearity, method detection limits, method quantification limits, precision (intra- and inter-day), recovery, and matrix effect. Samples of human AT from Portuguese women undergoing bariatric surgery at Hospital de São João, Portugal (CE146-09) were analysed for PAHs following the optimized methodology and 8 PAHs were found in all samples.

Acknowledgements: Sara Sousa would like to thank Fundação para a Ciência e Tecnologia (FCT) for the Ph.D. scholarship SFRH/BD/137516/2018 and COVID/BD/153231/2023. The authors thank the General Surgery Department of Hospital de São João (Porto, Portugal) for the human adipose tissue samples and to all patients that consent to participate.

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P05 Effect of mobile phase composition on the separation between phosphatidylethanol (PEth) and phospholipid background using LC-MS/MS

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Phosphatidylethanol (PEth) is a group of specific direct ethanol biomarkers with a substantially longer half-life than ethanol and other ethanol biomarkers, that can be used to distinguish between different drinking patterns, such as heavy and social drinking. More than forty PEth homologues have been detected in blood from heavy drinkers. PEth 16:0/18:1 is the most predominant PEth homologue and also the one most commonly determined by LC-MS/MS [1,2]. Since PEths are phospholipids, it is difficult to separate them from unwanted phospholipids during sample preparation. Therefore, to minimize possible matrix effects, it is important to separate PEth from other phospholipids during LC-MS/MS analysis[3].

In this study, we have investigated how the retention and chromatographic separation of eight PEth homologues and the phospholipid background are influenced by changes in mobile phase composition. Our findings show that the buffer concentration of the aqueous part of the mobile phase had a huge effect on both the retention of PEth homologues and the separation of PEths from unwanted phospholipids. By using a buffer-free mobile phase consisting of 0.025% ammonia in Type 1 water, pH 10.7, as solvent A and methanol as solvent B, all eight PEth homologues were separated from unwanted phospholipids using a Acquity BEH C18 column (50 x 2.1 mm I.D., 1.7µm particles). This information can be important for the development of reliable and robust bioanalytical LC-MS/MS methods for determination of PEth homologues[4].

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P06 Development of acid-chars for microextraction of micropollutants in water

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The present work aimed to develop and characterize acid-chars as potential adsorptive phases for bar adsorptive microextraction technique (BAµE), followed by high performance liquid chromatography with diode array detection to monitor trace levels of nine pharmaceutical compounds and hormones in water matrices. Both polar and nonpolar compounds were selected as model compounds, such as, 17-α-ethinylestradiol, clofibric acid, mefenamic acid, carbamazepine, diclofenac, estrone, gemfibrozil, triclosan and sulfamethoxazole, to represent different therapeutic classes. The acid-chars were prepared by H_2SO_4-mediated carbonization of sisal using two different concentrations, 9 M and 13.5 M. To study the potential of acid-chars as BAµE phase, the effect of different organic solvents to clean the acid-chars and the influence of the matrix pH were performed. For benchmarking the two lab-made acid-chars were tested along with a commercial powdered activated carbon. The carbon materials (acid-chars and commercial activated carbon) were characterized by N_2 adsorption isotherms and by the pH at the point of zero charge. The results obtained showed that, despite having incipient porosity, the acid-chars managed to obtain a response for all analytes under study and in some cases, the recoveries efficiencies overcame those attained with the commercial activated carbon used as control. Therefore, this work showed that acid-chars have potential as adsorbents for samples enrichment.

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P07 Application of a GC-MS metabolomics approach to reveal how sub-toxic concentrations of ciprofloxacin affect the brain

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Ciprofloxacin (CPX) is a fluoroquinolone antibiotic widely used for treating respiratory, urinary, gastrointestinal, and abdominal infections. Although several case reports correlate CPX with neuropsychiatric/psychological adverse effects[1], the putative neurotoxic pathways activated by therapeutic doses of CPX remain unclear. Here, we aimed to investigate the early metabolic alterations caused by CPX in SH-SY5Y dopaminergic-differentiated human neuroblastoma cells, through a non-target metabolomic approach using gas chromatography-mass spectrometry (GC-MS). For that, we performed an endometabolome analysis of cells exposed to pharmacologically relevant CPX concentrations (0.1, 1 and 10 μM) for 4, 24, 48 and 72h. Notably, none of the concentrations tested significantly reduced metabolic activity (MTT reduction assay) or caused apoptotic/necrotic cell death (Hoechst 33342/propidium iodide labeling). Our results revealed significant metabolic changes in control cells after prolonged exposure (48h and 72h). These seemed to be associated with the cells' dedifferentiation, as they began to lose their dopaminergic phenotype. As such, we considered the metabolic changes observed at 24h as the most relevant and informative. At this time point, CPX induced a significant dysregulation of several metabolites involved in the tricarboxylic acid cycle, amino acids biosynthesis and metabolism, glycolysis/gluconeogenesis and aminoacyl tRNA biosynthesis, even at the lowest concentration tested (0.1 μM). Overall, these data indicate that GC-MS-based endometabolome analysis provides valuable metabolic information, allowing the evaluation of the early neurotoxicity events associated with CPX exposure.

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P08 Quantification of urinary monohydroxyl-polycyclic aromatic hydrocarbons in firefighters participating in a controlled urban fire event

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Occupational exposure as a firefighter is carcinogenic to humans, being polycyclic aromatic hydrocarbons (PAHs) among the most health-relevant pollutants[1]. PAHs are formed during the incomplete combustion of organic materials and have toxic, mutagenic, and carcinogenic properties[2]. The characterization of Portuguese firefighters during firefighting at urban fires is inexistent. Biomonitorization represents a crucial tool to characterize exposure to PAHs regardless of the route of exposure (inhalation, ingestion, and dermal contact)[2,3]. The monohydroxylated PAHs (OH-PAHs) are biomarkers of exposure that are primarily eliminated through the urine[2,3]. This work characterizes the levels of 6 urinary OH-PAHs, 1-hydroxynaphthalene, 1-hydroxyacenaphthene, 2-hydroxyfluorene, 1-hydroxyphenanthrene, 1-hydroxypyrene, and 3-hydroxybenzo(a)pyrene in Portuguese firefighters participating in a controlled urban fire.
Firefighters collected the first-morning urine on the day of the fire event and in the day after. Samples were extracted and analyzed by liquid chromatography with fluorescence detection[4]. Concentrations were normalized with urinary creatinine levels determined by the Jaffe colorimetric method[4]. Urinary 1-hydroxynaphthalene and 1-hydroxyacenapthene were the predominant compounds (97.8–99.5% of total OH-PAHs), followed by 2-hydroxyfluorene (0.30–2.03%), 1-hydroxyphenanthrene (0.15–0.16%), and 1-hydroxypyrene (0.06–0.09%). 3-hydroxybenzo(a)pyrene, a biomarker of exposure to carcinogenic PAHs, was not detected. Levels of 1-hydroxypyrene, a biomarker of exposure to PAHs, were 1.5 times higher after firefighting than in the pre-fire spot urine samples (0.552 *versus* 0.377 µmol/mol creatinine, respectively); post-fire values were slightly higher than the benchmark level (0.5 µmol/mol creatinine) proposed by the American Conference of Governmental Industrial Hygienists[4]. Additional studies are needed to characterize firefighters´ occupational exposure during urban fires.

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P09 Chromatographic measurement of aldehydes and ketones diffusivities in compressed liquid ethanol

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The knowledge of diffusion coefficients (D_{12}) is fundamental for the accurate design and optimization of chemical processes limited by mass transfer kinetics[1]. These data are generally scarce in the literature specially for polar solvents, and reliable models and computational strategies are also lacking for such systems emphasizing the need of experimental D_{12} values[2].
In this work the measurements of D_{12} in compressed liquid ethanol of three aldehydes (butanal, pentanal and hexanal) and six ketones (propanone, butanone, pentan-2-one, pentan-3-one, hexan-2-one and hexan-3-one) were carried out over 303.15-333.15 K and pressures up to 150 bar using the Chromatographic Peak Broadening (or Taylor-Aris) technique[3]. The experimental method was accurately validated and the obtained D_{12} values ranged from 1.39×10^{-5} to 2.68×10^{-5} $cm^2\ s^{-1}$ for aldehydes, and from 1.28×10^{-5} to 2.89×10^{-5} $cm^2\ s^{-1}$ for ketones. The general trends regarding pressure, temperature and Stokes-Einstein dependencies were analyzed, and the obtained diffusivities of the various isomers were compared. It was found that between the different ketones isomers the D_{12} values were indistinguishable. Moreover, between functional isomers the D_{12} values of aldehydes were found to be in average 7 % higher than the D_{12} values of ketones.

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P10 Caracterização cromatográfica de compostos bioativos obtidos a partir de casca, sementes e fibras remanescentes do aproveitamento industrial da polpa de abóbora

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A procura de fontes económicas adequadas para obtenção de compostos naturais com propriedades bioativas tem vindo a ser crescentemente investigada. Neste sentido, o extrato das cascas, fibras e sementes de três variedades de abóbora ('Butternut Squash', 'Common Pumpkin' e 'Kabocha Squash') cultivadas em Portugal foram avaliadas cromatograficamente quanto ao seu conteúdo de moléculas de alto valor acrescentado. A composição das amostras em tocoferóis foi determinada por HPLC-FLD, em ácidos orgânicos por UFLC–PDA e em compostos fenólicos por HPLC/DAD-ESI/MS. As cascas apresentaram o perfil mais diversificado de compostos fenólicos, sendo tentativamente identificados oito diferentes compostos na variedade 'Common Pumpkin'. O (-)-epicatechin ([M-H]- em *m/z* 289) foi o composto maioritário em todas as amostras, que revelaram conter entre um a cinco flavonoides. Para além disso, verificou-se a presença do ácido fenólico 7 4-O-(6'-O-glucosyl-4''-hydroxybenzoyl)-4-hydroxybenzyl alcohol ([M-H]- em *m/z* 405). Relativamente aos tocoferóis, o α-tocoferol foi encontrado em todas as amostras, sendo esta a isoforma biologicamente mais ativa da vitamina E. Nas amostras que apresentaram a isoforma β, esta foi detetada em maior quantidade. Foi ainda encontrado o δ-tocoferol e nenhuma das amostras apresentou γ-tocoferol. No que respeita aos ácidos orgânicos, foram identificados os ácidos oxálico e málico em todas as amostras e quase todas apresentaram ácido cítrico e fumárico. Os ácidos quínico, ascórbico e chiquímico não foram detetados. Estes resultados demonstram a riqueza em compostos naturais bioativos dos subprodutos do processamento industrial da polpa da abóbora e corroboram, assim, a importância da valorização dos mesmos, promovendo processos industriais mais sustentáveis.

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P11 Caracterização fenólica (HPLC-DAD-ESI/MS) e bioativa de extratos ricos em moléculas conservantes

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Com o intuito de valorizar subprodutos industriais e promover processos sustentáveis, o presente trabalho teve como objetivo explorar as cascas, sementes e fibras resultantes do processamento da abóbora como fonte de compostos bioativos com capacidade conservante. Assim, foram avaliados os subprodutos de três variedades cultivadas na Argélia, nomeadamente 'Gold nugget pumpkin', 'Butternut Squash' e 'Musqueé de Provence'. A composição fenólica foi avaliada por HPLC-DAD-ESI/MS e o potencial conservante dos extratos foi demonstrado através da análise da atividade antioxidante, por dois métodos biológicos (TBARS e OxHLIA), e antimicrobiana, contra oito estirpes de bactérias e duas de fungos. Todas as amostras apresentaram capacidade de inibição microbiana e elevada atividade antioxidante, com destaque para as sementes, especialmente da variedade 'Gold nugget pumpkin' que apresentou um valor de IC_{50} inferior ao do controlo positivo Trolox no ensaio TBARS. As sementes também apresentaram melhor atividade antibacteriana do que os restantes subprodutos, protegendo contra sete das oito estirpes bacterianas testadas, na concentração máxima de 10 mg/mL. Relativamente ao perfil fenólico, conforme apresentado na Figura 1, foram tentativamente identificados sete compostos, sendo estes o flavan-3-ol (-)-epicatechin ([M-H]- at *m/z* 289; pico 1), o ácido cítrico ([M-H]- at *m/z* 191; pico 2), o ácido fenólico 4-O-(6'-O-glucosyl-4''-hydroxybenzoyl)-4-hydroxybenzyl alcohol ([M-H]- at m/z 405; pico 3), e quatro flavonoides nos restantes picos. Curiosamente, as cascas e as fibras apresentaram maior quantidade e heterogeneidade de compostos fenólicos totais do que as sementes. Estes resultados provam o potencial dos subprodutos de abóbora como fontes económicas e promissoras de compostos conservantes, favorecendo processos industriais mais sustentáveis.

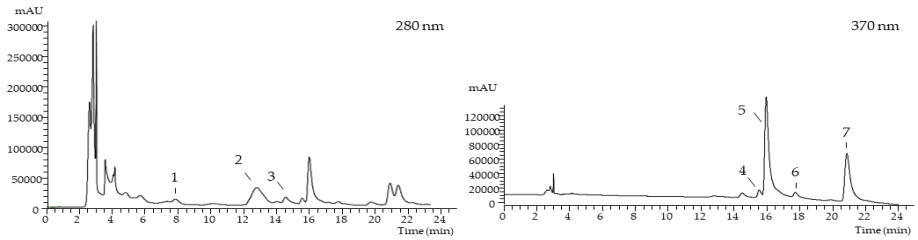

Figura 1: Cromatograma do extrato de casca de abóbora 'Gold nugget pumpkin'.

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P12 Analysis of the insecticide Emamectin in cork trees after treatment

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The flathead oak borer (*Coroebus undatus*) is a beetle in the Buprestidae family whose larvae grow inside the cork tree (*Quercus Suber*), causing significant damage to the cork itself, and compromising its economic value. Currently, the insecticide Emamectin is being field tested to fight the oak borer. In an effort to understand the mobility of this insecticide and check if the cork is bellow maximum residue limits, an analytical method was developed to quantify emamectin in cork removed from treated trees. The method involves griding the frozen samples and sieving through a 1 mm mesh. Extraction is performed with acetonitrile in an ultrasound bath, followed by drying under nitrogen and derivatization with 1-methylimidazole, triethylamine and trifluoroacetic anhydride[1], to generate a single fluorescent derivative. HPLC-FLD was used in isocratic mode, with water/acetonitrile as eluent. Abamectin, a structurally similar compound to emamectin, was used as internal standard, added before the extraction to correct any volumetric errors as well as to check the derivatization efficiency. Calibration was performed by extracting several spiked cork samples at known concentrations. The R^2 was 0.97 and the limit of quantitation was 20 µg *per* kg of cork. The absolute extraction efficiency was between 73-80% and the relative standard deviation between same concentration extractions was at most 10%.

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P13 Removal of naproxen from water using adsorbents obtained from low-cost materials

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The continuous growth of world population together with the strong urbanization has triggered an increasing demand for freshwater which has resulted in a serious deterioration of water bodies[1]. Water pollution with pharmaceutical drugs is becoming a relevant problem. The concentration of non-steroidal anti-inflammatory drugs, estrogens, personal care products, among others, in waterways is reaching hazardous levels, posing a threat to the environment and human health. Moreover, conventional cleaning and degradation processes applied on wastewater treatment plants are inefficient to eliminate or remove these compounds. Adsorption is a treatment process considered as effective process used to remove micropollutants such as pharmaceutical drugs from wastewaters[2,3]. This work will present the main experimental results obtained for the removal of naproxen, a representative anti-inflammatory drug, from water by adsorption using activated carbon obtained from olive stone. From the raw material, four different types of activated carbon adsorbent were prepared and characterized. The equilibrium adsorption isotherms were measured using the batch method. The most significant adsorption parameters were optimized, such as the solution pH, mass of the adsorbent, contact time and temperature. The physicochemical characterization of the pyrolyzed material shows a considerable superficial area of 608 m^2/g when compared with other natural biomass-based materials. The adsorbent with the better performance allowed, using a contact time of 24 hr and a solution pH of 4.5, a removal efficiency of 100%. The Langmuir model was used to better described the adsorption behavior with the highest maximum adsorption capacity value of 35.2 mg naproxen/g adsorbent. The kinetics of the adsorption is well described by a pseudo-second order model.

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P14 Firefighters' exposure to $PM_{2.5}$ and PM_1 bound polycyclic aromatic hydrocarbons during a prescribed fire

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Portugal is among the most severely affected European countries by wildfires[1-2]. The characterization of Portuguese firefighters' occupational exposure to fine particulate matter (PM) during firefighting remains inexistent. Fine particles, *i.e.*, PM with aerodynamic diameter below 2.5 µm, can penetrate the deepest regions of the human respiratory system, and even cross the alveolar surface membranes, contributing to the development and/or exacerbation of cardiorespiratory diseases[3-4]. PM can have absorbed/adsorbed on its surface other health-relevant pollutants *e.g.*, metals and polycyclic aromatic hydrocarbons (PAHs). This work reports firefighters' exposure to $PM_{2.5}$ and PM_1 bound PAHs during a prescribed fire performed in the winter of 2021 in the North of Portugal.
$PM_{2.5}$ and PM_1 were simultaneously collected on aluminum filters with a DEKATI low-pressure impactor (DLPI+, Dekati®, Finland) during a prescribed fire (2.5 h). PM levels were determined by gravimetry. PAHs were extracted from filters and quantified in a C18 column by liquid chromatography equipped with a photodiode array and fluorescence detectors online. Limits of detection ranged between 0.065-37.2 µg/L. $PM_{2.5}$ (0.897 mg/m^3) and PM_1 (0.846 mg/m^3) contained 158.5 and 376.3 ng/m^3 of total PAHs, respectively. Firefighters' exposure during the fire event was below the international recommendation for the threshold limit of 0.2 mg/m^3 for a time-weighted average during a normal 8-hours workday[5]. The (possible/probable) carcinogenic naphthalene, benz(a)anthracene, benzo(b+j)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-c,d)pyrene were detected and altogether accounted for 5 and 21% of total PAHs in $PM_{2.5}$ and PM_1, respectively. Further studies are needed to better characterize firefighters´ exposure to PM-bound PAHs during prescribed fires.

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P15 Determination of multi-class contaminants in seafood by gas-chromatography mass spectrometry

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Multi-class quantification of contaminants is of major importance in seafood safety, with the number of analytes under consideration always expanding due to an increase in anthropogenic pollution of aquatic environments. Thus, sensitive and accurate quantification places stringent demands upon both analytical instrumentation and sample preparation. In this study, a gas chromatography-mass spectrometry method for quantification of pesticide residues, bisphenols, musk fragrances and UV-filters in four distinct seafood matrices was optimized. The sample preparation was based on the QuEChERS procedure combined with dispersive liquid-liquid microextraction (DLLME) with in situ acetylation. This method was validated under SANTE/12682/2019 guidelines for different seafood samples – algae, mussel, lean and fatty fish[1]. Matrix-induced signal enhancement was observed for the most of analytes, with the highest effects observed in mussel samples, thus matrix-matched calibration were used for all type of matrices. The linear interval was 1 to 500 µg /kg with a $r^2 > 0.99$ for all the analytes in all matrices. Suitable recovery (70–120%) and precision (RSD <20%) were achieved for all musk fragrances (cashmeran, celostolide, galaxolide, and tonalide) and UV-filters (benzophenone-3, 2-ethylhexyl 4-methoxycinnamate, 2-ethylhexyl salicylate, and isoamyl-4-methoxycinnamate) under study in all the matrices. Similar values were also obtained for most pesticide residues (alpha-HCH, atrazine, lindane, vinclozolin, chlorpyrifos-methyl, alachlor, fenitrotion, linuron, aldrin,chlorpyrifos, fipronil, alpha-chlordane, gamma-chlordane, ethion, p,p'-DDT, bifenthrin, mirex, permethrin and deltamethrin) and for 6 bisphenols (A, B, E, F, Z and AF) in algae, mussel, and fish muscles. However, lower values ranging between 58 and 66% were observed for bisphenol AP in fish muscles and for hexachlorobenzene and prochloraz in algae.

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P16 New insides into the analysis of phthalates esters

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Phthalate esters (PE's), better known as phthalates, are a group of chemical compounds widely used since 1960 as plasticizing agents in order to impart flexibility, durability and longevity to plastics.[1] Given their unique physicochemical properties, some phthalates and their metabolites have a severe toxic effect on human health, primarily in the reproductive, endocrine and respiratory systems.[2,3] Several studies have led the EU and the USA, among other countries, to intervene and regulate these compounds[4]. The control must be rigorous with very low levels of detection (ppb or lower), so it is important to define methodologies that respond to this need. Traditionally, the analysis of PEs is performed using 1D gas chromatography techniques, *e.g*. GC/MS and GC/MS/MS.
However, these have shown several problems both in identification and quantification, mainly due to co-elutions between different PE`s, and also with compounds in the matrix. Another major problem in identifying these compounds in real matrices is their ubiquity around us, including in the analytical chemistry laboratory.
In this work, 26 phthalates and 8 phthalate substitutes were separated using a non polar column and detected using a triple quadrupole in multiple reaction monitoring mode, taking into account all possible contaminations during the analyte preparation, extraction and injection process.
In the future, this project will apply classical and alternative 2D analytical methodologies (GCxGC and/or MD-GC and/or LC-GC) in order to obtain better separation, detection and sensitivity for PEs in complex food matrices, such as wine and olive oil.

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P17 Gas chromatography coupled to mass spectrometry (GC-MS) as a tool for the analysis of secondary metabolites in shrimp (*Litopenaeus vannamei*)

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The Aquacombine project centers on cultivating and biorefining a type of salt-tolerant plant that can produce more food and plant material for bioenergy and biochemicals on marginal land [1]. Among these plants are species of the *Salicornia* genus, halophytes that can grow on saline lands without freshwater for irrigation [2]. When grown as a vegetable, only the fresh tips are used, while the woody part of the plant is considered a residue. Thus, the need to value these residues and minimize their environmental impacts becomes evident through their use in different applications, such as health, food, and feed production for aquaculture. The present work focuses on this last topic and evaluates the effect of ingestion of *Salicornia ramosissima* at different percentages on the profile of secondary metabolites produced by aquaculture shrimp (*Litopenaeus vannamei*). For this, two methods were used to guarantee that all compounds in the shrimp were identified. The first method used the hexane extract, which was then derivatized through silylation. The second method, direct silylation of shrimp biomass, was performed. In both methods, GC-MS was used to perform the analysis. With the results obtained, it can be verified that the two methods used allow the identification of the same compounds. Furthermore, the results showed that the ingestion of *S. ramosissim*a does not disturb the biochemical profile of shrimp, which suggests that its incorporation into the daily shrimp diet does not affect its nutritional value. These results will be presented and discussed in the communication.

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P18 Methodology development using Ultra High-Performance Liquid Chromatography with Tandem Mass Spectrometry and Solid Phase Extraction for the analysis of urinary phthalates metabolites

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Phthalates are dialkyl or alkyl esters of the ortho-benzene dicarboxylic acid (phthalic acid) used to increase the flexibility and the softness of plastics. These compounds are not chemically bound to the polymer, so are constantly released. Food and beverages are considered the main source of population exposure to high molecular weight (HMW) phthalates. For low-molecular weight (LMW) phthalates other lifestyle-dependent exposure pathways seem to be more relevant[1]. The preferable matrix to assess human exposure to phthalates is urine by measuring their urinary polar metabolites[2].
Eleven metabolites were targeted in the present study. The precursor, ionisation mode, and the products were determined to obtain higher specificity and sensitivity. After, ion source parameters (heat block temperature, desolvation line temperature, interface voltage, drying gas flow, and nebulizing gas flow) were optimised. The dwell time was also tested for best compromise through the sensitivity and the relative standard deviation. Finally, eluents and its composition and isocratic and/or gradient elution were studied. For the solid phase extraction, the cartridge composition, and their specific steps (conditioning, washing, and eluting) were optimized. Moreover, the loss of metabolites by retention in the syringe filter (PTFE and Nylon) and the adsorption in the glass vial were also considered. Different solvents (acetonitrile, methanol, and ultra-pure water) or mixtures with different proportions were used for extract redissolution.
Recoveries ratios were calculated by comparing the area for the sample fortified before the extraction (pre-spiked sample) with the area of the sample fortified after the extraction (post-spiked sample).

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P19 Evaluation of fatty acid profile from wild *Columba oenas*

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Food obtained through hunting contributes to the social and cultural values linked to the consumption of food items coming originally from the environment. Since this meat are less processed, their consumption is associated with a better nutritional quality[1]. Pigeon belongs to the Columbidae family, their meat is considered a delight and their popularity has been increasing in United States of America, China, and certain countries of Europe. Nevertheless, the knowledge comprising the nutritive value and chemical composition of pigeon meat continue to be limited. Previous reports characterized pigeon meat as highly nutritive, with high protein content and low cholesterol[2].
In this work the fatty acid (FA) profile of fifteen samples of wild pigeon muscle collected in Portugal was evaluated using gas chromatography – flame ionization detection (GC-FID). The total lipid content was extracted, for that 3 g of each wild pigeon muscle was used. The lipid residue obtained was then dissolved in organic solvent and the FA profile was determined according to base-catalyzed transesterification method[4]. The profile of 36 FA was analyzed, C13:0 was used as an internal standard and the samples were analyzed in duplicate.
The samples analyzed presented a lipid content values between 22 and 39 mg/g. The main fatty acids found in wild pigeon muscle were palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9c), linoleic (C18:2 n-6c) and arachidonic (C20:4 n-6) acids. The results demonstrate the suitability of this method for efficient isolation of FAs from wild pigeon muscle, prior GC-FID analysis.

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P20 Determination of benzophenone-type UV filters in environmental waters by bar adsorptive microextraction

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The occurrence of pharmaceuticals and personal care products in environmental matrices has been a topic of high interest in the scientific community since many are emerging environmental contaminants. These compounds can resist treatment in wastewater treatment plants and be later detected in the environment. A good example of this group of substances are benzophenones, which are additives used in cosmetics, pharmaceuticals, and personal care products as ultraviolet filters. These filters can be found in environmental matrices such as wastewater, lakes, rivers, and coastal areas at low concentration levels (µg/L)[1,2].

The aim of this study was to monitor 8 benzophenones in environmental matrices through the development, optimization, and validation of bar adsorptive microextraction followed by high-performance liquid chromatography with a diode array detector (BAµE/HPLC-DAD) analysis[3].

For the optimized experimental parameters, recoveries between 60 and 85% were obtained, with limits of detection and quantification between 0.1 and 1.0 µg/L and between 0.33 and 1.33 µg/L, respectively.

The proposed methodology proved to be an alternative strategy for the analysis of benzophenones in environmental matrices, presenting as main advantages the use of small amounts of sample and solvent, easy handling, simplicity, and excellent analytical performance.

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P21 Study of the lipophilic profile of *Caulerpa prolifera*

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Many reports have shown that extracts and compounds from *Caulerpa* spp. presented a diverse range of bioactivities like insecticidal, antimicrobial, antifouling, anti-inflammatory, and cytotoxic, indicating a potential to isolate added-value compounds from species of this genus.[1]
Caulerpa prolifera is the only native species of *Caulerpa* in Europe, occurring in the Mediterranean Sea, subtropical and tropical Atlantic Ocean. It has invaded various non-native habitats, with the westernmost record of this species in European waters being in the Azores (NE Atlantic).[2] The most interesting compound isolated from this species is caulerpenyne which presents antibacterial, antitumor, and anti-inflammatory activities, among others.[3] Despite its potential, the lipophilic composition of this species has never been explored. In this regard, the present work aimed to study a dichloromethane extract of *Caulerpa prolifera*, identify possible added-value compounds, and increase both phytochemical knowledge about this macroalga and its economic value.
The dry algal material was extracted with dichloromethane by maceration in 3 cycles of 72 h each, and the sample was concentrated with the rotavapor. Then the extract was silylated and analyzed by GC-MS. A total of 24 compounds were identified, belonging mainly to the fatty acid organic family (49%), including saturated, mono-, and poly-unsaturated fatty acids. Palmitic acid was the most abundant compound identified, with an abundance of 4.66 ± 0.55 mg/100 mg extract. Other compounds isolated are sterols and linear terpenoids. Some of these constituents, *e.g*. unsaturated fatty acids and sterols, have a significant economic value due to their beneficial health effects, showing great potential of this macroalga.

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P22 Liquid chromatography strategies for one health assessment

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The production of Food and the intensive animal food production, represent a huge impact in human, animal and environment health leading to increasing concerns in a "One Health" perspective. In animal farms, the continued use and release of pharmaceuticals can result in undesirable residues of those compounds in the food chain and in the environment through wastewater or use of manure. For instance, the uncontrolled use of antibiotics may contribute for the so-alarming problem of antimicrobial resistances and their dissemination. The protection of consumers is one of the main concerns in European Union, and for that purpose the control of veterinary drug residues is mandatory to ensure that all animal products in the food chain do not represent a threat. As a sustainable alternative, evaluation of environmental matrices for possible persistent pharmaceuticals pollutants can also provide information to be used on the definition of levels that might pose risk for human, animal and environmental health[1]. Advances and new developments in liquid chromatography coupled with mass spectrometry, namely UHPLC-QTRAP-MS/MS and UHPLC-TOF-MS, can provide the deeper knowledge of the occurrence of persistent pharmaceuticals and levels of contamination in food products and in the environment[2]. This work aims at evaluating the multi-detection methods recently developed, and to be used as Food Safety tools[3] in line with the “One Health” approach: determination of antibiotics in food samples of animal origin (fish, meat, milk and eggs) and analysis of environment samples for the presence of different pharmacologically active compounds (water, algae and sediments).

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P23 Encapsulation of anthraquinone dyes from *Rubia tintorum* L. by freeze and spray-drying for application in the textile industry

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Natural dyes have been used since the 1990s to replace synthetic ones due to their biodegradable nature, being anti-allergenic, non-carcinogenic and low toxicity. Nowadays, the dyeing with natural colorant from plants, such as the garança (*Rubia tinctorum* L.) is carried out at three technological levels, artisanal, semi-industrial and industrial, by companies with good environmental practices. For this, it is essential the stabilization and storage of natural dyes, since they have less stability, to pH, light and washing, when compared with the synthetic ones.
The encapsulation of anthraquinones by freeze-drying and spray-drying was studied on the colour stability, using maltodextrin and Arabic-gum as carrier agents[1]. In the extracts, encapsulated and non-encapsulated, the colour parameters (CIE Lab), total phenolic compounds content (TPC), alizarin content and chemical profile were evaluated by HPLC-DAD combined with HRMS.
The results showed that the anthraquinone extracts encapsulated by freeze-drying, exhibited higher encapsulation efficiency (EE) and solubility.
The chromatographic profile indicated that the majority dyes are glycosides, namely lucidin primeveroside, ruberythric acid and rubiadin primeveroside[2].
Regarding temperature and pH stability, temperature was found to be the variable with the most significant effect on colour degradation. As to pH stability, the colour of the plant extracts at pH 4 is closer to the non-encapsulated extract[3].
The encapsulation is a promising process since it allows improving the stability of natural colorants, because allow them to be obtained in powder form, and an easier use, transport and storage.

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P24 Análise de substâncias de abuso utilizando novas configurações "LC Tips" de microextração em fase sólida e cromatografia gasosa-espetrometria de massa: estudo de prova de conceito.

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O consumo de substâncias de abuso é considerado um problema de saúde pública de grande importância, uma vez que limita a vida dos seus consumidores e desvia recursos de várias áreas da sociedade. Os problemas relacionados com o seu consumo e os seus efeitos negativos consequentes, justifica a sua monitorização, bem como o desenvolvimento de novos métodos analíticos, que permitam uma melhor análise das mesmas em contexto clínico, legal ou epidemiológico.
Neste sentido, foi estudada e avaliada a adequabilidade de novos métodos de preparação de amostras, baseadas em microextração em fase sólida (SPME), com configuração "LC Tips", para a deteção de substâncias de abuso em matrizes biológicas.
O estudo foi realizado para os analitos cocaína, cocaetileno, fentanilo e norfentanilo, tendo sido avaliadas as variáveis: tipo de fibra, agitação e tempo de extração. A análise quantitativa e qualitativa foi realizada por cromatografia gasosa hifenada à espetrometria de massa (GC-TOF/MS). A matriz biológica utilizada foi urina.
Os dados experimentais obtidos indicam a técnica de SPME com configuração "LC Tips" como um método promissor, seletivo e eficaz para o enriquecimento das substâncias em estudo, cumprindo os princípios da química analítica verde e performance analítica, sendo, no entanto, necessários estudos adicionais de otimização do método. Os resultados operacionais demonstraram que a metodologia é operacionalmente simples, amiga do ambiente e com um custo-benefício vantajoso, sendo o investimento inicial na aquisição dos dispositivos SPME "LC Tips" compensado pela sua capacidade de reutilização e baixo consumo de amostra e solventes orgânicos durante o ciclo analítico.

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P25 Amanitin determination in bile samples by UHPLC-MS/MS and UHPLC-ToF-MS

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The consumption of mushrooms is very popular and normally harmless for consumers. However, there are some species comprising toxins responsible for harmful or even fatal effects. Amanita mushroom species are considered the most dangerous due to the presence of amanitin toxins - highly toxic cyclopeptides. The erroneous misidentification of these mushrooms is the main cause of poisoning that normally occurs in forest regions where the consumption of wild mushrooms is very common. To diagnose potential cases of amanitin poisoning and follow the necessary emergency procedures, there is a need for rapid methods able to detect those toxins in biological samples[1].

This study presents the development and validation of analytical methodologies to determine α- and β-amanitin in bile, a matrix used to evaluate the levels of contamination in the organism. An ultra-high performance liquid chromatography (UHPLC) was optimized with two separated mass spectrometry detection technologies: a Low-Resolution as a triple quadrupole (UHPLC-MS/MS) and a High-Resolution Time-of-Flight detector (UHPLC-ToF-MS). The developed method was fully validated, for both MS detection methods, in accordance with the ICH guidelines[2] and the CIR 808/2021[3]. The parameters evaluated were linearity, specificity, robustness, recovery, precision, limit of detection (LOD) and limit of quantification (LOQ). Both methods fulfilled all the validation criteria and, in terms of LODs and LOQs the lowest values were achieved for UHPLC-MS/MS with LOD< 4 $ng.mL^{-1}$ and LOQ< 9 $ng.mL^{-1}$. The possibility of using the developed methods represents the availability of having efficiently tools to help in the rapid respond to this fatal health problem.

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P26 Casting light on the chemical characterization of Acacia pods

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Invasive species impose a strain on natural ecosystems by contributing to the loss of certain native species. Acacia species are amongst the most aggressive invasive species in Portugal.
In this work, *Acacia retinodes*, *A. longifolia*, *A. melanoxylon*, *A. pycnantha* and *A. dealbata* pods were studied concerning the extraction of compounds for potential industrial application. The Acacia pods were collected in 2021 from different regions, according to their geographical distribution, lyophilized and frozen at -80 °C until analysis. The extraction was made according to the methodology described by Puga *et al.*[1]. Different phenolic compounds were identified using high-performance liquid chromatography-diode array detector (HPLC/DAD) and liquid chromatography-electrospray ionization high-resolution tandem mass spectrometry (LC-ESI-HRMS/MS) using a quadrupole time-of-flight instrument (Q-TOF). Prior to any treatment, the fresh pods were also analysed by Near-infrared spectroscopy (NIR).
So far, 20 compounds have been analysed and identified, including simple phenolics, hydroxybenzoic acids, hydroxybenzoic aldehydes, hydroxycinnamic acids, hydroxycinnamic aldehydes, furans, flavonoids, flavanols, flavones.
The principal component analysis performed using analytical data and NIR spectra produced similar findings, allowing us to conclude that the Acacia pods present a distinct profile of compounds that may be easily distinguished by vibrational spectroscopy.
The compounds found in the Acacia pods seem to have the potential for harvest, with a focus on prospective applications being the goal of upcoming research.

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P27 Vitamin E profile determination by HPLC-DAD-FLD and fatty acid composition by GC-FID in coffee pulp

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Vitamin E, especially the α-tocopherol isoform, is recognized for its antioxidant properties, protecting fatty acids and cellular constituents from oxidative damage. Fatty acids, such as polyunsaturated fatty acids, are essential for brain function, physical health, and well-being throughout life[1]. Seeds and nuts are well-known sources of vitamin E and fatty acids, but these compounds may also be found in green coffee beans and by-products of the coffee industry[1,2,3]. Thus, the aim of this study was to determine the vitamin E profile and fatty acid composition of Colombian coffee pulp (*Coffee arabica*), a by-product of coffee cherry depulping.

First, lipid content was determined using the Soxhlet method (AOAC 991.36). After lipid extraction according to Alves et al. (2009), vitamin E and fatty acid methyl esters were analysed by RP-HPLC-FLD-DAD and GC-FID, respectively[2,4].

The results show that coffee pulp has a low fat content (1.52 % dried sample). Linoleic acid and α-linolenic acid are the most abundant polyunsaturated acids (21.51 and 16.85% of total fatty acids, respectively). Vitamin E content of coffee pulp, as far as we know, has not previously been described. Three tocopherols were found in coffee pulp: α-, γ-, and δ-tocopherol. The lipid fraction of coffee pulp has more vitamin E (13.24 mg/100 g dried sample) than green coffee beans and silverskin, a by-product of roasting[2,3].

The lipid fraction of coffee pulp can be considered a source of vitamin E and polyunsaturated fatty acids, and its consumption may be beneficial to health.

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P28 Study of the insulin release profile from locust bean gum microparticles

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Insulin is a hormone produced by the pancreas, with the main function of regulating the blood levels of glucose. Current therapies used for administration of this protein are associated with pain and discomfort, leading researchers to study alternative pathways like pulmonary administration[1]. Microparticle formulations from polysaccharides can protect and deliver insulin to reach the deep lung[2]. Gas-chromatography (GC) derivatization can follow the polysaccharide composition of the carriers, while high performance liquid chromatography (HPLC) allows to quantify insulin release.
In this work, locust bean gum (LBG) was subjected to a microwave (MW) assisted partial acid hydrolysis, followed by separation with ultrafiltration (membrane cut-offs 300, 100, 50, 30, and 10 kDa). The fractions were used for MPs assembly, adding 10% insulin (w/w) followed by spray-drying at 150°C. Neutral sugar composition was studied with gas-chromatography. Insulin release profile was studied using HPLC.
Microwave treatment allowed material depolymerization with recovery of mainly retentate (>300 kDa) or low molecular weight material (<10 kDa). All fractions showed the prevalence of mannose (75.9±1.8%) and galactose (21.2±1.1%), consistent with the galactomannan structure. Retentates were used for microparticle assembly, yielding 65.2±4.3%. Most of the losses were accounted in the polysaccharide's moiety, as the insulin quantification reveled 10-14%. The HPLC analysis showed a quicker and total insulin release from MPs after 20 min when using material of lower dimensions (10 kDa fraction). The remaining retentate fractions required 40 min for a full release. All conditions showed a controlled and fast delivery profile, with potential to deliver insulin in post-prandial conditions.

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P29 Development and optimization of a new method to determine antidepressants in oral fluid by microextraction by packed sorbent and analysis by GC-MS/MS

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Between 2013 and 2016, consumption of antidepressants doubled in Portugal, with around 30 million packages of medicines dispensed annually. Portugal has one of the highest rates of mental illness in Europe[1]. Therapeutic drug monitoring is well established for a small number of drugs, namely for those where a direct relationship between concentration and pharmacological effect exists. This work aims to develop a methodology for the detection of antidepressants (fluoxetine, venlafaxine, citalopram, sertraline, paroxetine and metabolites) in 250 µL of oral fluid samples by microextraction by packed sorbent (MEPS) and gas chromatography-tandem mass spectrometry.
The MEPS technique was optimized using the Design of Experiments approach. The number of strokes was the only significant factor for some of the analytes under study. The final extraction procedure was: mix 250 µL of sample with acetonitrile, centrifuge, decant and evaporate; dissolve the residue with 1 mL of 25 mM phosphate buffer (pH 2.5), add the internal standard and homogenize; conditioning with 250 µL of MeOH and 250 µL of 0.1% formic acid in H_2O; loading 150 µL of sample (12 strokes); washing 4x50 µL with 0.1% formic acid in H_2O; drying by aspiration of air 4x50 µL; elution 6x100 µL with 1% ammonium hydroxide in MeOH; evaporate to dryness, derivatize for 2 min in a microwave oven at 800 W and inject 2 µL. Recoveries between 12-93% and detection limits of 10-75 ng/mL were obtained. This is the first method that uses MEPS in the determination of antidepressants and metabolites in oral fluid samples.

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P30 Determination of essential oils compounds responsible for anti-cellulite actions by GC-MS: pre and post encapsulation in β-cyclodextrin nanoparticles

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Essential oils (EO) have been used for a long time specially for perfume, food and beverage industry. Their application to treat cellulite, a skin condition commonly considered an unacceptable aesthetically cosmetic problem that resembles an orange peel, is still little researched.
The main goal of this work was to characterize five EO blends and optimize an encapsulation procedure into β-cyclodextrin (β-CD) nanoparticles. The obtained EO-β-CD complex will later be used to functionalize textiles that can be further applied for cellulite treatment.
A GC-MS analytical equipment from Agilent Technologies with a HP-5 MS capillary column (30m x 0.25-mm I.D., 0.25-µm film thickness) was used for the characterization of the EO blends. Injection port and the transfer line temperatures were set to 250 °C and 280 °C, respectively. The oven temperature started at 40 °C for 5 min, after which a 5 °C/min rate up to 250 °C was applied and held for another 5 min. Sample extracts were injected (1 µL) in split mode (50:1). Quantification was performed in SIM mode.
Five EO blends were characterized, and the main compounds found were β-myrcene, limonene, 1,8-cineol, γ-terpinene, linalool, citronellal, mentone, L-borneol, mentol and linalool acetate. The blend that presented greater quantities of these compounds, was the one encapsulated in β-CD. The optimized encapsulation procedure complexed 13.6 ± 1.7 % of total EO, of which 1.0 ± 0.1 % was on the surface and 12.7 ± 1.7 % was inside the β-CD.

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P31 Quantification of amino acids from Algae by HPLC

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Algal protein has been reported as a sustainable source of non-animal and non-allergenic proteins for application in the food industry. Algae proteins have advantages such as non-allergenic proteins, complete amino acid composition, higher productivity, simple and low production cost (1,2). 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, four different species, such as *Arthrospira platensis* (*Cyanobacteria*), *Chlorella vulgaris* and *Tetraselmis chuii* (*Chlorophyta*), and *Gracilaria gracilis (Rhodophyta*) 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 were accessed using an High-Performance Liquid Chromatography (HPLC) equipped with a diode array detector (DAD) using a reversed phase C18 column according to Sheng et al. 2017 with modification (3). Furthermore, the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein standards allowed the characterization of different proteins according to molecular weight. Total nitrogen was also evaluated by the Kjeldahl method. Protein determination in the Kjeldahl procedure provide results overestimation by other non-protein nitrogen components in algae comparable to HPLC protein analysis. For amino acids analysis, the HPLC method is a suitable and automated technique that has good chromatographic separation of 15 amino acids and contribute to more accurate protein determinations in microalgae and macroalgae.

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P32 Separation of betulinic and oleanolic acids by analytical and preparative liquid chromatography: experiments and modeling

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Betulinic and oleanolic acids are two triterpenic acids that due to their diverse nutraceutical and pharmacological properties (e.g., antioxidative, anti-inflammatory, anti-viral and anti-tumoral properties[1,2]) have attracted considerable research interest in the last few years. Nonetheless, pure betulinic and oleanolic acids are difficult to obtain since they are structurally similar and occur simultaneously in different natural matrices[3]. As a result, their prices increase greatly with increasing purity.

Accordingly, the separation of these two triterpenic acids using a triacontyl stationary phase (Acclaim C30) was addressed in this work. Methanol, water, acetonitrile, ethanol, isopropanol, ethyl acetate, acetone and mixtures thereof were tested, and the best mobile phase to carry out the separation was found to be methanol/acetonitrile 50/50 (%, v/v) at 23 ºC. The equilibrium and kinetic parameters of pure betulinic and pure oleanolic acids were obtained by the method of moments and were successfully validated by predicting unary and binary breakthrough curves. Simulated moving bed phenomenological simulations demonstrated that betulinic and oleanolic acids can be both obtained with purities of 99.2 % and productivity of 56.2 kg/($m^3_{adsorbent}$ day) using the packing material of the Acclaim C30 column with a one column *per* section configuration with columns of 7.5 cm long. The solubility of each solute was measured in methanol/acetonitrile 70/30, 50/50, and 30/70 (%, v/v) modified with water. The obtained results showed that adding 65 % (%, v/v) of water it is possible to recover as precipitate 98 % of the dissolved solutes in all the tested methanol/acetonitrile mixtures.

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P33 Mapping the diversity of mono- and multi-varietal commercial Portuguese virgin olive oil in the phenolic composition

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Portugal is the fourth highest producer of olive oil in Europe, after Spain, Italy, and Greece[1]. The virgin olive oil (VOO), a key ingredient in the Mediterranean diet is traditionally produced by crushing the olive fruit in a hammer mill to form a paste followed by separation and centrifugation for clarification and purification of the olive oil[2]. The VOO is mostly composed by fatty acids but also by a minor unsaponifiable fraction rich in phenolic compounds with associated beneficial health effects, responsible for the prevention of cardiovascular diseases[3]. The major phenolic compounds in the VOO are oleuropein, hydroxytyrosol and tyrosol. Nevertheless, their abundance in the VOO depends on several factors such as the cultivar, the growing region, and the degree of fruit ripeness[1]. For the present work 31 samples of commercial VOO, mono- and multi-varietal, obtained from different Portuguese regions were extracted using the Oleum procedure[4]. The methanolic extracts were characterized for their phenolic composition, namely for the hydroxytyrosol and tyrosol contents using liquid chromatography, HPLC-DAD. For the daily recommended intake, 1.5 tablespoons of olive oil (20 g), the sum of hydroxytyrosol and tyrosol ranged between 2 and 21 mg/ 20 g. For 80 % of the samples the amount of hydroxytyrosol was ≥ 5 mg/ 20 g of VOO, which is in accordance with the EFSA health allegation[5]. Such information could be labeled for consumer information and olive oil valorization.

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P34 Bioactive sterols from the macroalga *Codium tomentosum*: Sustainable extraction with eutectic solvents

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Macroalgae have been attracting attention due to their wide range of secondary metabolites, some of them with unique bioactivities. Among the variety of interesting compounds, sterols found in macroalgae are described as having anti-inflammatory, antibacterial and antiproliferative activities, which make them very promising for high-value applications, such as nutraceuticals or pharmaceuticals[1]. However, the exploitation of sterols from macroalgae in such fields has been hindered by the lack of eco-friendly and efficient extraction methods. Eutectic Solvents (ES) have been suggested as an eco-friendly alternative to hazardous solvents for the selective extraction of natural bioactive compounds, addressing the principles of Green Chemistry[2].
In this work, the conductor-like screening model for real solvents (COSMO-RS) was used as an initial screening tool to predict the potential of individual compounds in eutectic mixtures to extract the mentioned compounds, aiming at decreasing the number of experiments necessary to develop an efficient extraction process. ES composed of terpenes, fatty acids and some alcohols were found to be the most suitable solvents for sterols extraction. Solid-liquid extractions of *Codium tomentosum* were performed using the selected solvents and their ability to obtain sterols rich extracts was evaluated by gas chromatography coupled to mass spectrometry (GC-MS) and compared with the predictions made by COSMO-RS and with results from the extraction with conventional solvents.

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P35 Occurrence of plastic-related chemicals in soil samples from the Natural Park of Montesinho

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The presence of (micro)plastics in terrestrial environments has been reported.[1] The hazard posed by these pollutants is related to their ability to sorb/desorb environmental contaminants such as heavy metals, flame retardants, or pesticides, increasing the bioavailability and toxicity of these chemical compounds in soil.[1,2] The aim of this study was to develop a Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction method followed by Gas Chromatography coupled with an Electron Capture Detector (GC-ECD) for the simultaneous determination of 28 plastic-related chemicals (PRC) (polybrominated diphenyl ethers (PBDE), polychlorobiphenyls (PCB), pyrethroid and organochlorine pesticides) in soil samples. In October 2021, 21 samples were collected near beehives from Natural Park of Montesinho (NPM) (Portugal), and were stored at room temperature, in glass containers, and kept from light until analysis.

The extraction and quantification of PRC were based on the QuEChERS approach developed by Fernandes et al. (2013)[3]. Briefly, 5 g of soil was weighed into a centrifuge tube, and 3 mL of deionized water plus 7 mL of acetonitrile were added and vortexed. The QuEChERS salts were added to the tube, which was then vortexed and centrifuged. The supernatant was transferred into a glass vial containing PSA, $MgSO_4$, and C18. The vial was shaken and centrifuged. Finally, the extract was dried, recovered with n-hexane, and injected in GC-ECD. Matrix-matched calibration standards were prepared, and the method was validated in terms of linearity, matrix effect, recovery percentage, the limit of detection (LOD), the limit of quantitation (LOQ), and precision.

The results obtained showed that the optimized analytical techniques presented good performance in the simultaneous analysis of PRC, with LOQs ranging from 2.15 to 11.87 μg kg^{-1} and LODs ranging from 0.64 to 3.56 μg kg^{-1}. PCB 101 was quantified in two samples at a concentration of 20.26 and 13.88 μg kg^{-1}. The flame retardants BDE 154 and BDE 100 were quantified in two samples (3.48 and 24.41 μg kg^{-1}) and one sample (8.33 μg kg^{-1}), respectively. This work demonstrates that PRC can be detected in remote locations where human activity is thought to have a little environmental impact. In the case of the NPM the existence of this cocktail of compounds can ultimately put the bees at risk.

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P36 UHPLC-DAD-MS^n analysis of phenolic compounds bioavailability throughout *in vitro* simulated gastrointestinal digestion

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Phenolic compounds, one of the most widely distributed and structural diverse plant secondary metabolites families, have been the focus of several studies due to their vast range of biological activities (such as antioxidant, anti-inflammatory and/or antiproliferative). Since they are commonly present in human diet, phenolic compounds could be responsible for human health beneficial effects, preventing some disorders [1–4]. Notwithstanding, these health effects are strictly dependent on their bioavailability, which consists in the amount of each ingested compound that reaches the target tissue where it can have a promising biological effect [5]. Therefore, compound's structure, human enzymatic activity and gut microbiota are some of the numerous factors that influenced phenolic compounds bioavailability, and consequently their human health beneficial effects [5].
In this vein, the bioavailability of phenolic compounds from different classes, particularly, flavonols (rutin), flavanones (naringenin and naringin), dihydrochalcones (phloretin) and tannin monomeric units (phloroglucinol), were evaluated in an *in vitro* simulated gastrointestinal digestion and further analyzed and quantified through ultra-high performance liquid chromatography with diode-array detection and coupled to electrospray ionization tandem mass spectrometry (UHPLC-DAD-MS^n). Most of them showed a bioavailability >70% in intestinal digestion phase and seemed to be absorbed, reaching the systemic circulation. Thus, these results could be a future remark to evaluate the human health effects of promising phenolic compounds combination, or of plant-based extracts with a similar composition or even extracts enriched with them.

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P37 Determinação de potenciais biomarcadores de infertilidade feminina: estudo piloto

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Devido à sua elevada incidência, a infertilidade tornou-se uma questão de saúde pública proeminente, representando um desafio significativo para a medicina reprodutiva moderna. Algumas condições clínicas que levam à infertilidade feminina incluem a síndrome de ovários poliquísticos (PCOS), endometriose, e falência ovárica prematura (FOP). O fluido folicular (FF) é a matriz biológica que tem mais contacto com o oócito, podendo ser utilizado como um preditor da sua qualidade[1]. A volatilómica surge como um método não invasivo, direto, acessível e simples para a caracterização de várias doenças. Este estudo teve como objetivo determinar o padrão volatómico do fluido folicular de pacientes com PCOS, endometriose, e POF, e encontrar potenciais biomarcadores destas condições clínicas. Para analisar os compostos orgânicos voláteis (VOCs) presentes em amostras de FF de mulheres inférteis foi utilizada a microextração em fase sólida em modo *headspace* e posterior análise por GC-MS. Foram identificados 136 VOCs em 52 amostras, correspondendo a 15 pacientes com PCOS, 8 com endometriose, 12 com POF, e 17 controlos. Devido à sua prevalência em todas as amostras, apenas 37 compostos foram considerados, e a análise estatística multivariada revelou alterações significativas nos níveis de certos metabolitos de acordo com cada condição clínica. Os perfis bioquímicos analisados revelaram vias metabólicas comprometidas, bem como a presença de compostos fortemente ligados com infertilidade[2]. Esta metodologia abre a porta para a investigação das diversas vias metabólicas relevantes para o correto funcionamento do sistema reprodutor, bem como a melhoria das ferramentas de diagnóstico existentes.

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P38 Application of bar adsorptive microextraction (BAμE) to monitor trace levels of estrogens in environmental and forensic context

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The present work aimed to monitor trace levels of estrogens (estrone, estradiol and 17α-ethinylestradiol) in environmental and forensic matrices, using a novel analytical approach, bar adsorptive microextraction, followed by high performance liquid chromatography with diode array detection (BAμE/HPLC-DAD). The method was developed and optimized by evaluating several important parameters, namely the type of sorbent phase, equilibrium time, agitation speed, ionic strength, and pH, as well as the back-extraction conditions. Under optimized experimental conditions [microextraction - sorbent phase: HBL polymer, equilibrium time: 16 h (990 rpm), ionic strength: 5 % NaCl; back extraction: ACN (15 min. under sonication)], high recoveries (70.7 - 77.9 %), low analytical thresholds (0.6 ppb < LOD < 1.9 ppb) and good linear dynamic ranges (5.0 - 80.0 ppb; r^2 > 0.9973) were achieved for the three target analytes. The validated methodology was later applied to real matrices (wastewater and urine), showing good performance and proving to be a promising alternative to monitor trace levels of estrogens, given the high selectivity and sensitivity exhibited, great simplicity, easy handling, and low cost.

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P39 Volatile profile of 15 monovarietal white wines produced from grapes cultivated in a hot and dry region of Portugal

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Climate change trends and impacts could influence drastically the economy and the way that we produce or adapt cultures in near future. The higher air temperatures can modify the characteristics of wine, that depends on many factors, including the conditions they're grown in. Nevertheless, some varieties can be more adapted for these climate changes and produce high quality wines. The WineClimAdapt project (project code PDR2020-101-031010), study the adaptability of white grape varieties in the hottest and driest region of Portugal. In this context, fifteen varieties were used to produce monovarietal white wines. The white wines were vinified in the INIAV winery in duplicate and the volatile profile of the wines produced were screened by GC-MS and GC-FID.
Concerning the 35 compounds identified by GC-MS and quantified by GC-FID all exhibited significative differences when the wines were compared together.
From the 35 analysed compounds, the ones more relevant to distinguish the different wines were: ethyl 3-hydroxybutanoate; ethyl decanoate, butanoic acid, hexyl acetate and *cis*-3-hexenol. The monovarietal wines that presented a higher differentiation in their volatile profile were 'Riesling', 'Petit Manseng', 'Sarigo', 'Alvadurão' and 'Parellada'. This work pointed out the absence of varietal volatile compounds in all the wines and these results could be related with the hot and dry weather conditions in grape production. In general, the volatile profile is similar and the changes are detected in the concentration of each volatile compound.

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P40 Chromatographic profile of free sugars in fruits from five different varieties of *Ficus carica* L.

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Ficus carica L. is one of the first domesticated fruit species, originating from Western Asia and also established in the Mediterranean region. Nowadays, the fruits are consumed worldwide and represent a significant production that exceeds 1 million tons, with Turkey, Egypt, Algeria, Iran, Morocco, Spain, and USA providing about 80% of global production. This nutritious fruit is appreciated for its taste, color, and sugar content. Sugar sweetness is one of the most appreciated quality attributes as it determines the perception of flavor, stage of ripeness, and directly impacts consumer approval. However, a challenge present in the production of this fruit is its high perishability due to its rapid decomposition after harvest, generating high amounts of biowaste[1,2]. Given this reality, the present work studied five different fig varieties (Pasteliere, Dauphinie, Boujassote Noire, Marseille and Longue d'Aout) and 3 different parts of the fig (pulp, peels, and hole fruit), from a private company (Quinta da Mô de CIMA) in order to characterize and quantify Total Free Sugars (TFS) using liquid chromatography with refractive index detector (HPLC-RI). Regarding the results obtained, the pulp (91.44±0.24 mg/ g dw), as expected, was the part presenting the highest amounts of TFS, followed by hole fruits (87.78±0.77 mg/ g dw) and finally peel (79.72±0.43 mg/ g dw). In all samples, glucose could be identified as the main sugar, followed by fructose and then sucrose.

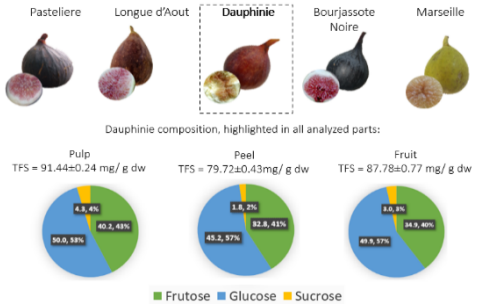

Figure (1): Varieties analyzed with emphasis on those with the highest amounts of free sugars.

Given the results obtained, of the five varieties, Dauphinie (**Figure 1**) was the one presenting the highest sugar content, being the variety more suitable for sweetened fig products for the food industry, such as jams, requiring less externally added sugars such as white sugar.

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P41 The use of HPAEC-PAD to evaluate alginate oligosaccharides to be used as clean label alternative in meat products

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Alginate is an anionic polysaccharide from brown seaweed constituted by D-mannuronic and L-guluronic acids. Due to its anionic properties, soluble oligosaccharides and low molecular weight polysaccharides from alginate are potential clean label alternatives to tripolyphosphates in meat products, since mimics its size and charges. Microwave assisted partial hydrolysis of sodium alginate in water at 120 °C (MW120) and at 150 °C (MW150) was performed to obtain the low molecular weight polysaccharides. For comparison, acid hydrolysis with HCl 0.1 M 80 °C for 36 h and H_2SO_4 1 M 100 °C for 2.5 h was also carried out. The hydrolysis efficiency was evaluated by high-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD)[1] for detection of monosaccharides, oligosaccharides and low molecular weight polysaccharides and by *m*-phenylphenol colorimetric method[2] for quantification of the uronic acids.
HPAEC-PAD showed that MW hydrolysates presented similar chromatographic profiles, while acid hydrolysis presented a more pronounced abundance in saccharides with lower degrees of polymerization. Furthermore, HCl 36h treatment originated significantly more monosaccharides and oligosaccharides than MW fractions. This indicates that the MW-treatment originated carbohydrates with higher molecular weight when compared with acid hydrolysis. These results show that HPAEC-PAD technique is a suitable tool to monitor alginate depolymerisation, enabling the selection of the most fitting strategy to prepare sodium alginate hydrolysates as potential clean label ingredients.

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P42 Mycotoxins as agricultural & livestock biosecurity threats: Chromatographic assessment tools for maize

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Fungal and mycotoxic control at a primary stage in the food chain is crucial to maintain the nutritional quality of feed and is one of the essential points that a good biosecurity program must establish to ensure safe feed and protect animal health. Fungi produce mycotoxins, which are harmful for health and productive performance of animals, as well as for public health. In this matter, application of rapid and reliable tools in a Biosecurity Context towards the identification of regulated and emerging mycotoxins along the food chain is essential. A comprehensive understanding of the role of these fungi mycotoxins is, therefore, vital to fulfil the breaches that will allow to perform proper risk assessment plans and accurate risk management strategies.
The present work was focused on the validation of analytical methodologies based on a QuEChERS approach followed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-QTrap-MS/MS) detection for the determination of regulated, non-regulated and emerging mycotoxins, in samples representative of maize agricultural fields and livestock farms. Validation parameters, including linearity, limits of detection (LoD) and quantification (LoQ), repeatability, reproducibility, and recovery were assessed, and compared among published chromatographic methods. Performance criteria for 12 mycotoxins, including aflatoxins, ochratoxin A, zearalenone, fumonisins, T-2 and HT-2 toxins, and citrinin were evaluated according to the specific requirements for confirmatory methods stated in Commission Regulation nº 401/2006[1]; and, for non-regulated and emerging mycotoxins (n=11), European method validation guidelines were followed[2,3]. Application of the validated chromatographic methods to real samples was ultimately performed.

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P43 Is spelt wheat a superior source of fructans than common wheat? A multivarietal survey by high-performance anion-exchange chromatography

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Fructans are fructose oligomers and polymers that can be naturally found in wheat. While fructans have health benefits for most people as prebiotics and fiber, they can cause gastrointestinal side effects in a significant portion of the population. In particular, as fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) for people suffering from irritable bowel syndrome and inflammatory bowel disease (including Crohn's disease and ulcerative colitis). In recent years we have witnessed a growing interest in ancient wheat species, such as spelt wheat, in view of the perception of a superior nutritional value or a beneficial health effect when compared to modern species. Here, we have analyzed the fructan contents as well as its degree of polymerization (DP) in a genetically-diverse set of wheat varieties from different consumed species as common wheat (*Triticum aestivum* L.) and spelt (*T. aestivum* ssp. *spelta* L.). Our results showed an important variation of fructans content within and between species as the following relationship: Common wheat > Spelt wheat. Also, a substantial part of fructans (>50%) showed a degree of polymerization ≤ 6. Despite not having a higher content of fructans, spelt wheat may, on the contrary, be a suitable alternative to common wheat for low FODMAP diets.

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P44 Determinação de canabinóides em amostras de urina por microextração em seringa empacotada e cromatografia gasosa-espetrometria de massa

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A canábis é a substância ilícita mais frequentemente consumida a nível mundial. Em laboratórios de análise de drogas. A urina é uma das amostras mais utilizadas para a deteção de canabinóides. Nesta matriz biológica é possível detetar metabolitos do Δ9-tretahidrocanabinol (THC), principal composto psicoativo presente na cannabis, o ácido 11-nor-9-carboxi-Δ9-tretahidrocanabinol (THC-COOH) e ainda o 11-hidroxi-Δ9-tetrahydrocannabiol (11-OH-TCH) que são utilizados como marcadores de consumo desta droga de abuso. Este trabalho descreve o desenvolvimento e validação de um método para a determinação de cannabidiol (CBD), canabinol (CBN), THC, 11-OH-THC e THC-COOH em urina com recurso à MEPS e à cromatografia gasosa acoplada à espectrometria de massas. Após otimização, a metodologia foi validada seguindo normas internacionais de validação para bioanálise. Foi obtida uma linearidade de 1-400 ng/mL para o THC e CBD, de 5-400 ng/mL para CBN e 11-OH-THC e de 10-400 ng/mL para THC-COOH, com um coeficiente de correlação em todos os casos superior a 0,99. As precisões e exatidão inter-dia, intra-dia e intermedia obtidas tiveram coeficientes de variação abaixo dos 15% e uma exatidão inferior ou igual a 15% para todos os compostos em estudo. As recuperações obtidas variaram entre 26% a 85%. Os resultados obtidos permitem afirmar que a técnica proposta apresenta uma excelente sensibilidade (1-10 ng/mL). O método desenvolvido foi ainda aplicado a amostras de indivíduos com suspeita de consumo de canábis. É necessário destacar que o procedimento descrito é o primeiro trabalho que recorre à MEPS combinada com a GC-MS para quantificação de canabinóides em amostras de urina.

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P45 Otimização de uma metodologia por microextração em seringa empacotada e cromatografia gasosa-espetrometria de massa em tandem para a identificação de arilciclohexaminas em amostras de cabelo

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Nos últimos dez anos o consumo de novas substâncias psicoativas (NSP) tem vindo a aumentar, sendo que novas substâncias têm sido introduzidas a cada semana. O seu consumo constitui um problema social e de saúde pública, pelo que a análise toxicológica se reveste da maior importância. Neste documento descreve-se o desenvolvimento e otimização de um método simples, rápido, sensível e com baixo consumo de solventes orgânicos para a determinação da ketamina e do seu principal metabolito norketamina em amostras de cabelo. O procedimento incluiu a utilização da microextração em seringa empacotada (MEPS) bem como a cromatografia de gases acoplada à espetrometria de massa em tandem (GC-MS/MS). Prévio ao processo de *clean-up*, foi otimizado o procedimento de hidrólise. Os principais parâmetros que influenciam a extração foram previamente otimizados com recurso à ferramenta estatística de desenho experimental. As condições finais foram as seguintes: (1) acondicionamento com 5 ciclos de 250 µL de metanol e 4 ciclos de 250 µL de água desionizada; (2) 15 ciclos de aspiração da amostra; (3) lavagem com 50 µL de ácido acético a 0,1% em água desionizada e 50 µL de metanol a 10% e (4) eluição com 100 µL de 3% de hidróxido de amónio em metanol.
Os limites de deteção obtidos foram de 0,01 e 0,05 ng/mg para a ketamina e norketamina respetivamente, tendo sido utilizados 50 mg de cabelo. A eficiência de extração variou entre 32 e 60%. É importante salientar que este é o primeiro método descrito que permite a determinação de ketamina e metabolito com recurso à MEPS e GC-MS/MS em amostras de cabelo.

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P46 Preliminary chromatographic data of extracts and fractions of Panamanian geisha coffee

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Panamanian Geisha coffee has been internationally recognized for its high scores in specialty coffee tastings and for reaching high prices in coffee auctions. In studies carried out in our laboratory it has been found that this variety of coffee has a lower caffeine/chlorogenic acid ratio in comparison with other varieties of coffee (1). In order to determine the phytochemical constituents of roasted and ground Panamanian geisha coffee, the raw material was subjected to several processes of aqueous extraction, trying to reproduce the traditional way of preparing coffee for human consumption. For this study, we used the Italian coffee pot method and the "puchero" method. To facilitate the study of their components, the initial aqueous extracts were fractionated according to solubility in a methanolic, ethyl acetate and chloroform extract (2). For their study, the different extracts were subjected to a gas/mass system (GC-MS) or HPLC/Mass in order to identify their most significant components. Caffeine was the major compound in the methanolic extract, the other constituents being long-chain polyunsaturated fatty acids and various chlorogenic acids. Phytosterols, such as β-sitosterol and stigmasterol, were identified in the ethyl acetate extract. On the other hand, the chromatographic study of the chloroform extract is inconclusive.
In conclusion, these are the preliminary results obtained for the extracts of geisha coffee, being the first study that aims to know the phytochemical characteristics of this coffee variety. In addition, NMR studies are being carried out to identify minority compounds present in the prepared fractions.

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P47 Molecular, morphological and chemical diversity of two new species of Antarctic Diatoms, *Craspedostauros ineffabilis* sp. nov. and *Craspedostauros zucchellii* sp. nov.

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The current study focuses on the biological diversity of two strains of Antarctic diatoms (strains IMA082A and IMA088A) collected and isolated from the Ross Sea (Antarctica) during the XXXIV Italian Antarctic Expedition. Both species presented the typical morphological characters of the genus *Craspedostauros*: cribrate areolae, two 'fore-and-aft' chloroplasts and a narrow 'stauros'[1]. This classification is congruent with the molecular phylogeny based on the concatenated 18S rDNA-*rbc*L-*psb*C alignment, which showed that these algae formed a monophyletic lineage including six taxonomically accepted species of *Craspedostauros*. Since the study of the evolution of this genus and of others raphe-bearing diatoms with a 'stauros' is particularly challenging and their phylogeny is still debated[2], we tested alternative tree topologies to evaluate the relationships among these taxa. Metabolic fingerprinting approach was implemented for the assessment of the chemical diversity among the two closely related species IMA082A and IMA088A. This approach has been shown to be a promising method in the classification and taxonomy of filamentous fungi, yeast and microalgae providing a high differentiation at order, genus and species levels, reinforcing morphological and molecular data[3]. In conclusion, combining: (1) traditional morphological features used in diatoms identification, (2) phylogenetic analyses of the small subunit rDNA (18S rDNA), *rbc*L and *psb*C genes, and (3) metabolic fingerprint, we described the strains IMA082A and IMA088A as *Craspedostauros ineffabilis* sp. nov. and *Craspedostauros zucchellii* sp. nov. as new species, respectively.

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P48 Optimal separation of polar anionic pesticides from fruits and vegetables with unique HPLC column selectivity

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Analysis of polar pesticides presents multiple challenges including adequate retention, separation of critical pairs, and reproducibility, to name a few. In addition, food matrices can add additional challenges due to the presence of complex matrix components including pigments, fats, and sugars that can interfere with the analyte of interest.
In this study, we are presenting a unique HPLC selectivity that provides optimal separation of various anionic polar pesticide classes including Glyphosate, Chlorate, Perchlorate, Ethefon, Phosphoric Acid-based pesticides, and N-AcGlu pesticides. The study demonstrates robust polar pesticide analysis from real sample matrix.

P49 Contents of pottery vessels from the megalithic tomb of Zambujeiro, Portugal

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Organic residue analysis applied to the archaeological materials relies on the advances in chromatographic and mass spectrometric techniques. Chemical analysis can help to uncover the original contents of the vessels and past pottery use. Organic molecules can preserve well inside the porous structure of the ceramic, thus the information obtained can be used to identify the presence of natural products or food remains.
The Great Dolmen of Zambujeiro, is an archaeological site located on the left bank of Ribeira de Valverde stream, near the village of Valverde, in the municipality of Évora. It is the largest chambered megalithic tomb in Alentejo region of Portugal, constructed between the 4th and mid-3rd millennia BC. Multiple burials were accompanied by various goods, including amber beads, chert arrowheads, and remains of pottery vessels. Organic residue analysis of 27 selected ceramic sherds from 24 distinct vessels was performed using gas chromatography coupled with mass spectrometry (GC-MS).
The analysis revealed organic content in all analyzed vessels, in a good preservation state, as unsaturated fatty acids and monoacylglycerols (MAGs) were detected in most samples. Remains of fats and oils were recognized by the distribution of saturated and unsaturated fatty acids, dicarboxylic acids, and fatty alcohols. Furthermore, in several vessels of different forms, residues of degraded beeswax were recognized by a characteristic chemical profile. These results give an insight into funerary practices and provide direct evidence for the exploitation of bee products at the time Zambujeiro was used as a burial site.

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P50 Influence of the maturation stages on the chemical composition and biological activity of cardoon seeds

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Cynara cardunculus L., usually known as cardoon, is a Mediterranean herbaceous plant with diversified industrial applications. It is used in traditional medicine due to its several health-promoting effects, mainly associated with its chemical composition. Cardoon is also considered a functional food due to its richness in compounds with high added value and health benefits (e.g., phenolic acids, flavonoids, fiber, inulin)[1]. In the present study, the influence of the harvest stage of cardoon seeds was analyzed. Seeds samples of cultivated cardoon (*C. cardunculus* L. var. *altilis*) were collected in Greece at different maturation stages (samples S1 - S4). The individual profiles in tocopherols, free sugars, organic acids, and fatty acids were determined using chromatographic methodologies. Its hydroethanolic extracts were characterized in terms of phenolic composition and cytotoxic, anti-inflammatory, antioxidant, and antimicrobial activities. The polyphenolic profile was analyzed by HPLC-DAD-ESI/MS. Antioxidant capacity was measured through two cell-based assays, namely TBARS and OxHLIA. The cytotoxic potential was determined against four tumor cell lines and a non-tumor cell line (PLP2). For the anti-inflammatory activity, it was determined the extracts' capacity to inhibit the formation of NO production. Finally, the antimicrobial activity was assessed by the microdilution method. Six phenolic compounds were tentatively identified, with their content increasing with the increasing maturation state. Mature seeds also demonstrated high content in lipids (23 g/100 g) and tocopherols (29.62 mg/100 g), as well as relevant cytotoxic (GI_{50} of 97 – 216 µg/mL), antioxidant (TBARS; IC_{50} = 5 µg/mL), and anti-inflammatory potential (IC_{50} = 148 µg/mL). Significant antibacterial and antifungal activities were also demonstrated, particularly for samples S3 and S1, respectively. Further studies are needed to better understand which compounds are responsible for the observed bioactivities and the mechanisms responsible for the corresponding effects.

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P51 Evaluation of biogenic volatile organic compounds emitted from Mediterranean shrubs

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Currently, one of the major environmental concerns is the frequent occurrence of forest fires, particularly under extreme atmospheric conditions. Some studies suggest that higher temperatures lead to a greater emission of biogenic volatile organic compounds (BVOCs), produced and accumulated in different plants, becoming extremely flammable gases in the event of forest fires. Consequently, in the presence of an ignition source, BVOCs can contribute to the spread of forest fires, leading to catastrophic events, as was the case with the 'Pedrogrão Grande' tragedy[1,2] (Portugal) in 2017. Thus, it becomes relevant to study the composition of BVOCs even more in depth, especially the terpenoid fraction, consisting of compounds such as α-pinene, 1,8-cineole and thymol, since they are among the most abundant monoterpenes in trees and shrubs, and even some sesquiterpenes, such as *trans*-caryophyllene[3]. Therefore, it is important to develop and apply methodologies that allow the identification of the main BVOCs present in trees and shrubs, highlighting the use of analytical tools such as solid phase microextraction in the headspace mode, an easy-to-use, solvent-free technique combined with gas chromatography-mass spectrometry (HS -SPME/GC-MS)[4,5].
The present work aims to identify the main BVOCs emitted from several common shrubs in Portugal, namely *Cistus Ladanifer*, *Erica Arborea*, *Lavandula Stoechas* and *Thymus Vulgarius*, using the HS-SPME/GC-MS approach. It is also our intention to compare the performance, advantages and limitations of HS-SPME in comparison to other alternative techniques, namely bar adsorptive microextraction in the headspace mode (HS-BAμE)[6].

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P52 Genetic information influence on phenolic composition and bioactivities of *Ceratonia siliqua* L. seeds

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Ceratonia siliqua L., commonly known as the carob tree is native to the Mediterranean countries and is widely known and consumed. Due to its chemical constituents, flavoring properties, and nutritional benefits, it has the potential to be of significant importance to the food industry[1]. The carob bean is made up of 90% pulp and 10% seeds. Those seeds are widely used in the food industry as a thickening agent (E-410). However, knowledge about phenolic composition and its relation to biological properties is scarce. This study used seeds of thirteen carob varieties harvested in Algarve, Portugal. The phenolic composition of the hydroethanolic extracts was analyzed by HPLC-DAD-ESI/MS. The antioxidant, anti-inflammatory, cytotoxic and antibacterial properties of the extracts were also analysed. The phenolic composition was analyzed by HPLC-DAD-ESI/MS. Cytotoxic activity was evaluated by the colourimetric method of sulforhodamine B. Anti-inflammatory activity was determined by inhibition of NO production in murine macrophages. The antibacterial activity was evaluated through the method of successive microdilutions and the antioxidant activity through the TBARS and CAA assays. Seventeen phenolics compounds were tentatively identified, being (Epi)catechin dimer type β dimer and apigenin-*O*-hexosyl-pentoside the most abundant ones (3.08 – 11.67 mg/mL). All the varieties studied exhibited the capacity to inhibit TBARS formation. The extract obtained from the *Gasparinha* variety was the only one that inhibited the reactive oxygen species formation in the CAA assay. For the cytotoxic activity only *Cavi*, *Cardeira* and *Pé Comprido* varieties demonstrated the ability to inhibit the proliferation of the tumor cell lines tested, without showing a hepatotoxic effect. All extracts presented a broad-spectrum microbial growth inhibition without an efficient bactericidal power. These findings highlight Carob seed as a rich source of structurally diverse biomolecules with potential application as additives in food formulation development. However, further studies are needed to understand the correlation between phenolic compounds and the bioactive properties associated with carob seed tissues.

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P53 Valorização dos efeitos antioxidantes e toxicidade "in vitro" dos extratos de *Bauhinia thonningii*

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Bauhinia thonningii (Milne-Redhead) é uma planta da medicina tradicional de Africa Occidental, onde as suas folhas são empregadas para tratar doenças inflamatórias e infeciosas[1] .
Metodología: A: Extractos de *Bauhinia thonningii.* A preparação dos extratos das folhas, casca e raiz foram realizados com a seguinte sequência de solventes: hexano a quente (extração em Soxlhet), acetona e etanol a temperatura ambiente. Para o analise e estudo dos compoentes dos extratos de acetona e etanol de folhas, casas e raiz foi realizada uma extração previa pela sua solubilidade em hexano a quente, clorofórmio (para os extratos de acetona) e acetona (para os extratos de etanol). Depois de realizar esta separação os componentes destes extratos foram separados e identificados pela técnica de HPLC – MS/MS.
B: Atividade recetora do radical DPPH. No teste de DPPH foi usada a metodologia descrita por Pombal et al 2017[2] . Os extratos foram avaliados a diferentes concentrações (de 0,48 ate 125 µg/ml), e foi usada a quercetina como estândar.
C: Avaliação da capacidade de retenção do óxido nítrico. Diferentes concentrações de cada um dos extratos foram usados para medir a capacidade de retenção do radical NO[2].
D: Capacidade de captura do ânion superóxido em um sistema não enzimático. A atividade inibitória contra o ião $^{\bullet}O_2^-$ foi avaliada por um sistema não enzimático[3]. A placa que continha o estrato ou a Quercetina foi incubada durante 5 minutos, a seguir foi realizada as medidas a 560 nm.
D: Teste de inibição da peroxidação lipídica. Foi usado o homogeneizado de ovo como fonte biológica de lípidos e avaliamos a capacidade dos extratos de *Bauhinia tonningii* para reducir a peroxidação lipídica[4].
D: Toxicidade em *Artemia salina* Leach. Foi avaliada a toxicidade "*in vitro*" para *Artemia salina* como bioteste geral. Artêmias de 48 horas foram expostas a uma concentração de 1000 µg/ml do extrato a avaliar e foi determinada a mortalidade a las 24 horas de incubação. Os resultados foram dados como percentagem de mortalidade para a concentração usada[5].
Resultados
A: Atividade recetora do radical DPPH. Para Bauhinia thonningii, os extratos de folhas, raízes e casca obtidos com etanol e acetona desenvolveram atividade antirradicalar contra o DPPH, foi obtida uma inibição de 60% a 70%. Estes resultados são muito parecidos aos obtidos com o estândar usado, Quercetina (77,9±4,3%).
B: Capacidade de retenção do óxido nítrico. A concentrações baixas, os extratos BACA-Hex, BACA-Acet e BARA-Acet mostram uma atividade antirradical contra o ião NO com valores entre 50% e 60%, em comparação com a atividade máxima registada com o padrão de Quercetina (74,8%).
C: Capacidade de captura do ânion superóxido. A atividade de inibição frente ao ião $^{\bullet}O_2^-$ foi semelhante tanto para a Quercetina (53,0±0,8%), como para os extratos em acetona e etanol obtidos a partir das folhas de *Bauhinia tonningii* (BAFA-Acet 50,1±3,8% e BAFA-Eta 51,9±2,5%).

D: Inibição da peroxidação lipídica. Os resultados obtidos mostram uma capacidade inibitória media de 90% para quase todos os extratos a máxima concentração usada. Só para os extratos de hexano de folhas e raízes da planta, a atividade foi inferior ao do valor do padrão curcumina (71±0,65; 84,4±1,15 e 97,2±0,3% de inibição respectivamente).

E: Toxicidade em *Artemia salina* Leach. Na avaliação da toxicidade dos diferentes extratos a uma concentração de 1000 µg/ml,foi observado que o promedio de mortalidade entre os extratos foi de xx%, sendo o extrato BAFA-Hex o que apresenta maior % de mortalidade (59%).

Tabela 1 Valores máximos de eficácia inibitória (Emax) e concentração inibitória 50 (CI_{50}) para os extratos de folhas (BAFA), casca (BACA) e raiz (BARA) de *Bauhinia tonningii* frente aos radicais DPPH e óxido nítrico.

Extratos	DPPH		Óxido nítrico	
	Emax (% de inhibición)	CI_{50} (µg/ml)	Emax (% de inhibición)	CI_{50} (µg/ml)
Quercetina	77,9 ± 4,3	8,5	74,8 ± 3,5	Nd
BAFA-Hex	29,8 ± 1,5*	*Nd*	33,9 ± 4,1*	-9,7
BAFA-Acet	69,8 ± 0,5*	14,3	38,7 ± 3,6*	Nd
BAFA-Eta	66,6 ± 0,9*	61,4	41,7 ± 0,4*	-35,9
BACA-Hex	11,5 ± 1,2*	*Nd*	57,0 ± 1,2	1,8
BACA-Acet	69,5 ± 0,8*	3,3	50,4 ± 2,5*	Nd
BACA-Eta	65,5 ± 0,9*	3,2	46,5 ± 3,3*	Nd
BARA-Hex	20,8 ± 2,1*	*Nd*	38,4 ± 1,7*	Nd
BARA-Acet	73,0 ± 0,7	37,9	51,5 ± 1,2*	103,0
BARA-Eta	72,2 ± 0,4	17,7	45,9 ± 2,5*	-216,0

*(p<0.05 *vs* Quercetina). Nd = no determinado

Conclusões. *Bauhinia tonningii* é uma planta de interesse biológico, já que as atividades antioxidantes e o perfil toxicológico são descritos a través deste estudo.

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P54 Identification of microplastics in water and food using pyrolysis GC with high resolution Orbitrap mass spectrometry

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Introduction: Microplastics are small particles made from synthetic polymers with a diameter typically ranging between 5 mm and 1 µm. Microplastics may consist of not only the pure synthetic polymer, but also include residuals of the monomer, plasticizers, flame retardants, and many other toxic additives that can have a negative impact on human health. Among the analytical techniques are FTIR and Raman spectroscopy, and microscopy. However, for microscopy-based analysis, the number of samples that can be screened is limited. Pyrolysis gas chromatography-mass spectrometry (py-GC-MS) is a promising alternative for surveillance and identification of microplastics where throughput is critical. This analytical approach enables time-saving detection of bulk amounts of micro- and nanoplastics below the lower size limit of the microscopy techniques.
Materials and Methods: For stormwater analysis, the sample (1 L total volume) was spiked with deuterated polystyrene (D5-PS). The sample was filtered to collect particulates. It was then wrapped in aluminum foil dried in an orbital incubator at 50 °C weighed and placed in a pyrolysis cup. The milk and steak samples were freeze dried and milled. After that, 1 g of each sample was spiked with D5-PS extracted by pressurized liquid extraction in pre-cleaned 34 mL ASE cells on a Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor. Extraction was performed with dichloromethane at 180 °C and 1,500 psi over three extraction cycles. The extracts were weighed and 80 µL transferred to a pyrolysis cup. A pyrolizer (Frontier Laboratories) was mounted on a Thermo Scientific™ Orbitrap Exploris™ GC 240 mass spectrometer. The mass spectrometer was operated in full-scan mode using 60,000 mass resolving power. Lock mass corrected data was processed using Thermo Scientific™ Compound Discoverer™ software and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software. A double-shot method was used for the analysis of the food and environmental samples. Double-shot methods are useful when very complex samples are analyzed, as the TD step eliminates a significant part of the matrix. Polymer standards were also analysed.
Results: A series of polymer standards were subjected to pyrolysis to find characteristic fragmentation products that can be used for polymer identification in unknown samples. The resulting pyrograms were screened to find the pyrolysis products known from the literature. To simplify further data treatment during the analysis of samples, a targeted processing method was created. The processing method included all compounds previously identified, with each compound´s presence confirmed using a minimum of three representative ions extracted from the TIC using a mass extraction window of ±5 ppm. During the data processing, benzene, naphthalene, and fluorene were found in the stormwater sample. Styrene, allylbenzene, α-methylstyrene, and toluene were revealed in two remaining samples (milk and steak).
Conclusions: The study demonstrates that Py-GC-Orbitrap is a robust tool for the confirmation of the presence and identity of microplastics in different sample types. High selectivity and sensitivity were achieved in combination with a targeted screening approach using both Compound Discoverer software and Chromeleon software. The presented method allows for target microplastics analysis in different sample types, including complex food and environmental matrices.

P55 Assessment of the phenolic profile and antioxidant properties of strawberry fruit ('*Fragaria* x *ananassa* Duch') bio-residues

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The fruit "*Fragaria* x *ananassa* Duch" (strawberry) is known to contain high amounts of minerals and sugars besides being a valuable source of phenolic compounds and antioxidant agents, which makes it attractive to be used as raw material by the food industry. However, the waste generated during its production, in addition to that generated by the final consumers, increases the percentage of unused food[1]. In this context, the objective of the present study was the evaluation of strawberry bio-residues regarding their phenolic profile, specifically non-anthocyanin and anthocyanin compounds by ultra-fast liquid chromatography, equipped with a diode array detector coupled to an electrospray ionization mass detector (LC-DAD-ESI/MSn); as also assess its antioxidant capacity with the inhibition of lipid peroxidation (TBARS) and oxidative hemolysis (OxHLIA) assays, for the development of new products with high added value[2-3]. Regarding the phenolic profile, 29 phenolic compounds were identified, among which 17 non-anthocyanin compounds (flavan-3-ols and ellagic acid derivatives) with total concentration of 11.85 ± 0.05 µg/mL and 12 anthocyanins (*O*-glycosylated pelargonidin, cyanidin, and peonidin derivatives) with total concentration of 9.235 ± 0.005 µg/mL. The extract showed high antioxidant properties, when compared to Trolox (standard), presenting IC_{50} = 249 ± 12 µg/mL for TBARS assay and IC_{50} = 143 ± 7 and 424 ± 14 g/mL for 60 and 120 min, respectively, in OxHLIA assay. These results demonstrate the richness of these residues in high added value compounds, with high commercial value, as also its functionalization properties, proving its suitability to be used as ingredients or even as raw material for the development of new products.

Acknowledgements: The authors thank to FCT (Portugal) for financial support through national FCT/MCTES funds to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021), and for the CEEC-Institutional contracts of C. Pereira, M.I. Dias, and L. Barros. To FEDER, through the POCI of Portugal2020 for the financial support through the project "IntegraValor" (POCI-02-0853-FEDER-045654).

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P56 Impact of chromatographic-based platforms in the progress of society, science, and technology

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Chromatography was born roughly one century ago but suffered an outstanding technological improvement in innovation, research, and development since then, which made it fundamental to advances in knowledge at different levels, with a relevant impact on the well-being and health of individuals. Chromatography boosted a comprehensive and deeper understanding of the complexity and diversity of human-environment interactions and systems, how these interactions affect our life and the several societal challenges we are currently facing that affect it, namely those related to the sustainability of our planet and future generations. From life sciences, which allowed us to identify endogenous metabolites relevant to diseases mechanisms, to the OMICS field, nanotechnology, clinical and forensic analysis, drug discovery, environment, and "foodprint", among others, it is outstanding the wide range of applications of today's chromatographic techniques. This is fueled by a great variability of powerful chromatographic instruments currently available, with very high sensitivity, resolution, and identification capacity, that provide a strong basis for an analytical platform able to support the challenging demands of the postgenomic and post Covid-19 eras. Within this context, we aim to address the great utility of chromatography in helping to cope with several societal-based challenges, such as the characterization of disease and/or physiological status, and the response to current agri-food industries' challenges of food safety and sustainability, or the monitoring of environmental contamination. These are increasingly important challenges considering the climate changes, the tons of food waste produced every day and the exponential growth of the human population.

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P57 The significance of chromatography on disease diagnostics. The case study of prostate cancer

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Chromatographic techniques have become an indispensable tool with broad applications in several impact fields including agricultural, environmental, pharmaceutical and forensics, in addition to systems biology and biomedical research. With recent advances in chromatography, MS instrumentation, and hyphenated systems, making increasingly significant contributions to clinical applications, especially in cancer biomarker discovery and verification, allowing quantitative measurements of thousands of secondary endogenous metabolites, even in complex clinical specimens, such as plasma/serum, human blood, tissue, and urine. Despite spectacular advances in molecular medicine in the post-genomic era, cancer mortality remains unacceptably high constituting a major public health problem worldwide being the second leading cause of death only surpassed by cardiovascular diseases. Prostate cancer (PCa) is the second most frequent malignant tumour, the fifth leading cause of cancer death among men worldwide (the leading cause of cancer death among men in 46 countries), and the most frequently diagnosed cancer in 105 of 185 of the world countries [1]. Given the sheer burden of PCa, new and innovative strategies for cancer diagnosis and care are urgently required. In this context endogenous volatile metabolites (EVOMs), present in different biological fluids, emerge as a promising non-invasive approach. Therefore, the aim of this study was to establish the urinary volatilomic fingerprint of PCa patients to identify and define a set of molecular biomarkers for the diagnosis of PCa. Chromatographic data were submitted to advanced statistical tools as a powerful way to define a pool of potential PCa biomarkers which can be used, after validation, on PCa diagnosis.

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P58 Evaluation of different sample preparation procedures on extraction efficiency of five opium alkaloids from poppy seed tea

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Poppy seeds are used for the preparation of relaxing teas for insomnia and anxiety due to their opium alkaloids (OAs) content. Lack of knowledge of the concentration in teas and uncontrolled use has caused cases of intoxication. In the present work, an effective methodology based on a micro-solid phase extraction (µSPEed®) followed by gas chromatography-tandem mass spectrometry (GC-MS) has been developed, optimized, and validated to quantify five OAs in poppy seed tea. For the optimization of the µSPEed® procedure, nine cartridges of different chemical nature (C4, C8, C18, silica, APS, PFAs and polymeric: PS/DVB-RP, SCX and SAX), pH, cycles, and elution solvent, were evaluated. The method was successfully validated according to SANTE/12682/2019 and ICH Q2(R1) guidelines and applied to study the transfer of OAs from poppy seeds to tea, through the evaluation of three factors (water temperature, infusion time, and seed amount) at two levels. The optimal conditions were achieved at 90 ºC for 5 min using 4 g of seeds, corresponding to a transfer rate of 71% for morphine, 96% for thebaine and 100% for codeine, papaverine and noscapine, respectively. These conditions were used to quantify the OAs in four teas produced with different seeds. A high amount of morphine (1563 µg/L) was found in one sample indicating that the seeds used had twice the concentration of the maximum limit legislated by the EU (20 mg/kg) and highlighting the need to warn the population of this dangerous practice

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P59 The Madeira wine aroma - from glass to consumer. A chromatographic-based approach

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From centuries, wine has a fundamental role in the culture and habits of different civilizations. Amongst numerous wine types that involve specific winemaking processes, fortified wines possess an added value and are greatly honored worldwide. Chemically, wine is a fascinating and very complex matrix constituted by several hundreds of chemical compounds/groups – water, ethanol, glycerol, organic acids, carbohydrates, and to minor extent terpenoids, pyrazines, higher alcohols, ethyl esters, fatty acids, nitrogenous compounds, sulphur compounds, furanic compounds, among others [1,2]. The final quality of wine depends on several factors and parameters namely the grape varieties, geographical region, terroir and climatological conditions, vinification process, including fermentation conditions (must composition, dominant yeasts, pH, temperature) and aging [1,2].
Amongst numerous wine types that involve specific winemaking processes, fortified wines possess an added value and are greatly honored worldwide. The description of the most important characteristics of the main worldwide fortified wines—Madeira, Port, Sherry, Muscat, and Vermouth will be considered. The chemistry of fortified wines flavor, the origin of typical aromas (primary, secondary and tertiary), and the influencing parameters during the winemaking process will be highlighted in addition to some specificities of worldwide fortified wine, mainly its volatile composition with particular emphasis on aroma compounds. The vinification processes, the evolution of volatile organic compounds (VOCs) during the aging processes, and the most important odor descriptors will be also considered. Given the worldwide popularity and the economic relevance of fortified wines, much research should be done to better understand accurately the reactions and mechanisms that occur in different stages of winemaking, mainly during the oxidative and thermal aging.

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P60 Análise cromatográfica da composição química de conservas à base de trigo

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A importância de uma dieta variada para a saúde, prevenindo doenças e/ou deficiência de nutrientes é incontestável[1]. Embora o consumo de alimentos frescos seja altamente recomendado, muitas vezes não é possível, e os alimentos enlatados são uma opção para a sua substituição, ajudando a garantir a ingestão necessária de nutrientes que devem ser ingeridos diariamente[2].
No presente estudo, dois produtos em conserva de trigo germinado foram caracterizados por cromatografia em termos da sua composição em ácidos gordos (GC-FID) e em açúcares livres (HPLC-RI). Um dos produtos continha trigo orgânico germinado e alho preto, e o outro trigo orgânico germinado, cebola, abóbora, cenoura, pimentão vermelho, nabo e alho. Ambos continham caldo vegetal.
Relativamente aos ácidos gordos, para ambas as amostras, o ácido oleico foi o que mais se destacou, seguido do ácido linoleico (**Fig. 1, à esquerda**). Em termos de açúcares livres, a maltose foi o açúcar mais abundante em ambas as amostras e a sacarose e a frutose foram detetados em menores quantidades na conserva de trigo e alho preto e na de trigo e vegetais, respetivamente (**Fig. 1, à direita**). Em conclusão, estas conservas de trigo germinado mostraram uma composição química importante para a manutenção de uma dieta variada e equilibrada.

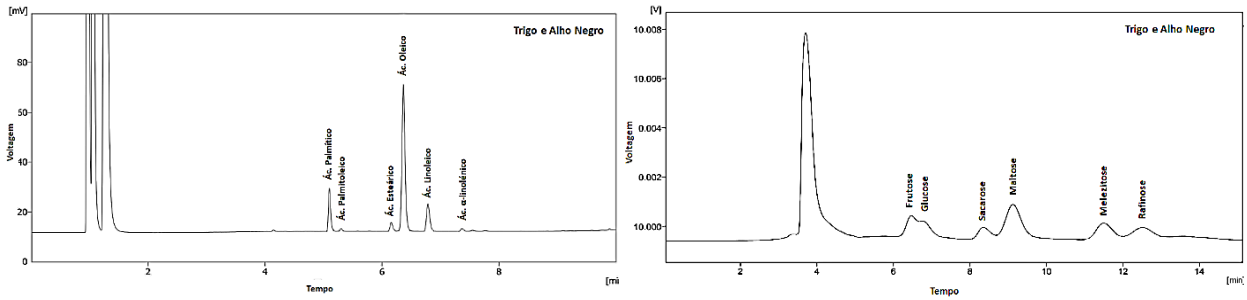

Fig. 1. Perfil cromatográfico dos ácidos gordos (à esquerda) e açucares (à direita) da amostra de trigo e alho negro.

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P61 Chemical profile and proximate composition of '*Fragaria* x *ananassa* Duch' fruit bioresidues

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'*Fragaria* x *ananassa* Duch' fruit (strawberry) is widely known for its pleasant organoleptic characteristics (e.g., flavor, smell) and health benefits, being used in several applications in food industry. However, due to their physical fragility, a considerable amount is damaged along the distribution chain, which justify its valorization for the development of novel food products[1-2].
In this context, the objective of this study was to evaluate the centesimal composition (AOAC methods) and the chemical profile of these bioresidues, namely in fatty acids by gas chromatography with flame ionization detector (GC-FID), in sugars by high performance liquid chromatography with refraction index detector (HPLC-RI), in tocopherols by HPLC-fluorescence, and in organic acids by ultra-fast liquid chromatography with diode array detection (UPLC-DAD).
The bioresidues presented high levels of water and carbohydrates, when compared to the other macronutrients studied (protein, fat, and ash), as expected. Regarding the chemical profile, two individual sugars (fructose and glucose), six organic acids (mainly citric and malic acid), and fourteen fatty acids (predominance of α-linolenic acid) were detected. Moreover, regarding tocopherols (vitamin E), three isoforms were found, with a predominance of α-tocopherol. The results obtained in this study demonstrate the importance of recovering bioresidues from the food industry for the development of new food products with relevant nutritional profile and rich composition in fatty acids, tocopherols, organic acids, and sugars.

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P62 From cork stoppers to sparkling wine – The impact of different cork stoppers in the aroma profile

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Sparkling wine is an alcoholic beverage consumed worldwide in celebrations and festivities and it is stored in bottles using cork stoppers. Cork is a natural vegetable tissue with superior characteristics such as impermeability to liquids and air, compressibility, resilience and chemical inertness.[1] When converted into stoppers it contributes to an improved preservation of the sparkling wine, keeping the quality of these beverages in the best possible way.[2,3] Therefore, it is very interesting to know how wines develop under different stoppers.

Nowadays many cork stoppers are made from microagglomerate (1-3 mm particles), agglomerate corks (3-7 mm particles), and corks with and agglomerate body plus one, two or three natural cork disks obtained from thin cork planks.[4] The differences between these corks are striking – while agglomerate and particularly microagglomerate stoppers use up to 50% of glue in their composition, corks with natural disks have a higher percentage of natural cork and, importantly, the fraction of cork in contact with wine is composed of high-quality natural cork, allowing an extended contact with this type of material.[4]

In this work, several samples of sparkling wine bottled with different types of stoppers were analyzed to study the differences resulting from different bottling stoppers (one agglomerate + 2 discs stopper and two different microagglomerate stoppers). The samples were extracted using HS-SPME, and the separation was carried by GC/MS using a polar and nonpolar capillary column.

The PCA results (**Figure 1**) revealed differences in the volatile composition of the sparkling wines when closed with different stoppers. The compounds that differ between bottling are VOCs such as esters and alcohols.

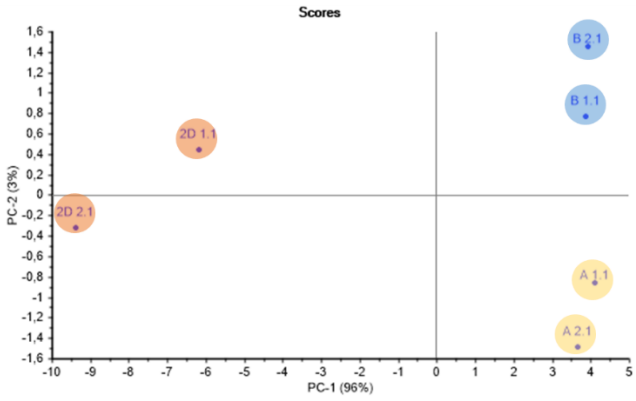

Figure 1: Differences in the volatile composition of the sparkling wine when closed with different stoppers. In red is represented the samples bottled with two discs stoppers and in blue and yellow are represented two different types of microagglomerated stoppers.

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P63 Analytical quality by design approach for selecting the optimal RP-HPLC conditions for the determination of clobetasol in cream formulation

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Pharmaceutical companies are increasingly concerned with the life cycle of the pharmaceutical product (PP). In this context, one of the most important factors is the stability of the PP. To predict the stability of the PP, forced degradation studies are performed, and the initial analytical methods are often unable to provide optimal separation of degradation products (DP). In this work, the Analytical Quality by Design (AQbD)[1] approach was applied to develop a simple, rapid, and stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the effective analysis of clobetasol in a cream formulation. To this end, the analytical target profile was defined, and an Ishikawa diagram was constructed for initial risk assessment. The initial mobile phase composition (42.5:10:47.5, v/v 0.05 M Na_2HPO_4 buffer pH 5.5:methanol:acetonitrile) and flow (1.0 mL/min) were used to analyze the degraded samples. The worse scenario tested was basic degradation, have resulted in a lack of resolution between the active component and the DP. A two-level full factorial design was then used to model impact of the CMVs (acetonitrile % and flow rate) as function of the CAAs (N, tailing factor, R, and RT)[2]. The optimal method was carried out using a Merck C18 column (150 x 4.6mm, 5µm), set at a temperature of 30°C, a volume injection of 20 µL, a detection at 240 nm, and a mobile phase of 0.05 M Na_2HPO_4 buffer pH 5.5:methanol:acetonitrile (40.5:10:49.5, v/v) eluted at an isocratic flow rate of 0.8 mL/min. AQbD enabled a systematic understanding and acquisition of robust analytical method that play an important role in the development of PP.

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P64 Analytical method for determination of levamisole in fish feed by LC-MS/MS

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Levamisole has been considered a promising anthelmintic and immunostimulant drug for fish farming. In aquaculture, the main route for drug administration is oral through the medicated feed. However, incorporating drugs into the feed is a challenging step to assure the goal treatment dose with the drug. In this way, it is important to have an incorporating process with high efficiency (avoiding drug loss), suitable homogeneity of drug concentration among the feed pellets, and with minimum leaching rate of the drug from the feed to the water. Thus, aiming to evaluate the drug incorporating process is necessary to have a suitable analytical method to assess the drug concentration in the medicated feed. For so, this study aimed to optimize and validate an analytical method involving a simple extraction procedure associated with LC-MS/MS analysis to determine levamisole in fish feed. The sample preparation step was optimized using a two-level (2^3) full factorial design. The optimum conditions were achieved by using 2.5 mL of methanol/water (80/20 v/v), both with 1% ammonium hydroxide. The LC-MS-MS analysis was performed with a mobile phase composed of methanol and water (85:15 v/v) containing 0.5% formic acid in isocratic mode at a flow rate of 0.4 mL min^{-1}. The electrospray interface source was set to operate in positive mode. The proposed analytical method presented high extraction efficiency (100.2 ± 2.5%) and was validated,[1,2] being considered selective, accurate (recovery >98.4 and <98.9%), precise (RSD <4.87), and linear in the range of 125 to 750 mg kg^{-1}.

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P65 The chromatographic analysis techniques and fatty acids to unlock secrets of meagre migrations

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Meagre (*Argyrosomus regius)* is a sciaenid fish, with distribution along the Northeast and Central-West Atlantic from the coast of Sweden to the Gulf of Guinea and in the Mediterranean and Black Sea[1]. However, at a finer spatial scale, the distribution, movements and population dynamics of the wild stocks of meagre remain scarcely known, particularly on what concerns the identification of the feeding areas at sea that, together with the spawning areas such as the Tagus estuary, constitute critical areas for the management of the stocks.
In Portugal, the meagre is targeted by both commercial and recreational fisheries. During the last decade, the Tagus estuary and the adjacent coastal zone represent between 60 to 70% of the Portuguese meagre landings with a total annual volume around 132 t[2].
In the framework of MIGRACORV project (https://migracorv.pt/) this study is to evaluate if fatty acids (FA) signature can be used to assess possible differences among adults *versus* juveniles wintering areas. To achieve this goal, FA signature of the heart was used to assess whether juvenile and adult meagre share the feeding areas at sea. This methodology was only possible because the phospholipid FA are genetically controlled and can be used as a natural marker[3].
Using various sample preparation[4,5] (ASE, SPE) and chromatographic analysis techniques[4] (GC/MS, GC-FID, HPTLC) we analyzed and identified the FA profile in the phospholipid classes and results point out to variations between juvenile and adults regarding proportion of phospholipids classes and present similar values in total proportion of FA profile in phospholipids.

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P66 Optimization of a GDME-HPLC-DAD methodology for the extraction of volatile organic compounds from wood-based panels

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In recent years, the increase of the time spent indoors raised concerns about indoor air quality and the consequences that exposure to poor air quality could have on human health [1, 2]. From the many indoor air pollutants, the emission of volatile organic compounds (VOCs) from wood-based panels (WBPs) is a topic of interest, given their use in furniture and building materials.
WBPs is a broad term used to refer to wood products made from fibres, particles or veneers, and can be categorized as particleboards (PBs), oriented strand boards, medium-density fibreboards (MDFs), plywood, etc. The fibres or particles are bonded together by an adhesive, creating a mixture that will solidify and gain the desired shape through heat and pressure [3].
In this work, an ingenious sample preparation technique for VOCs is presented. Gas-diffusion microextraction (GDME), previously used with other liquid and solid samples [4-6], was used to extract volatile carbonyls from PBs and MDFs towards their HPLC-DAD analysis. Different cleaning procedures of the experimental GDME apparatus were tested, to avoid possible contaminations between extractions. Furthermore, extraction parameters such as temperature, time and volume and concentration of acceptor solution were studied and optimized, as well as the derivatizing reagent used (2,4-dinitrophenylhydrazine and 4-hydrazinobenzoic acid). For the HPLC-DAD analysis, the separation of the analytes was tested using different gradient elution methods and chromatographic columns.

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P67 Pesticide multiresidue determination in tomato using dilution of QuEChERS raw extracts and UHPLC-MS/MS

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Tomato is one of the most cultivated and consumed vegetables in the world, with a high pigment and water content. Pesticide residues may be present in food samples because they are used to control pests and diseases that can affect crops[1]. Because of the presence of matrix components that can affect analyte signal response, pesticide residue determination requires sensitive and selective analysis, as well as an efficient sample preparation. The QuEChERS method and its modifications are applied to a wide variety of matrices and compounds. Various sorbents and different combinations can be used during the cleanup step, depending on the type of matrix, chromatographic technique and the compounds to be analyzed[1]. However, some sorbents may remove some pesticides resulting in lower recoveries, increasing time and reagent costs. Given the increased sensitivity of current instrumentation, extract dilution is an appropriate solution for minimizing matrix effects and increasing method robustness. Therefore, the aim of this study was to evaluate the dilution of QuEChERS uncleaned extracts to reach low limits of quantification. The extraction method was performed using the QuEChERS acetate-buffered[2]. After centrifugation, the supernatant was filtered (0.2 µm) and diluted 10 fold (1+9, v/v) with mobile phase for analysis. The analyses were performed in a Waters Acquity UHPLC system with Xevo TQ triple quadrupole mass spectrometer equipped with an electrospray source and a column Acquity UPLC® BEH C18 (50 × 2.1 mm, 1.7 µm). The mobile phase consisted of (A) water/methanol (98:2, v/v) and (B) methanol, both containing 0.1% (v/v) formic acid and ammonium formate 5 mmol L^{-1}. The electrospray ionization operated in positive and negative mode (ESI±) using selected reaction monitoring mode (SRM). The method was validated according to SANTE Guide protocol for 129 pesticides. Most compounds showed recoveries from 70 to 120%, with RSD < 20%. The limit of quantification (LOQ) ranged from 0.01 to 0.04 mg kg^{-1}. The proposed method was applied to 16 commercial tomato samples. Ten pesticides were found in 10 samples, with concentrations between 0.011 and 0.238 mg kg^{-1}, and four pesticides are not authorized for this crop in Brazil. A simple and rapid sample preparation procedure has been established for determination of pesticide residues in tomato by UHPLC-MS/MS analysis. The results demonstrate that the proposed method is suitable and effective for easier implementation in the routine analyses.

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P68 Optimization of a semi-preparative liquid chromatography method to produce analytical standards of dicaffeoylquinic acid isomers from yerba mate

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Dicaffeoylquinic acid isomers are increasingly studied for their beneficial biological effects in humans. However, their commercial analytical standards are high-cost, which limits research. This study aimed at optimizing a semi-preparative high-performance liquid chromatography (HPLC-SP) method to purify analytical standards of three dicaffeoylquinic acids isomers (3,4-dicaffeoylquinic (3,4DQA) 3,5-dicaffeoylquinic (3,5DQA) and 4,5-dicaffeoylquinic (4,5DQA)) from an inexpensive natural source - yerba mate (*Ilex paraguariensis)*. Concentrated extracts with dicaffeoylquinic acid isomers were injected into the (HPLC-SP) system. The following chromatographic parameters were optimized through a 2^3 central composite design: the final percentage of methanol of the chromatographic method (A, 30 to 60%), the time to reach the final percentage of methanol (0 to 30 minutes) and the injection volume of the concentrated extract (100 to 900 µL). The yield (mg) of each compound was assessed as a response. For 3,4-DQA, the best injection volume was 738 µL of the extract, with the mobile phase reaching 36% A at 24 minutes, yielding 13.6 mg. For 3,5-DQA, the best injection volume was 900 µL, with mobile phase reaching 45% A at 15 minutes, yielding 141.5 mg. For 4,5-DQA, it was 738 µL, with mobile phase reaching 36% A at 6 minutes, yielding 13.6 mg. Thus, our data indicate efficient chromatographic conditions by HPLC-SP to obtain high yields of analytical standards of 3,4-DQA, 3,5-DQA, 4,5-DQA from yerba mate, in laboratory or industrial scale, providing an alternative for laboratories to study their presence in foods and drugs, their beneficial effects on the human organism, and the underlying mechanisms.

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P69 HPLC-DAD-(ESI)-MS/MS analysis as the first step to metabolic fingerprinting of medicinal herbs: the case of underexploited *Euphorbia* species

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The genus *Euphorbia* comprises more than 2,000 species widely distributed in Asia, Africa and Latin America. They have been extensively used in folk medicine to treat disorders such as abdominal pain, skin diseases, tumors, wart, among others[1]. Although the well-recognized ethnopharmacological relevance of *Euphorbia* species, most of them have not been studied yet, such as *Euphorbia hirta* and *Euphorbia jokinii.* In this context, screening the bioactive molecules potentially responsible for the observed medicinal effects is an initial, but key step for discovering novel active compounds and understanding the mechanisms of action underlying their biological activities. Thus, this study aimed at determining the phenolic compounds composition of *E. Hirta* and *E. jokinii.* Both plants were freeze-dried, milled, and extracted with ethanol:water (60:40 v/v) for 3 hours at 45 °C. After centrifugation, the extracts were freeze-dried, re-suspended in ethanol:water (20:80 v/v), filtered and injected into the HPLC-DAD-(ESI)-MS/MS system. The phenolic profile of *E. Hirta* and *E. Jokinii* revealed mainly the presence of flavonoids and galloyl derivatives. Thirty-two compounds were tentatively identified in *E. Hirta* and twenty-two in *E. Jokinii.* Quercetin 3-*O*-rhamnoside was the major compound in both species, with *E. Hirta* showing the highest content (10.5 mg/g *vs* 8.2 mg/g extract). Our results indicate that *E. Hirta* and *E. Jokinii*, yet underexploited *Euphorbia* species, are interesting sources of flavonoids. Further studies should evaluate the relationship between these compounds and the biological activities of these plants, as well as establish possible related mechanisms of action.

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P70 Análise cromatográfica de metabolitos secundários em frutos de castanheiro da Índia

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Em Portugal, o castanheiro da Índia (*Aesculus hippocastanum*) é uma árvore amplamente cultivada como ornamental. Entre a comunidade científica, esta espécie é reconhecida pelos glicosídeos esteroidais, dentre os quais a aescina tem sido descrita como o principal constituinte ativo[1]. No entanto, esta planta apresenta também outros compostos bioativos, incluindo flavonóis glicosilados[2]. Portanto, este trabalho teve como objetivo caracterizar o perfil fitoquímico de três partes distintas do fruto desta árvore, o qual consiste numa cápsula subglobosa de paredes carnudas, com 1 a 3 sementes. Após recolha e preparação do material vegetal, foram preparados extratos hidroetanólicos por sonicação da mistura a 400 W durante 40 min. As etapas de separação e identificação de compostos fenólicos e saponinas foram realizadas por HPLC-DAD-ESI/MS^n, utilizando diferentes gradientes de eluição[3]. Foi possível identificar 31 compostos fenólicos nos três extratos, incluindo ácidos fenólicos (derivados dos ácidos cafeico e *p*-cumárico), flavan-3-óis (derivados de (+)-catequina e (-)-epicatequina) e flavonóis (quercetina *O*-glicosilada, derivados de isoramnetina e kaempferol). Enquanto os flavonóis e os flavan-3-ols predominaram nos extratos da semente, o extrato obtido a partir do pericarpo do fruto apresentou quantidades comparáveis destes constituintes fenólicos. Também foi possível identificar as quatro principais aescinas no extrato dos cotilédones da semente, nomeadamente aescina Ia e Ib (β-aescina) e isoaescina Ia e Ib (α-aescina). Este estudo evidenciou que as diferentes partes do fruto não comestível do castanheiro da Índia apresentam um perfil fitoquímico diferente em termos qualitativos e quantitativos. Estudos futuros serão importantes para avaliar as atividades biológicas dos extratos deste fruto de interesse farmacêutico.

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P71 Comprehensive HPLC-DAD-(ESI-)MS/MS characterization and comparison of bioactive molecules from different parts of *Ganoderma lucidum* sporocarps

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Ganoderma lucidum is a well-known medicinal mushroom, both historically and currently. Driven by the ethnopharmacological prospect and the crescent body of scientific evidence that associates *G. lucidum* intake with health, the interest in its secondary metabolites has been further fostered. Whereas most research on medicinal mushrooms has focused on the comprehensive identification and yields of metabolites throughout their different growth phases, the distribution of those compounds along the sporocarps (fruiting bodies) in the mushroom's antler growth phase remains poorly investigated. This study aimed to directly compare the chemical composition of the exterior skin and interior flesh of *G. lucidum* sporocarps. Dried samples provided by Käapa Biotech (Finland) were homogenised and subjected to ultrasound-assisted extraction with ethanol:H_2O (80:20, v/v) at an amplitude of 47% for 15 min. Extracts were analysed in an HPLC-DAD-(ESI-)MS/MS system. Sixty-two compounds were tentatively identified in both extracts and comprised primarily lanostane-type triterpenes, besides six phenolic compounds. Among the triterpenes, 20 lucidenic and 16 ganoderic acids were found, with Lucidenic acid F and Ganoderic acid D being the major compounds in the flesh and skin. The overall chemical profile was the same regardless of the sporocarp part analysed, but the proportion among the compounds was considerably different. Interestingly, whereas the total triterpene content of the outer part exceeded 3 times that of the inner part, both presented equivalent amounts of total polyphenols. Our results indicate a similar profile but a higher concentration of compounds in the skin when compared to the interior biomass of fruiting bodies. This is the first time a study has examined the variations of triterpenic components between different parts of *G. lucidum* sporocarps.

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P72 Profiling the volatile exometabolome of *Pedobacter lusitanus* NL19

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Microbial metabolomics provides information about microbial metabolites produced by an organism, in a specific environment/experimental condition. *Pedobacter lusitanus* NL19 (NL19), a new species recently described by our group, whose genome encodes the information for the production of several Secondary Metabolites (SMs). However, the compounds produced are different depending on the composition of the culture medium. This is particularly notable when the microorganism is grown in 25% PC, a modified Tryptic Soy Broth (TSB) culture medium, in which the peptone of casein concentration is reduced to 25%. Furthermore, the profile of the SMs produced is different from that of the phylogenetically close strain, *Pedobacter himalayensis* MTCC6384 (MTCC6384) cultivated in the same conditions. The aim of this work was to investigate the differences in the volatile exometabolomes of these two species in PC 25% and TSB 100%. For this, a combined methodology HS-SPME/GC×GC-ToFMS was employed. 320 compounds were identified, and the relative abundance of the produced compounds/CFU was higher in the MTCC6384 strain. In this strain we have also observed a greater abundance of compounds that are involved in the biosynthesis of monoterpenoids, degradation of aromatic compounds, degradation of valine, isoleucine and leucine and biosynthesis/degradation of fatty acids. Preliminary results show that a decrease of nitrogen concentration from 100% to 25% resulted in a strong impact on the energy-producing metabolism, mainly in NL19 which may be due to the activation of accessory pathways responsible for energy production, probably due to the stress induced by starvation.

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P73 SE-HPLC and RP-HPLC as powerful tools for analyzing the gastrointestinal delivery of collagen hydrolysates obtained from codfish skins using chitosan-TPP hydrogels

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Approximately 70% of the fishery industry's production is waste, including heads, skins, bones, and scales. The valorization of these by-products may result in new raw materials for applications in various industries. Collagen is a ubiquitous protein with many applications, mainly derived from mammals. However, considering some health and religious restrictions, using marine collagen in codfish skin is a new alternative.[1,2] In this work, collagen was extracted with acetic acid and a mixture of urea and propanoic acid (1:2).[1] Additionally, bioactive collagen peptides were obtained recurring to enzymatic hydrolysis with alcalase.[3] Peptide size was evaluated using size exclusion (SE-HPLC), with the prevalent molecular weight ranging between 1-3 kDa and 3-5 kDa. The hydrolysates were encapsulated in chitosan-tripolyphosphate hydrogels,[4] accounting for 38.3% and 39.2% encapsulation efficiencies for peptides extracted with acetic acid and with the eutectic solvent, respectively. Finally, gastrointestinal simulation allowed the evaluation of the release of peptides from the hydrogels and the effect of digestion on the hydrolysates. Thus, it was possible to observe that the peptides were primarily delivered in the intestine, releasing approximately 87% of acetic acid-based peptides and 58% of urea with propanoic acid-based peptides. It is noteworthy that encapsulation can rely on peptides' polarity. Therefore, RP-HPLC was a powerful tool that showed hydrolysates' heterogenicity. Although enzyme action in the human tract did not significantly alter collagen peptides in size, it did in their bioactive properties, so encapsulation is still the most suitable alternative to apply them as possible nutraceuticals to replace conventional drugs.

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P74 Method validation for the analysis of pesticides in water samples using Solid Phase Extraction and Gas Chromatography coupled to Mass Spectrometry (SPE-GC-MS) - Matrix effect assessing in water sources

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The Council Directive 2020/2184 of 16 December 2020 regarding the quality of water intended for human consumption states a parametric value of 0,10 g/L for each individual pesticide and that the analytical methods should allow the quantification of pesticides at 30% of the parametric value. The aim of this work was the implementation and validation of an analytical method for the analysis of several pesticides in raw water and in water for human consumption, according to the documents referred above, namely: Biphenyl, Molinate, Trifluralin, Simazine, Atrazine, Lindane, Terbuthylazine, Diazinon, Dimethenamid-P, Chlorpyrifos-methyl, Alachlor, Metalaxyl-M, Malathion, S-Metolachlor, Chlorpyrifos-ethyl, Pendimethalin, Chlorfenvinphos.
Several mass spectrometer parameters were optimized to get the best conditions for each pesticide. Two different ions (one for quantification and one for qualification) were selected for each pesticide. Many compounds showed significant ion enrichment effects (more than 6 times for Diazinon). For all compounds surrogate labeled compounds were used for results correction. This method showed excellent linearity ranges for all pesticides (between 17 and 165 g/L), with correlation coefficients greater than 0,9992. Recovery studies in several matrices with different fortification levels were performed using solid phase extraction with recoveries between 89% (Chlorpyrifos-ethyl) and 113% (Pendimethalin) with RSD lower than 14% (Chlorpyrifos-ethyl). The Method Quantification Limits obtained for these compounds were between 0,011 µg/L (Pendimethalin) and 0,32 µg/L (Dimethenamid-P). The expanded uncertainty (k=2) of the analytical method was below 28.3% (Chlorpyrifos-ethyl).

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P75 Optimization of fabric phase sorvent extraction and UHPLC conditions for quantification food bioactives

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The objective of this work was to validate an emerging analytical methodology, the fabric phase sorvent extraction (FPSE) coupled to ultra-performance liquid chromatography equipped with a PDA detection system (UPLC-PDA) for the extraction and analysis of free polyphenols from food samples. Several analytical parameters influencing the efficiency of the extractive process and chromatographic separation were optimized. Regarding the FPSE extraction, optimization included the extraction time, ultrasound-assisted agitation (US), extraction temperature, nature of the extraction solvent, and back-extraction time and solvent. Concerning the chromatographic conditions, the methodology was optimized to obtain a fast chromatographic separation and analysis of eight polyphenols abundant in many food samples.

The optimal extraction conditions for the extraction of the selected polyphenols include a 10-min US-assisted extraction at room temperature followed by a 15-min US back-extraction. Overall, methanol was the best solvent for this step, although the effectiveness of the procedure depends on the nature of the polyphenols to be extracted. Satisfactory results were obtained for the different analytical parameters of the method with R^2 values greater than 0.9, values for inter- and intra-day precision less than 15%, low detection and quantification limits, and recovery percentages that varied between 55 and 90%. The applicability of the method to food matrices was evaluated using saffron, fresh orange and lemon juices, as well as commercial juices, as real samples.

Overall, FPSE revealed a great potential in the extraction of free phenolic compounds from the selected food samples. In addition, it proved to be a fast and sensitive analytical approach, using small volumes of sample and extraction solvent, in the order of µL.

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P76 Liquid chromatography approaches for phosphopeptide enrichment

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Protein phosphorylation is engaged in many biochemical pathways, including cell differentiation, cell cycle control, metabolism, and apoptosis, thus, it is systematically linked to a wide range of disorders, such as cancer and dementia. Phosphoprotein enrichment is a key step for overcoming analytical challenges associated with phosphoproteins' nature, which include: 1) their dynamic modification patterns, 2) sub-stoichiometric concentrations, and 3) poor mass spectrometric sensitivity.[1]

Among the several phosphopeptide affinity enrichment approaches, ion metal affinity chromatography (IMAC) has become the most widely employed. Despite ongoing advances in IMAC materials for phosphopeptide enrichment, unbiased and quantitative recovery of phosphopeptides in complex biological mixtures remains a challenge. For example, the aminoacidic composition of peptides has a tremendous impact on their affinity for the various metal ions employed in IMACs. As a result, quantifying phosphopeptide concentration while maintaining high selectivity is indeed complex.[2]

In this study, we compared three distinct approaches for phosphopeptide enrichment (an optimized in-house method, a commercial kit (Thermo Scientific REF: A32993), and the EasyPhos protocol) [3] towards a standard sample. Comparing the in-house method against the other two approaches, this method showed the highest number of unique phosphosites. Despite this result, an ICP-MS analysis of the enrichment indicated that only 40% of the phosphorous initially found in the sample was recovered. Taking these findings into perspective, ICP-MS analysis must be applied to the other methodologies. However, mass spectrometry evidence suggests that they are underperforming approaches for phosphoprotein enrichment. Thus, quantitative phosphoproteomics conclusions should be carefully analysed by more than one technique.

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P77 Determination of MOSH and MOAH by GC×GC-TOFMS

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Nowadays across the European Union food contamination by mineral oils from production processes and packaging is becoming a serious problem for public health institutions and governments.
In this study comprehensive two-dimensional gas chromatography (GC×GC) in combination with time-of-flight mass spectrometry was evaluated, in order to find a robust one run analytical method. LECO GC×GC system takes advantage of a dual-stage, quad-jet thermal modulator positioned between the two columns and a secondary oven allows independent temperature control of the second dimension column, combined with high acquisition rate, full range TOF mass spectra.
The combination of two different polarity columns led to effective separations between compound families, identifications within families were easily reached by high acquisition rates TOFMS systems and ChromaTOF software classification capabilities defined chromatogram regions to locate clearly each compound family.

P78 Can musts sulphitation be a preservation strategy to keep the 'Vinho Verde' musts character?

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High-volume mass-market white wines production method by means of harvest-deferred fermentation from desulphited musts allows an efficient business management by avoiding the seasonality in wine sector. This technology has been used in the production of wines from Vinhos Verdes Appellation (VVA - Portugal). This Appellation presents a diversity of varieties and wine styles, and is known for producing light and fresh wines, but also mineral, complex and structured ones, with, in general, low ethanol content. The light and fresh VV wines are characterized by herbaceous, citrus, tropical fruits, orchard fruits and floral notes. Thus, the goal of this work was to unveil based on physical-chemical data if sulphitated musts from VVA preserve the organoleptic potential that allows the production of high-volume wines with the typical light and fresh VV character. A set of musts were produced at industrial scale from grapes harvested from different sub-regions of VVA, which were then sulphited under controlled conditions. For comparison purpose, sulphitated musts from Beira Atlântico and Trás-os-Montes Portuguese regions were also characterized. Free volatile and glycosidically-linked compounds were determined by two dimensional gas chromatography (GC×GC-ToFMS). The physical-chemical parameters currently used in musts quality control were also determined. Statistical tools were applied by combining all data domains. The aroma potential of musts was performed based on the construction of aroma networks[1].

A total of 145 volatile compounds were putative identified, which varied from 136 to 142, in must from Cávado and Lima sub-regions, respectively. Regarding the glycosidically-linked fraction, 29 compounds were putatively identified, which varied from 20 to 24 in must from Cávado and Amarante sub-regions. Clustering analysis unveiled the formation of 3 main clusters, one of which includes all VVA musts, which allows to infer that geographical region is the main distinguishing factor. VVA musts were characterized with higher total acidity, and lower °Brix, potency alcoholic strength and density, compared with the samples from other regions. Moreover, esters, monoterpenic and sesquiterpenic compounds detected in VVA musts may contribute with citrus, floral, orchard and tropical fruits aromas, which are relevant aromas for sensory characteristics of VV wines. Thus, must sulphitation, a methodology used to extend its preservation beyond the harvest season, seems to keep the particular musts character, which is extremely important for the consistency of light and fresh high-volume VV wines.

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P79 Universal sample handling for liquid biopsies

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Proteomics of liquid biopsies using mass spectrometry is gaining traction as a driving factor in the medical shift to personalized medicine. However, such approaches rely on time-consuming and labour-intensive sample processing methods. Furthermore, considering the simplicity with which samples may be collected, processing and treatment are crucial pre-analytical processes for liquid biopsies proteome analysis that is frequently overlooked[1]. We address these challenges by developing a unique alternative technique for storing liquid biopsies at room temperature in a portable filter-aided sample preparation (FASP) container that is ready to process utilizing a quick ultrasound-based approach[2,3]. The innovative technique was compared to the usual approach in urine samples using liquid chromatography paired with high-resolution mass spectrometry. The novel suggested storage approach eliminates the requirement for freezing and simplifies space, preservation, transport, and subsequent processing of the complete proteome. It was also shown how the use of ultrasonic energy accelerates the traditional FASP technique by processing. This technology may be adapted to several types of liquid biopsies and has a high potential to become the standard method for proteomics pipelines for clinical proteomics in hospitals since it is simple to use and does not require any special skills from the operator.

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P80 Development of chromatographic tools to identify and characterized anthocyanin-fatty acid adducts

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Anthocyanins are naturally occurring polyphenolic compounds widespread in our diet that have attracted the scientific community due both to their health-promoting properties and appealing colors (from orange to blue). For those reasons, research on the application of anthocyanins has attracted great interest over the last years from the food, nutraceutical, and cosmetic industries. However, some drawbacks have been limiting their industrial applications such as chemical instability to pH, temperature, light, and low solubility in lipophilic media, among others. To expand technological applications of anthocyanins in cosmetic products and oil-based foodstuffs it is imperative to increase their lipophilicity which could be achieved by structural modification of anthocyanins for example by esterification of anthocyanins with saturated and unsaturated fatty acids (1-5). In this work, reactions between malvidin-3-glucoside and different fatty acids were performed by enzymatic catalysis, and the resulting products were identified, separated, and characterized by HPLC-DAD/ESI-MS-MS techniques. The optimized chromatographic conditions were achieved by using a silica reversed-phase C8 column (150 × 2.1 mm, i.d., 5 µm pore size) with solvents water/formic acid 9:1 (v:v) (A) and acetonitrile/formic acid 9:1 (v:v) (B) under the following gradient: 0-20 % B over 5 min, 20-100 % over 10 min and isocratic 100 % B for 15 min at the flow-rate of 0.4 mL/min (Figure 1).

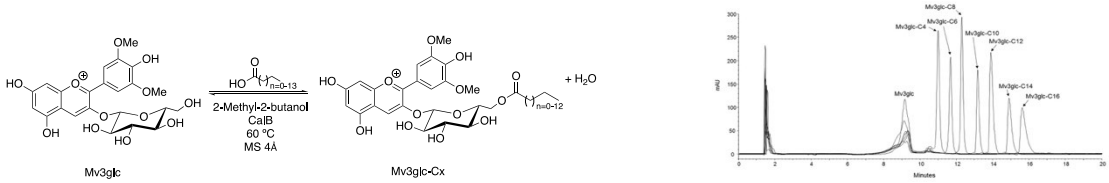

Figure 1. Optimized HPLC chromatograms of individual enzymatic esterification reactions recorded at the maximum wavelength for Mv3glc-fatty acid adducts.

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P81 Aplicação do controlo de qualidade interno e avaliação de resultados em toxicologia forense

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A garantia da qualidade dos resultados do Serviço de Química e Toxicologia Forense (SQTF) do INMLCF,IP é assegurada pelo cumprimento do programa de controlo de qualidade interno (e, sempre que possível, pela participação em ensaios interlaboratoriais).
O Controlo de Qualidade em laboratórios forenses constitui uma ferramenta essencial para garantir a confiança dos seus clientes. Paralelamente, enquanto laboratório forense de caráter essencialmente analítico, o seu produto corresponde à execução de ensaios laboratoriais e à correspondente emissão dos resultados obtidos. Assim, para um produto deste tipo, as ações de Controlo da Qualidade dividem-se em ações de âmbito interno (orientado para o controlo da precisão e cujos critérios dependem do laboratório) e externo (voltado para o controlo da exatidão, normalmente dependente duma intervenção externa)[1].
Este trabalho pretende abordar as ações de âmbito interno, como são os conceitos de controlo de qualidade interno e de avaliação dos resultados aplicadas a ensaios realizados por cromatografia, bem como a sua correspondente aplicação efetiva no Laboratório[1].
O Controlo de Qualidade Interno deve definir um conjunto de mecanismos capazes de garantir a verificação da conformidade de parâmetros predefinidos para aceitação dos resultados dos ensaios e a sua metodologia deve ser adaptada aos diferentes tipos de procedimentos de ensaio[2].
A avaliação e o correspondente cálculo dos resultados revelam-se tão importantes quanto analisar amostras representativas e executar adequadamente a técnica de análise selecionada. Avaliar e calcular corretamente os resultados e expressá-los de forma adequada tem uma influência crítica no valor probatório do resultado apresentado em cada relatório emitido[1].
As principais medidas adotadas pelo SQTF para garantir a qualidade dos seus resultados, baseadas em referências internacionais, preveem a inclusão de controlos internos da qualidade. Paralelamente, todos os métodos analíticos possuem critérios de aceitação/rejeição de resultados, os métodos quantitativos utilizam padrões internos e os controlos são representativos da matriz das amostras a analisar.
Com esta política, o SQTF pretende a manutenção da qualidade dos serviços prestados, reconhecendo o impacto que têm para a sociedade em geral e para a Administração da Justiça em particular[3,4].

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P82 Peptide fraction identification by SE-HPLC and LC-MS/MS analysis of the body mucus from Portugal coastal fish *Halobatrachus didactylus*

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The mucus covers the fish's body, working as a protective barrier. Besides physical protection, mucus provides molecules that protect the fish from pathogens damaging [1,2]. These include antimicrobial peptides secreted in the mucus, which play an essential role in defense against microbial pathogens since these belong to the innate immune system[2,3]. In this study, two adult *Halobatrachus didactylus* individuals were captured from the wild in Sesimbra. Then, mucus collection was performed by scraping the dorsal-lateral body of the fish with a sponge. Our objective was the identification of new peptides with bioactive potential in mucus samples by chromatography analysis. Size exclusion high-performance liquid chromatography (SE-HPLC) analysis performed on mucus samples from the two individuals revealed a similar profile with an intense highlight peak which resulted in a distribution of about 775 Dalton. With interest in that peak, the two mucus samples were pooled for fractionation by SEC. The resulting fraction was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify the most probable peptide sequences. Identification from databases did not provide reliable results, indicating a lack of information on the matrix analyzed. We resorted to de novo sequencing with good results using PEAKS Studio software. Five identified peptides were selected according to their bioactivities predicted *in silico*. Furthermore, the five identified peptides were synthesized, and the molecular size was validated by SE-HPLC analysis. Overall, this chromatographic approach enabled the identification of promising peptides, which bioactivities will be evaluated *in vitro* in future work.

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P83 Assessment of a capsule for easy urine proteome collection at home

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Urine has become one of the standard samples for diagnosis of multiple conditions, as well as for proteomics-based biomarker discovery studies. In recent years, our group has developed an approach for Bladder cancer (BCa) diagnosis and patient monitoring based on proteomics, using the dysregulation of the urinary proteome to obtain information regarding the patient's state, such as tumor diagnosis and stage. However, monitoring is impaired by the lack of regular sample collection at the hospital. Thus, our goal was to develop a new cheap and easy to use method of urinary proteome collection, so that patients could collect their samples at home. Thus, the urine proteome was collected using syringe filters and was further handled with a standard shotgun proteomics protocol. Multiple types of filter membranes were tested, and with the optimized conditions, a proof of concept was conducted with BCa patient samples, in which the Filter Aided Sample Preparation, FASP protocol was used as a control for the results obtained with our new approach. Our results demonstrate that the urine proteome can be separated and then treated with our filter approach. Further research is needed to obtain the same digestion efficiency than other standard protocols.

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P84 GC-MS in the optimization of microwave-assisted extraction of linear diterpenes from *Bifurcaria bifurcata*

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Linear diterpenes (LD) are quite exclusive terpenoids found in a few brown macroalgae families, for instance in Sargassaceae, and notably in *Bifurcaria bifurcata*[1]. LD have been associated with anti-inflammatory, antiproliferative and antimicrobial properties[2] which make them promising compounds for nutraceutical or pharmaceutical applications. However, most of the studies published so far, dealing with the extraction of these compounds, use conventional extraction methods, with extended extraction time and large amounts of organic (often toxic) solvents, making them unviable for those high-added value applications.
Despite the advantages of microwave-assisted extraction (MAE), namely shorter extraction times, as well as reduced energy and solvent consumptions[3], MAE has not yet been exploited in the extraction of LD from macroalgae. In this vein, this study envisaged to optimize the MAE of LD from *B. bifurcata*. Temperature, extraction time, solid:liquid and ethanol:water ratios were optimized using response surface methodology based on a Box-Behnken design. Gas chromatography coupled to mass spectrometry (GC-MS) was used as a rapid and feasible procedure to analyse and quantify the LD of the extracts obtained from MAE of *B. bifurcata*.
LD content on a dry weight basis (mg LD/ g DW) and per amount of extract (mg LD/g ext) were evaluated by GC-MS and compared with conventional extractions. Under MAE optimal conditions (T=30°C, 5 min, solid:liquid ratio of 1:13 and %EtOH of 78%) an extract with a diterpenes content of 381.8 ± 13.7 mg per g of extract was obtained, showing the promising results of this extraction methodology and GC-MS analysis.

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PRIZES AWARDED UNDER THE 12ENC

The following prizes were Prizes awarded to young researchers participating in 12ENC - XIV WARPA:

AWARD: ORAL COMMUNICATION CATEGORY

ANDREIA BENTO-SILVA

Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Portugal

was awarded with **best oral communication** at 12º Encontro Nacional de Cromatografia, presenting the work:

Unraveling the metabolites of N-ethylpentylone in mice serum and urine

SARA SILVA MARQUES

LAQV/REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

was awarded with **best oral communication** at 12º Encontro Nacional de Cromatografia, presenting the work:

Encapsulation efficiency measurements on polymeric and lipid drug-delivery nanocarriers: are we there yet?

ANA RITA CIRCUNCISÃO

LAQV/REQUIMTE, Departamento de Química, Universidade de Aveiro, Portugal

was awarded with an **honorable mention for oral communication** at 12º Encontro Nacional de Cromatografia, presenting the work:

Liquid and gas chromatography as a tool for the characterization of Fucus vesiculosus-rich extracts as potential food ingredients

AWARD: FLASH CATEGORY

MARLENE CONCEIÇÃO PEREIRA MACHADO

REQUIMTE/LAQV, Faculty of Pharmacy, University of Porto, Portugal

was awarded with **best flash communication** at 12º Encontro Nacional de Cromatografia, presenting the work:

Comparison of microwave assisted and conventional solid-liquid techniques for the chlorogenic acids extraction from silverskin: an analysis by HPLC-DAD

ANDREIA ALEXANDRA RIBEIRO FREITAS

INIAV & LAQV/REQUIMTE, Portugal

was awarded with an **honorable mention for flash communication** at 12º Encontro Nacional de Cromatografia, presenting the work:

Multi-detection of pharmaceuticals in environment matrices by UHPLC-ToF-MS

SÓNIA DOS SANTOS FERREIRA

Departament of Chemistry & CICECO, University of Aveiro, Portugal

was awarded with an **honorable mention for flash communication** at 12º Encontro Nacional de Cromatografia, presenting the work:

Salt pan brine water glycans: complementary characterization by GC-FID and HPAEC-PAD

AWARD: POSTER CATEGORY

MARISA HENRIQUES MARIA

Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal

was awarded with **best poster communication** at 12º Encontro Nacional de Cromatografia, presenting the work:

Effect of mobile phase composition on the separation between phosphatidylethanol (Peth) and phospholipid background using LC-MS/MS

LUIS BOTELHO DE CARVALHO

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon, Portugal

was awarded with an **honorable mention for poster presentation** at 12º Encontro Nacional de Cromatografia, presenting the work:

Universal sample handling for liquid biopsies

SAMUEL PATINHA

CICECO-Aveiro Institute of Materials & Departamento de Química, Universidade de Aveiro, Portugal

was awarded with an **honorable mention for poster presentation** at 12º Encontro Nacional de Cromatografia, presenting the work:

GC-MS in the optimization of microwave-assisted extraction of linear diterpenes from Bifurcaria bifurcate


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