10º Encontro Nacional de Cromatografia

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10th Chromatography Meeting

Título

10º Encontro de Cromatografia

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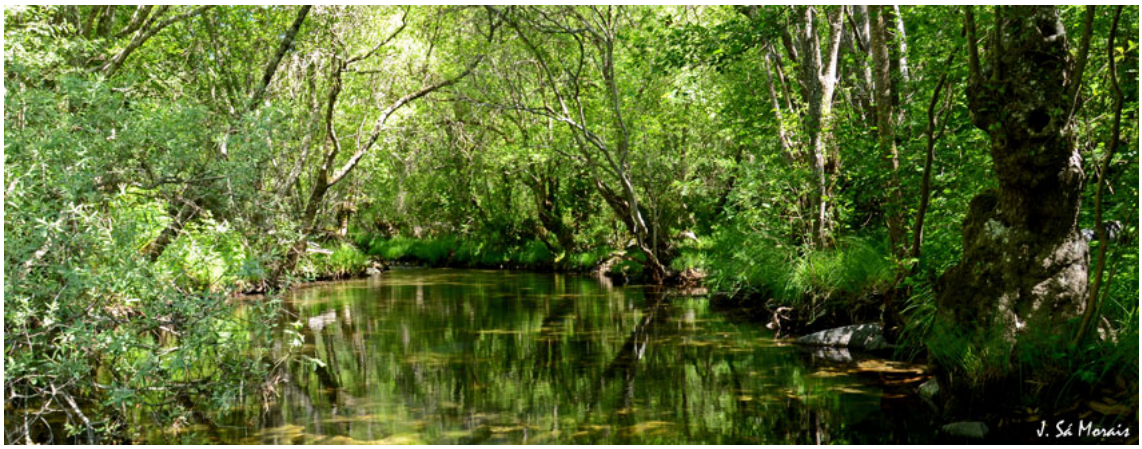

Courses / Cursos

Susana Cardoso

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Aspetos úteis da técnica de UHPLC-DAD-ESI-MSn na identificação de compostos bioativos de origem vegetal

Os extratos de origem vegetal são atualmente reconhecidos como uma excelente fonte de compostos bioativos com aplicações comerciais em diversas áreas, incluindo a alimentar, cosmética e farmacêutica. O rastreamento destes extratos é efetuado por monitorização química e biológica, realizadas em paralelo. Atualmente, a técnica hifenizada de UHPLC-DAD-ESI-MSn é uma ferramenta essencial na deteção e quantificação rápida de compostos naturais biologicamente ativos em extratos complexos, em particular por fornecer informação estrutural sem ter de recorrer ao seu isolamento. Neste curso serão abordados os aspetos fundamentais a considerar na análise de compostos de origem vegetal (com foco em carotenoides, clorofilas e compostos fenólicos), tendo em consideração as suas especificidades químicas.

Sónia Santos

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GC-MS aplicada à análise de metabolitos secundários: aspetos teóricos e práticos

Nos últimos anos a Cromatografia gasosa acoplada à espectrometria de massa (GC-MS) tem sido uma ferramenta fundamental na caracterização de metabolitos secundários, tais como compostos lipofílicos ou mesmo fenólicos. Pretende-se com este curso fornecer as ferramentas necessárias para a utilização desta técnica, orientando como selecionar instrumentos e protocolos apropriados. Irão ser abordados os princípios, aspetos práticos e teórico-práticos, dando-se especial foco aos métodos de derivatização, condições de GC e à interpretação de espectros de massa. As principais vantagens e desvantagens irão também ser discutidas.

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Program / Programa

Time		December 4
8:00-9:00		• Registration
9:00-10:00		• Opening session in *Auditorium Dionísio Gonçalves*
Moderator / Moderador - Auditorium Dionísio Gonçalves ***Isabel C.F.R. Ferreira*** *(Instituto Politécnico de Bragança)*		
10:00-11:00	PL-01	In-tube SPME from open tubular column (in-tube SPME-LC) to directly coupled to mass spectrometry ***Maria Eugênia Costa Queiroz*** *Universidade de São Paulo, Brasil*
11:00-11:30		• Coffee Break and panel session
Moderator / Moderador - Auditorium Dionísio Gonçalves ***Sílvia M. Rocha*** *(Universidade de Aveiro)*		
11:30-12:00	IC-01	Different Strategies Based on Micro(extraction) Followed by GC-MS/MS and LC-MS/MS for the Determination of Personal Care Products in Cosmetics and Environmental Samples ***Maria Llompart*** *University of Santiago de Compostela, Espanha*
12:00-12:30	EC-01	LCMS Technologies: Introducing the Orbitrap for Ultrahigh Resolution Exact Mass and Unequivocal ID ***Daniel Ettlin*** *Thermo Unicam Sistemas Analíticos*
12:30-14:30		• Lunch
Moderator / Moderador - Auditorium Dionísio Gonçalves ***Nuno Mateus*** *(Universidade do Porto)*		
14:30-15:00	IC-02	Back to Basics: Considerations in eco-user-friendly/cost-effective micro-extraction techniques ***José Nogueira*** *Universidade de Lisboa, Portugal*
15:00-16:30		***Oral session 1A / Sessão Oral 1A***
	OC-01	A multiresidue targeting approach for pesticide detection in olive oil: the role of dual-layer solid-phase extraction based on molecular imprinting technology ***Raquel Garcia***
	OC-02	New brush-type chiral stationary phases based on xanthone derivatives for liquid chromatography ***Carla Fernandes***
	OC-03	Chromatographic techniques to assess the profile of biomolecules in different mycorrhizal mushroom species ***Filipa Reis***
	OC-04	Multicolumn based liquid chromatography processes for the separation of nadolol racemates ***António Ribeiro***
	OC-05	An expanded bed chromatography approach for improving human mesenchymal stem cells purification ***Ricardo Silva***

	OC-06	Chromatographic analysis of biological samples using monolithic columns ***Marcela Segundo***

Moderator / Moderador - Auditorium B
Marco Gomes da Silva *(Universidade Nova de Lisboa)*

15:00-16:30		***Oral session 1B / Sessão Oral 1B***
	OC-07	Avaliação da qualidade do ar em espaços de um edifício de ciências ***Sara Santos***
	OC-08	Engineered polymer particles for the valorization of phenolic compounds present in mixtures obtained through supercritical extraction ***Catarina Gomes***
	OC-09	Thermostability studies of oil-soluble cyanidin-3-glucoside dyes ***Marta Guimarães***
	OC-10	Adsorption equilibrium and kinetics of CO_2, CH_4 and N_2 on zeolite BETA with different cations and SiO_2/Al_2O_3 ratio ***Adriano Henrique***
	OC-11	Seawater degradation studies of gallic acid persulfate, a promising synthetic antifouling agent ***Cátia Vilas Boas***
	OC-12	BeerOmics: how can advanced gas chromatography help to understand beer aroma properties? ***Cátia Martins***
16:30-17:00		• Coffee Break + Poster session

Moderator / Moderador - Auditorium Dionísio Gonçalves
José Manuel F. Nogueira *(Universidade de Lisboa)*

17:00-17:30	EC-02	Nexera UC Online SFE-SFC-MS System ***José Manuel Macias*** *Izasa Scientific*
17:30-18:00		***Oral session II / Sessão Oral II***
	OC-13	Phenolic composition, antioxidant and biological activities of Portuguese vine shoot from *Touriga Nacional* and *Tinta Roriz* varieties ***Manuela Moreira***
	OC-14	Caracterização química e bioatividades de *Hibiscus sabdariffa* L. ***Inès Jabeur***
19:30		• Alheira Honor / Alheira Honra

Time		December 5
Moderator / Moderador - Auditorium Dionísio Gonçalves ***Marcela Segundo*** *(Universidade do Porto)*		
9:00-10:00	PL-02	Green Foodomics: new strategies towards the discovery of functional food ingredients with biological activity ***Elena Ibanez*** *Institute of Food Science Research – CIAL, Espanha*
10:00-10:30	IC-03	Multicolumn Continuous Countercurrent Chromatography for Downstream Processing of Biopharmaceuticals ***Paulo Mota*** *University Nova de Lisboa, Portugal*
10:30-11:00		• Coffee Break + Poster session
Moderator / Moderador - Auditorium Dionísio Gonçalves ***M. Beatriz Oliveira*** *(Universidade do Porto)*		
11:00-12:30		***Oral session IIIA / Sessão Oral IIIA***
	OC-15	Selective capillary coatings for in-tube SPME off-line or on-line with LC-MS/MS for bioanalysis ***Israel Souza***
	OC-16	Preparative separation of nadolol racemates by fixed-bed liquid chromatography using C18 columns ***Rami Arafah***
	OC-17	Hollow fiber microextraction (HFµE) - A new hybrid microextraction technique for trace analysis ***Alessandra Ide***
	OC-18	Tape Adsorptive Microextraction - A new analytical approach for sample enrichment ***Nuno Neng***
	OC-19	Multidimensional chromatographic techniques applied to chemical ecology ***Eduardo Mateus***
	OC-20	Síntese de fase estacionária monolítica e posterior imobilização térmica de polidimetilsiloxano (PDMS) sobre a superfície porosa para aplicação em cromatografia líquida capilar ***Carla Bottoli***
Moderator / Moderador - Auditorium B ***Helena Soares Costa*** *(Instituto Nacional de Saúde Dr. Ricardo Jorge)*		
11:00-12:30		***Oral session IIIB / Sessão Oral IIIB***
	OC-21	Permeation of caffeine, CQA and HMF from Coffee silverskin extracts on EpiSkin™ 3D model ***Diana Pinto***
	OC-22	NTME/GC-qMS: a powerful strategy for selection sets of cancer-specific VOMs with potential for cancer differentiation ***Priscilla Figueira***
	OC-23	Validation of a dSPE-HPLC methodology for the determination of biogenic amines in wines ***Juliana Ferreira***
	OC-24	Looking for new contributions in asthma biomarkers - a chromatographic-based approach ***José Câmara***

	OC-25	Gas-chromatography mass spectrometry analysis of ^{13}C-labeled fatty acids revealed new information about the ruminal biohydrogenation of linolenic acid ***Susana Alves***
	OC-26	Multidetection of antibiotics in edible tissues: evolution of analytical strategies ***Andreia Freitas***
Moderator / Moderador - Auditorium Dionísio Gonçalves ***M. Beatriz Oliveira*** *(Universidade do Porto)*		
12:30-13:00	EC-03	A new, fast, simple, and ultra-sensitive determination of semi-volatile organic compounds in water samples by GC-MS/MS Triple Quadrupole system ***Miguel Ángel Pérez*** *Bruker Applications Development Laboratory*
13:00-14:30		• Lunch
Moderator / Moderador - Auditorium Dionísio Gonçalves ***Victor Freitas*** *(Universidade do Porto)*		
14:30-15:00	IC-04	Polyphenols identification. Has LC-MS killed HPLC-DAD ***Celestino Santos-Buelga*** *University of Salamanca, Espanha*
15:00-16:30		***Oral session IVA / Sessão Oral IVA***
	OC-27	Chromatographic analysis of nutritional and bioactive compounds in vegetative parts of *Fragaria vesca* L. obtained by in vitro culture ***Maria Inês Dias***
	OC-28	Liquid by-products from canned fish industry as sources of omega-3 polyunsaturated fatty acids ***Ana Carvalho***
	OC-29	Fingerprinting of volatomic profile of lemon (*Citrus limonum*) based on a new analytical approach - NTME/GC-MS analysis ***José Figueira***
	OC-30	Perfil fenólico e bioatividades de maçã portuguesa da variedade "Bravo de Esmolfe" ***Tânia Pires***
	OC-31	The volatile profile for discrimination of lavender and heather honey, using solid phase microextraction and gas chromatography-mass spectrometry ***Soraia Falcão***
	OC-32	*Geranium robertianum* L. phenolic compounds: individual characterization of stems and leaves profile ***Marcelo Catarino***
Moderator / Moderador - Auditorium B ***Cristina Delerue Matos*** *(Instituto Politécnico do Porto)*		
15:00-16:30		***Oral session IVB / Sessão Oral IVB***
	OC-33	Ácido 4-hidrazinobenzoico como agente derivatizante para a determinação de aldeídos por HPLC-UV e LC-MS ***Pedro Brandão***
	OC-34	Effects of natural colourants on the fatty acids profile of different ice cream formulations ***Custódio Roriz***
	OC-35	High Throughput Bar Adsorptive Microextraction (HT-BAμE): A novel cost-effective tool for monitoring psychotropic drugs in biological matrices ***Samir Ahmad***

	OC-36	Caracterização do perfil fenólico de agrião por HPLC-DAD-ESI/MS e otimização da extração por alta pressão hidrostática utilizando a metodologia de superfície de resposta ***José Pinela***
	OC-37	Polyols based solvents for the extraction of phenolic compounds from *Juglans regia* L. leaves ***Vanessa Vieira***
	OC-38	Oncolytic virus purification using multi-column chromatography ***João Mendes***
16:30-17:00		• Coffee Break + Poster session
Moderator / Moderador - Auditorium Dionísio Gonçalves ***Manuela Pintado*** *(Universidade Católica)*		
17:00-17:30	EC-04	Aplicações de cromatografia iónica e abordagem às técnicas de preparação em linha de amostras ***Susana M. M. Pereira*** *MT Brandão Lda*
17:30-18:00		***Oral session V / Sessão Oral V***
	OC-39	Effects of e-beam irradiation on bioactive content of cherry tomatoes ***Joana Madureira***
	OC-40	Otimização da extração de antocianinas de cereja madura através da metodologia de superfície de resposta ***Carla Pereira***
18:00-19:00		Reunião Grupo de Cromatografia SPQ /Meeting

20:00	Meeting Dinner / Jantar do Encontro

Time		December 6
Moderator / Moderador - Auditorium Dionísio Gonçalves ***Manuel António Coimbra*** *(Universidade de Aveiro)*		
9:00-10:00	PL-03	Comprehensive two-dimensional liquid chromatography in food and natural products analysis ***Paola Dugo*** *Università di Messina, Itália*
10:00-10:30	IC-05	Separation and concentration of nutraceuticals, active compounds and essential oils from Agro-Food sources using supercritical carbon dioxide ***Juan Francisco Rodríguez*** *TQUIMA, Espanha*
10:30-11:00		• Coffee Break + Poster session
Moderator / Moderador - Auditorium Dionísio Gonçalves ***Alírio Rodrigues*** *(LSRE/LCM, Faculdade de Engenharia, Universidade do Porto)*		
11:00-12:00		***Oral session VIA / Sessão Oral VIA***
	OC-41	Efeito da radiação gama e feixe de eletrões na concentração de ergosterol em *Agaricus bisporus* (J.E. Lange) Imbach ***Ângela Fernandes***
	OC-42	Optimization of the extraction of triterpenes from *Ganoderma lucidum* ***Miguel Angel Prieto***
	OC-43	Unveiling the chemical composition of willow added-value lipophilic extractives by gas chromatography-mass spectrometry ***Patrícia Ramos***
	OC-44	Application of anti-hail net in apple orchards: effects on fruits chemical characteristics ***Carlos Gomes***
Moderator / Moderador - Auditorium B ***José Câmara*** *(Universidade da Madeira)*		
11:00-12:00		***Oral session VIB / Sessão Oral VIB***
	OC-45	Characterization of the volatile composition of encapsuled coffee ***Davide Mendes***
	OC-46	Increased productivity in impurity profile characterization of innovative pharmaceuticals ***João Pereira***
	OC-47	Characterization of phospholipids, including plasmalogens, in bivalves of the Portuguese coast using solid-phase extraction followed by gas-liquid chromatography ***Rui Bessa***
	OC-48	Characterization and Identification of Four Essential Oils by GC-MS ***Ana Marques***
Moderator / Moderador - Auditorium Dionísio Gonçalves ***Luís Pais*** *(Instituto Politécnico de Bragança)*		
12:00-12:30	EC-05	Successful generic approaches for heartcutting 2DLC with focus on user friendliness ***Isabelle François*** *Waters*
12:30-13:00		Closing Session / Sessão de Encerramento

PL	Plenary communication / Comunicação plenária
IC	Invited oral communication / Comunicação oral convidada
EC	Enterprise oral communication / Comunicação oral de empresa
OC	Oral communication / Comunicação oral

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IC - Invited oral communications / Comunicações orais convidadas

IC-01

Different Strategies Based on Micro(extraction) Followed by GC-MS/MS and LC-MS/MS for the Determination of Personal Care Products in Cosmetics and Environmental Samples

M. Llompart
University of Santiago de Compostela (Espanha)

Maria Llompart is an Associate Professor in the Department of Analytical Chemistry, Nutrition and Food Sciences of the University of Santiago de Compostela, Spain. She is an expert analytical chemist in the field of sample preparation (green chemistry) and chromatographic analysis. The primary focus of her research is the design and development of extraction procedures based on advanced techniques such as PFE, MAE, MSPD, SPME, USAEME, for the determination of persistent and emerging pollutants in complex matrices, including environmental, agricultural, food, and more recently, personal care products. Several alternative techniques developed in her lab include the ultrasonic assisted emulsification microextraction for liquid samples, the micro-MSPD for cosmetics, or the development of photo-SPME to study the photochemical behavior of environmental pollutants. She authored more than 130 scientific publications, and supervised 11 PhD theses. She has been the chair of several international conferences such as the 19th International Symposium on Advances in Extraction Technologies (ExTech2017, 27-30 June 2017) and the 9th European Conference on Pesticides and Related Organic Micropollutants in the Environment & 15th Symposium on Chemistry and Fate of Modern Pesticides (4-7 October 2016).

IC-02

Back to Basics: Considerations in eco-user-friendly/cost-effective microextraction techniques

José Nogueira
Faculdade de Ciências da Universidade de Lisboa (Lisboa, Portugal)

Dr. J.M.F. Nogueira (b. 1965) is Associate Professor with habilitation 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 Centre of Chemistry and Biochemistry (UL) and head of the Separation Science & Technology group. His main research activity focuses on the development and application of innovative analytical methodologies involving chromatographic, electromigration and hyphenated techniques. In this context, the development of novel analytical approaches, with emphasis to the modern sorption-based microextraction techniques, point out the main research activities in areas such as environment, water, food, phytochemistry, natural products, etc. He is author and co-author of dozens of peer-reviewed articles (h-index: 32) 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. Now, he is the Portuguese representative of the European Society for Separation Science.

IC-03

Multicolumn Continuous Countercurrent Chromatography for Downstream Processing of Biopharmaceuticals

José P. B. Mota
University Nova de Lisboa (Lisboa, Portugal)

Jose P. Mota is full professor of chemical and biochemical engineering at the department of chemistry of University NOVA de Lisboa (Portugal) and researcher at the Laboratory for Green Chemistry and Clean Processes - LAQV@REQUIMTE. He has authored over one hundred papers in the areas of separation science and transport phenomena. He has received 8 international awards and is a former member of the Scientific Council of Sciences and Engineering (CCCE) of the Portuguese National Science Foundation (FCT/MCTES) and Board of Directors of the International Adsorption Society (IAS). He is a member of the scientific committees of the Fundamentals of Adsorption series of conferences and international Symposiums on Preparative and Industrial Chromatography and Allied Techniques. He serves as an associate editor of Adsorption Science & Technology Journal and consulting editor of the Journal of Pharmaceutical Analysis.

IC-04

Polyphenols identification. Has LC-MS killed HPLC-DAD

Celestino Santos-Buelga
University of Salamanca (Espanha)

Research interests related with phenolic compounds in plants and foods, and particularly flavonoids, their analysis, synthesis and structural characterisation, influence on the quality of foods and beverages, and health implications. Visiting researcher at the Institut de Produits de la Vigne (Narbonne, France) and Lehrstuhl für Obstbau - Technische Universität München (Freising-Weihenstephan, Germany). Responsible in 24 competitive projects funded European and national entities. Supervision of 18 PhD Theses and 25 Master or DEA Theses. Co-editor in 3 reference books, author of 10 chapters in international books, over 220 articles in peer-reviewed journals (JCR) and more than 50 full articles in non-refereed publications. Associate Editor of the journal Phytochemical Analysis (John Wiley & Sons Ltd). "María de Maeztu" Award to the Scientific Excellence granted by the University of Salamanca in November 2011.

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IC-05

Separation and concentration of nutraceuticals, active compounds and essential oils from Agro-Food sources using supercritical carbon dioxide

Juan F. Rodriguez
Institute of Chemical and Environmental Technology, UCLM (Espanha)

Dr. Juan Francisco Rodríguez is Full Professor of Chemical Engineering and the Head of the Institute of Chemical and Environmental Technology (ITQUIMA) from 2007. With about 40 researchers, ITQUIMA is one of the reference research Institutes in the University of Castilla-La Mancha in funding, number of projects, scientific publications and technology transference. More than 170 scientific publications and 180 participations in international congress validate the quality of his applied research work. Participant in 3 FP7 projects and NANOLEAP in H2020, he has been the main responsible of more than 20 research projects funded by National and Regional institutions. From lab to pilot plant scale, he has managed more than 100 research projects funded by private companies. He is also the author of seven patents (national and EP). The development of extraction process using scCO2 from many different natural substrates, like olive tree leaves, citrus lantaniferus, paprika or brewery residues to obtain concentrated essential oils and nutraceticals have been between the main research activities of his research group. The green synthesis of biomaterials in supercritical carbon dioxide are also between his research lines. He has received several national and international research awards and one regional distinction for the creation of the best "spin-off" in the region of La Mancha. Several large pilot plant facilities including one for spray drying preparation of particulate materials are now in operation in ITQUIMA under his supervision.

10

EC - Enterprise oral communications / Comunicações orais de empresas

OC - Oral communications / Comunicações orais

PC - Panel communications / Comunicações em painel

PL
Plenary communications
Comunicações plenárias

PL-01

In-tube SPME from open tubular column (in-tube SPME-LC) to directly coupled to mass spectrometry (in-tube SPME-MS/MS)

Maria Eugênia Costa Queiroz

Departamento de Química da FFCLRP / Universidade de São Paulo, Brasil

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Recent trends in sample preparation have focused on miniaturizing the analytical systems, which should simplify automation, provide high-throughput performance, and enable online coupling with analytical instruments. Microextraction or on-line analytical systems require small biological samples, and extremely low or no amounts of organic solvent. Minimizing biological sample preparation steps reduces not only the sources of error but also the analysis time and cost. In this lecture, we are going to discuss about the new developments and future trends on IN-TUBE SPME from open tubular column (in-tube SPME-LC) to directly coupled to mass spectrometry (in-tube SPME-MS/MS).

PL-02

Green Foodomics: new strategies towards the discovery of functional food ingredients with biological activity

Elena Ibáñez*, A. Del Pilar Sánchez-Camargo, M. Herrero, A.Cifuentes

Foodomics Laboratory, Department of Bioactivity and Food Analysis, Institute of Food Science Research (CIAL-CSIC), Nicolás Cabrera, 9, 28049 Madrid, Spain

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Since its first definition in 2009 [1], interest in Foodomics has greatly increased. Although Foodomics covers different fields of research included in food science approached using advanced -omics technologies and associated to sustainability, in this presentation we will focused on those aspects directly related to green analytical chemistry (GAC) and novel functional food ingredients' development.

At present there is an enormous interest in providing new answers to one of the main societal challenges: sustainability. Many aspects can be considered in this framework, ranging from the rational use of resources to the modern concept of biorefinery involving biomass conversion processes and equipment to produce fuel, power, and added-value chemicals from organic material. Considering this framework, the extraction of high added-value products from microalgae and food by-products is of high interest since it can allow consolidating the idea of sustainable processes.

Nevertheless, for the development of these sustainable processes the 12 principles of Green Chemistry have to be closely examined, considering that effectively provide a framework for designing and/or improving materials, products, processes and systems from an environment protection perspective. New challenges researchers are facing are the development of fast, selective, efficient, sustainable, green (without using toxic organic solvents) processes, providing also with high yields and at lower costs. Processes able to meet these requirements are, among others, those based on the use of compressed fluids such as supercritical fluid extraction (SFE), gas-expanded liquids extraction (GXLs), pressurized liquid extraction (PLE) and subcritical water extraction (SWE).

In this presentation, new trends in the production of functional food ingredients (through the use of alternative solvents and design of biorefinery processes) and in the development of alternative tools to improve green sample preparation will be presented. Examples that will be discussed deal with the isolation and fractionation, through the use of integrated processes, of all valuable components of algae (mainly lipids and carotenoids but also sugars and proteins). Moreover, the use of Hansen Solubility Parameters (HSP) will be presented as an easy an affordable alternative for green solvent selection.

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PL-03

Comprehensive two-dimensional liquid chromatography in food and natural products analysis

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Chemistry of food and natural products is continuously involved in the assessment of quality and authenticity, with a special focus on the characterization of molecules with a possible beneficial effect (nutraceuticals) or a toxic effect on human health. In this context analytical methods should be capable to allow the determination of the main components of food and natural products samples, but can also be selective and sensitive enough to determine minor components.

Comprehensive two-dimensional liquid chromatography (LC×LC) has emerged in the last two decades as an interesting alternative to analyze complex samples. The LC×LC technique involves the combination of two or more independent or nearly independent separation steps, increasing significantly the separation power of the corresponding one-dimensional liquid chromatography (1D-LC) techniques.

In our research group, since over a decade, we developed several LC×LC methodologies using different column sets e.g. NP×RP, RP×RP and HILIC×RP and different instrumental set-ups, including the use of photodiode array, light scattering and mass spectrometry detectors. Besides the use of different column technologies, a dedicated software for data processing was employed for handling specific case studies.

In this contribution, selected applications of LC×LC on food and natural products will be presented. A combined approach for the characterization and authenticity assessment of pistachio nuts will be also discussed.

IC
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IC-01

Different strategies based on (micro)extraction followed by GC-MS/MS and LC-MS/MS for the determination of personal care products in cosmetics and environmental samples

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The use of cosmetics has increased all over the world, especially in developed countries, constituting main consumer products of daily use, with an average consumption rate of eight products per person. In this lecture, new extraction strategies based on (micro)extraction procedures in combination with GC and LC MS/MS (QQQ) analysis are proposed for the determination of cosmetic additives and ingredients commonly used in PCPs, such as preservatives fragrances or UV-filters. All these families of compounds are included in de EU Cosmetic Directive and subjected to usage restrictions. The proposed methods are based on pressurized liquid extraction, micro matrix solid phase dispersion, and solid phase microextraction. In all cases, the purpose is the development of microextraction procedures involving low sample and solvent consumption, low residue generation, and rapid, simple, and low cost approaches regarding the sample preparation step.

On the other hand, many cosmetic ingredients (fragrances, UV filters, preservatives, and others) are considered emerging pollutants, due to the high production and massive entrance in the environment, degradation difficulty and persistence, bioaccumulation, and demonstrated toxic effects in organisms (e.g. endocrine disruption) in some cases. Therefore, these chemicals must be monitored in the environment, and some of them have been included in monitoring programs (e.g. “Watch List” Directive 2013/39/EU). In this way, several analytical environmental approaches based on microextraction techniques will be proposed.

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IC-02

Back to basics: Considerations in eco-user-friendly/cost-effective microextraction techniques

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Over the past thirty years, the microextraction techniques had play an important role as modern enrichment approaches for trace analysis that follow the green analytical chemistry principles [1]. Unlike the active microextraction approaches (e.g. solid phase extraction), the main advantages of the passive microextraction techniques include the use of miniaturized devices, simplification, easy manipulation, strong reduction or absence of the use of toxic organic solvents, selectivity and sensitivity enhancement, as well as low sample volume, making them convenient for interfacing with chromatographic and hyphenated systems [2]. Some well-established examples are the passive liquid-based microextraction techniques, such as dispersive liquid-liquid microextraction (DLLME), single-drop microextraction (SDME) and hollow fibber liquid-phase microextraction (HF-LPME). On the other hand, passive solid-based microextraction or sorption-based techniques have also been proposed as effective alternatives for trace analysis, like solid phase microextraction (SPME), stir bar sorptive extraction (SBSE) and, more recently, bar adsorptive microextraction (BAμE) [3]. Despite all of this, one cannot simply use a single technique as a universal approach, but the most suitable technique should be selected according to the target analytes and matrix involved. Furthermore, some of these techniques are neither user-friendly, eco-friendly or cost-effective nor suitable for the routine work. In general, the liquid-based microextraction approaches (i.e. DLLME, SDME and HF-LPME) present fast kinetics, use very simple apparatus and are cost-effective. On the other hand, the solid-based microextraction techniques (i.e. SPME, SBSE and BAμE) are easier to manipulate, more environmental-friendly, allow automation although need a back-extraction stage, which is not attractive since requires time-consuming steps particularly if liquid desorption (LD) is implemented. Furthermore, this drawback is more pronounced if reusable devices are adopted, making the LD the limitative stage. For all these reasons, novel ideas and concepts are welcome, especially if using simple analytical strategies.
In this contribution, the main advantages and limitations of the most used microextration techniques will be discussed, as well as proposing basic concepts using eco-user-friendly and cost-effective approaches that simultaneously could be dedicated for routine analysis.

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IC-03

Multicolumn Continuous Countercurrent Chromatography for Downstream Processing of Biopharmaceuticals

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Continuous processing is well established in many industries. Presently there is also an increased interest in continuous processing for biopharmaceutical manufacturing. Process intensification by implementing continuous or semi-continuous downstream processes can contribute to significant cost-savings and improved throughput. Liquid chromatography is currently the core technique for purification of biopharmaceuticals, and its use is often integrated vertically within the downstream processing (DSP) strategy, as it easily fits into the early capture stage as well as into the final purification phase. Single-column batch chromatography, which because of its simplicity is routinely used by industry, isolates the pure part of the product peak one is interested in at the expense of yield because the impure side fractions, containing valuable product, must be discarded. Compared with a traditional batch setup, the capacity of the chromatography medium can be utilized to a greater extent in a multi-column simulated countercurrent chromatography setup, with reduced stationary phase requirements and buffer consumption, as well as shorter processing times, and potential cost savings as results. Multicolumn continuous chromatography, whose most efficient implementation is based on the simulated moving-bed (SMB) concept, captures the side fractions by internal recycling until the entire product has been extracted while new feed is continuously or cyclically injected. This not only gives significantly higher yields of purer product, but also enables to process more feed and thereby increase overall throughput.

In the first part of this talk we review the new SMB-based technologies that have emerged as alternatives to the traditional batch chromatography process in DSP of biopharmaceuticals, highlighting their advantages and summarizing recent applications. We then present a newly developed chromatographic platform based on a novel single-column device that mimics the operation of multicolumn chromatography through ingenious management and recycling of mixed fractions. The newly developed platform shares the benefits of SMB chromatography in that it not only gives significantly higher yields of purer product, but also enables processing more feed and thereby increasing the overall throughput. However, the proposed process uses a single chromatographic column.

Our process is based on the realization that the periodic state of an SMB process can be mimicked by a single-column chromatographic process with a recycle lag of $(N - 1)\tau$ time units, where N is the number of columns of the equivalent SMB unit and τ is the switching interval (time interval between consecutive switches of the inlet and outlet ports). The recycle lag is implemented in practice by means of a special type of plug-flow tube that includes a moving piston to compensate for the difference between inlet and outlet flow rates. The proper operation of the inlets and outlets of such device implements an approximate “first in, first out” method of organizing and manipulating the fractions of fluid collected from the chromatography column, where the oldest (first) amount fluid, or ‘head’ of the fraction, is the first to exit the plug-flow tube.

It is shown that the single-column chromatograph can mimic the operation and performance of recent multicolumn capture and polish processes designed for the efficient separation and purification of monoclonal antibodies, biosimilars, and viral vectors. Moreover, the single-column chromatograph can be easily integrated into the existing downstream processing platforms of complex biopharmaceuticals.

IC-04

Polyphenols identification. Has LC-MS killed HPLC-DAD?

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Over the last two decades LC-MS has turned into the technique of choice for the identification of polyphenols in plants and food. Indeed, LC-MS is a powerful technique that provides sensible structural information, especially when triple quadrupole or ion-trap instruments, capable of performing MS/MS or MSn analyses, and/or high-resolution, high mass accuracy analyzers are used. However, the unequivocal identification of some compounds is not always possible from mass spectral data. For instance, isomeric structures and sometimes compounds with the same nominal mass cannot be differentiated, and no information can be obtained about the nature of sugar and/or acyl moieties linked to phenolic aglycones.
Despite most LC-MS devices are equipped with diode array detectors (DAD), in recent years, publications where peak identification is only based on MS data are becoming more and more common. It is not infrequent to find papers offering incompletely characterized structures, when not wrong structural assignments, which in many cases could be avoided drawing on the information contributed by the absorption spectra and paying attention to the simple elution behaviour.
In this keynote, the need of taking advantage of all available resources will be highlighted for accurate compound characterization. Some examples will be presented on how HPLC-DAD can assist and complement MS, especially when the information obtained from MS data is insufficient for complete peak identification.

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IC-05

Separation and concentration of nutraceuticals, active compounds and essential oils from agro-food sources using supercritical carbon dioxide

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The residual streams generated by de vegetal oil industry of La Mancha Region can be a valuable source of bioactive compounds. Some of these bioactives have shown a large variety of biological activities such as antioxidant or antimicrobial properties. Between them tocopherols are valuable compounds because of their activity as vitamin E and capacity as anti-oxidant agent [1].

Viticulture and the wine industry produces also large amounts of by-products. The extract of these by-products contain several compound families of compounds among them polyphenols. Grapes anthocyanins possess strong biological functions such as anti-inflammatory and antioxidant activities. Resveratrol with antioxidant, fungicidal and bactericidal properties is other of the most appreciated active ingredients of viticulture byproducts [3].

The flowers and leaves of the aromatic herbs from La Mancha hills possess a variety of bioactive agents: polyphenolics, including carnosic acid, carnosol, rosemarinic acid, ursolic acid, etc. Among these, the extracts and essential components of rock rose leaves (Cistus ladanifer L.) are especially appreciated as source of those bioactive agents.

Using $scCO_2$ the group of Separation and Polymerization Technology has performed separation and purification of many natural products like Vitamin E from olive tree wastes, rum flavors from sugar cane and rum derivatives and Capssaicionids form paprika, as well as essential oils from natural plants of the region.

Although the direct extraction using $scCO_2$ of these natural compounds can imply some kind of separations between their constitutive components, Supercritical Fluid Chromatography (SFC) arises as a valuable tool to separate at preparative scale the individual compounds of the supercritical extracts. The use of preparative SFC for the isolation of small-medium quantities of active compounds of high added value from the mentioned natural sources looks very attractive for a region with high agriculture potential as La Mancha.

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EC
Enterprise oral communications
Comunicações orais de empresas

EC-01

LCMS Technologies: Introducing the Orbitrap for Ultrahigh Resolution Exact Mass and Unequivocal ID.

High Resolution towards unequivocal Identification: The Orbitrap Analyzer

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The number of users and areas of specialization in mass spectrometry has increased enormously in recent years. In the world of small molecules and Proteins, the new analytical procedures require sensitivity, selectivity and robustness within a short analysis time. Several different types of mass spectrometers are available, each one with their specific strengths and drawbacks. The decision on which one to use is usually based on the quality of the data combined to cost/time of the analysis.

Different types of “traditional” Mass Analyzers, like Triple Quads, Ion Trap, and Linear Traps will be presented. We will discuss the instrumental arrangements, its advantages, features and disadvantages in Quantitation and qualifying the molecules.

Recent publications have shown that high resolution mass spectrometry is a well-accepted and better alternative to the analysis molecules. There is a high need to add the possibility to perform data dependent MS2 experiments by making use of a resolving power of up to 140,000 FWHM, or even 500,000 FWHM. On that way, the mass analyzer Orbitrap represents one of the best alternatives currently available in the market [1]. This mass analyzer was first described in 2000 and has now reached the status of a mainstream mass spectrometry technique as it can support a wide range of applications from routine compound identification to the analysis of trace-level components in complex matrices

This presentation will discuss the application and use of the different technologies with some examples that will show the features of the different technologies.

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EC-02

Nexera UC Online SFE-SFC-MS System

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The Nexera UC system is capable of user-friendly simultaneous multicomponent analysis, with online automation of everything from sample pretreatment to separation and analysis. It will be of practical use in fields where easier, faster and more reliable automatic analysis of multiple samples is required, such as in the examination of residual pesticides in foods, and searches for disease biomarkers.
This system uses supercritical carbon dioxide as the mobile phase. Up to 48 samples can be placed simultaneously, and everything can be performed automatically, from pretreatment by an automatic extraction unit to separation by a chromatograph and detection by a mass spectrometer. As a result, complicated pretreatment operations are unnecessary. At the same time, the system is capable of stable analysis of delicate components for which reliable measurements are difficult because they oxidize or decompose on contact with air. Moreover, taking the analysis of residual pesticides in foods as an example, a single pretreatment that would take 35 minutes can be shortened to 5 minutes by this system. In comparison to conventional hands-on methods, it can improves the yield and reduce human error, so residual pesticides analysis can be achieved in substantially less time.

This system has been developed based on the achievements by the cooperative research project among Shimadzu Corporation, Osaka University, Kobe University and Miyazaki Agricultural Research Institute, under the JST (Japan Science and Technology Agency) research results development program called "Development of Systems and Technology for Advanced Measurement and Analysis”

1. The "UC" in Nexera UC stands for Unified Chromatography.
Unified Chromatography is a new separation technique where unify the sample preparation and various separation modes as UHPLC and SFC using supercritical fluid.

2. SFE: Supercritical Fluid Extraction.
Some extraction method could be transferred to uses supercritical fluid. It can be available as pretreatment method in solid sample analysis.

3. SFC: Supercritical Fluid Chromatography.
It is the chromatography using supercritical fluids as mobile phases. Thanks to this unique properties, it realize high speed and high separation analysis.

EC-03

A new, fast, simple, and ultra-sensitive determination of semi-volatile organic compounds in water samples by GC-MS/MS Triple Quadrupole system

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The proposed methodology has been developed for the fast and ultra-sensitive analysis of semi-volatile organic compounds (SVOCs) in accordance with current European regulation for the analysis of organic pollutants in water. The new developed method for sample preparation is an innovative, fast and miniaturized based on the principles of Dispersive Liquid-Liquid Micro Extraction. Quantitation limits below ppt levels have been reached for most of the compounds analyzed by GC-MS/MS TQ. The methodology has been validated, reporting detection limits, linearity, reproducibility, ruggedness, and recoveries values for different kinds of water: tap, river and sea. The methodology has been implemented successfully in different routine laboratories working under accreditation quality parameters.

The study was done with 59 different class of compounds, including all the priority substances reported in the European regulation for environmental (2013/39/EU) and water intended for human consumption (2015/1787/EU). The compounds under study included several types of pesticides, Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs). One internal standard was used to correct signal fluctuation with a matrix-matched calibration.

The calibration curves were done in the different matrices the compounds of interest showed correlation coefficients (r^2) higher than 0.994 with and relative standard deviation of the response factors (RSDRF) lower than 30%. The linear dynamic range studied started with 0.1 up to 500 pg mL^{-1}. For most compounds, lower limit of quantitation achieved was between 0.1 – 0.5 pg mL^{-1}.

Accuracy and recoveries values for spiked samples of real tap, river and sea water were calculated as average value for 3 different replicates. All the values achieved were in the range of 70 and 125%.

For precision values, each kind of sample was spiked at a low and a high concentration level, and extracted by four different operators over four consecutive days (inter-day precision). The RSD values for all replicates the two concentration levels were below 30% with an average value of 15% for the low concentration level and below 18% with an average value of 7% at high concentration.

EC-04

Aplicações de cromatografia iónica e abordagem às técnicas de preparação em linha de amostras.

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A Metrohm apresenta uma gama de cromatografia iónica que se consolidou no mercado mundial pela sua inovação na determinação de aniões, catiões e compostos polares, podendo ser utilizada em variadas aplicações.

Figure 1 *- Sistema de Cromatografia Iónica com três canais de determinação*

Durante esta apresentação irão ser expostos exemplos de aplicação nas seguintes áreas:

1 - Análise de água;
2 - Indústria Química;
3 - Farmacêutica;
4 - Indústria Alimentar e Bebidas;

O foco principal de aplicações na cromatografia iónica prende-se com a deteção de condutividade, mas nesta apresentação vão ser descritos métodos em que se utiliza a deteção de UV/VIS, a deteção amperométrica e a combinação com outras técnicas de análise (Ex: ICP-MS).
Seguem-se alguns exemplos dos métodos EPA que vão ser descritos:

Tabela 1 - Métodos EPA apresentados

Método EPA	Descrição dos métodos
218.7	Crómio hexavalente em água de consumo por IC com PCR e deteção UV-VIS
300.1	Oxihaletos e aniões comuns em água da torneira
314.0	Percloratos em água de consumo por IC
321.8	Bromatos em águas de consumo por IC/ICP-MS

Além disto vão ser apresentadas técnicas de preparação de amostra em linha, como por exemplo:

A -Ultrafiltração
B - Diálise
C - Diluição
D - Calibração em Linha

EC-05

Successful generic approaches for heartcutting 2DLC with focus on user friendliness

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Laboratories in the pharmaceutical and biopharmaceutical industry are often facing usage of (U)HPLC methods which are not readily compatible to mass detection. These can be traditional USP methodologies including phosphate buffers or the usage of mobile phases containing salts or other MS incompatible reagents. The current contribution will cover the implementation of heartcutting 2DLC as a generic tool in the laboratory to overcome these difficulties providing increased MS compatibility and extended sample information. The proposed configuration provides a high success rate as well increased user friendliness due to the At-Column dilution (ACD) approach which is utilized in the interface. By introducing a trap cartridge as well as an isocratic pump and two two-position, six-port valves to the interface design, the user has access to an instrument allowing regular 1DLC analyses as well as the opportunity of running 2DLC separations without instrument changes or modifications needed to the first dimension (HPLC or UPLC, second or minute-wide fractions) while mitigating loss of compounds.
In addition, an identical setup is applicable for samples requiring increased resolution on specific peaks or fractions by selecting an orthogonal dimension in the second dimension. The setup can also be used to eliminate ion suppression for target compounds eluting in the presence of sample matrix. By the selection of a diverse separation mechanism in the second dimension, compounds can be efficiently resolved from matrix components and hence sensitivity and robustness are significantly improved.
In this presentation, the benefits of the aforementioned approach will be illustrated with practical examples in the pharmaceutical and biopharmaceutical industry.

OC
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OC-01

A multiresidue targeting approach for pesticide detection in olive oil: the role of dual-layer solid-phase extraction based on molecular imprinting technology

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Nowadays, the assessment of food safety standards is of huge relevance, particularly the control of chemical contaminants, such as pesticides. Those compounds are commonly used in modern agriculture, which can lead to food and environment problems. In particular, pesticide analysis in fatty matrices is a very challenging task. Aiming to introduce a reliable and sensitive trace analysis of pesticide residues in olive oil, a new sample preparation methodology for the selective enrichment of pesticides residues belonging to organophosphorus and triazines classes has been developed. It comprises the use of a dual layer of "tailor-made" molecularly imprinted polymers (MIPs) Solid Phase Extraction (SPE) for the simultaneous extraction of both pesticide residues in a single procedure. Thus, this work has focused on the implementation of a dual MIP-layer SPE procedure (DL-MISPE) encompassing the use of two MIP layers as specific sorbents. To achieve higher recovery rates, MIPs amounts have been optimized and the influence of MIP packaging order has been also assessed. The optimized DL-MISPE approach has been used in the preconcentration of spiked organic olive oil samples with concentrations of dimethoate (dmt) and terbuthylazine (tbz) similar to the maximum residue limits (MRLs) and further quantification by HPLC. High recovery rates for dmt (95%) and tbz (94%) have been achieved with good accuracy and precision [1]. Thus, DL-MISPE is a reliable, robust, and sensitive sample preparation methodology that enables preconcentration of the target pesticides in olive oil samples, even at levels similar to the MRLs. It constitutes the first attempt on the development of a dual pesticide residue methodology for the trace analysis of pesticide residues based on molecular imprinting technology.

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OC-02

New brush-type chiral stationary phases based on xanthone derivatives for liquid chromatography

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Analytical and preparative enantiomeric separations by liquid chromatography (LC) using chiral stationary phases (CSPs) play more than ever a crucial role in chemical industry and academic research [1]. However, in spite of a large number of different types of CSPs described [2], the development of new CSPs continues to be a field of great importance.

Chiral derivatives of xanthones (CDXs), when anchored to a chromatographic support by covalent linkage through a spacer, possess the necessary attributes to constitute chromatographic selectors, similar to brush-type CSPs [3].

Herein we report the synthesis and evaluation of five new CSPs, based on CDXs (Figure 1) [4]. These CSPs are based on a different type of small molecules from those commercially available.

The enantioresolution performance of the CSPs was evaluated by LC using several chemical classes of chiral compounds, including drugs. A library of CDXs was also evaluated in order to explore the principle of reciprocity as well as the chiral self-recognition phenomenon. The enantioseparations were investigated under multimodal elution conditions. The CSPs showed high stability, reproducibility, versatility in the selection of the mobile phase composition and, in general, they showed enantioselectivity for CDXs and other chiral compounds.

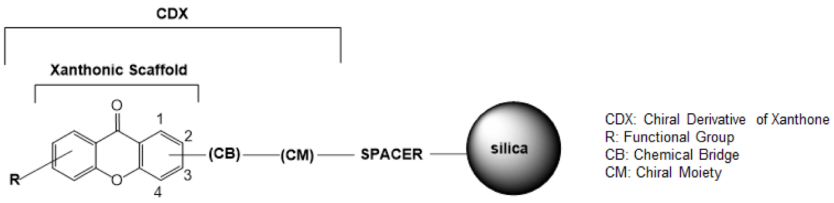

Figure 1. *Schematic representation of a stationary phase based on chiral derivatives of xanthones.*

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OC-03

Chromatographic techniques to assess the profile of biomolecules in different mycorrhizal mushroom species

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The consumption of wild mushrooms has been preferred compared to cultivated species in many countries, comprising a large number of species with excellent nutritional properties [1]. Moreover, many species have been reported as having bioactive properties, since they are rich in different biomolecules [2,3].

In the present work seven different wild mushrooms were chemically characterized by chromatographic techniques by using different detectors, in order to evaluate the presence of nutritional and/or bioactive molecules. The studied species were: *Amanita caesarea* (Scop.) Pers., *Cortinarius violaceus* (L.) Gray, *Lactarius volemus* (Fr.) Fr., *Leccinum molle* (Bon) Bon, *Leccinum vulpinum* Watling, *Suillus granulatus* (L.) Roussel and *Suillus luteus* (L.) Roussel. Some hydrophilic compounds, namely free sugars, were identified by HPLC-RI, and phenolic acids were assessed by HPLC-PDA. Regarding lipophilic compounds, fatty acids were determined by GC-FID and tocopherols by HPLC-fluorescence detection.

Mannitol and trehalose were the main free sugars detected. Gallic, protocatechuic and p-hydroxybenzoic acids were the main phenolic acids identified, as well as the related compound cinnamic acid. Mono- and polyunsaturated fatty acids were the prevailing fatty acids and generally, β-, γ- and δ-tocopherol were the vitamers of vitamin E detected in the samples. Since these species proved to be a source of biologically active compounds, the antioxidant properties were also evaluated. The antioxidant activity was measured through the reducing power, free radical's scavenging activity and lipid peroxidation inhibition of their methanolic extracts. All the species revealed antioxidant properties, being *S. granulatus* and *L. vulpinum* the most active species. Given the results obtained, other bioactivity assays are planned including the elucidation of the mechanisms of action involved.

Acknowledgements:
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OC-04

Multicolumn based liquid chromatography processes for the separation of nadolol racemates

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A few decades passed since the pharmaceutical industry realized the need to produce chiral drugs with reduced severe side-effects. To overcome this issue, the international agencies for drug safety become a real driving force, pushing more and more the industry towards the commercialization of drugs based on pure enantiomers. Traditionally, enantiomerically pure drugs are still produced in large-scale by organic asymmetric synthesis. However, it is also accepted that, in several cases, it is a too much time consuming production path. The direct resolution of racemic compounds, using multicolumn or fixed-bed liquid chromatography technologies, are nowadays, recognized at an industrial scale has a true alternative. The use of such technologies, like simulated moving bed (SMB) chromatography allows both high yields and purities of both enantiomers present in the racemic chiral compound. Also, these techniques can be applied to a wide range of racemic mixtures, since different stationary phases for enantiomer separation are now available. Nadolol is a pharmaceutical drug marketed as a mixture of four stereoisomers, used to treat cardiovascular diseases. This drug is a mixture of two pairs of racemates, therefore, its complete separation represents a challenging task. Recently, our research group reported the pseudo-binary separation of nadolol by SMB chromatography using both coated Chiralpak AD and Chiralpak IA immobilized chiral stationary phases [1,2]. In this work, it is proposed an alternative strategy, implementing a first achiral separation step, to be followed by two subsequent parallel chiral separation steps [3]. In this first achiral step, C18 columns are used to perform the separation of the two pairs of nadolol enantiomers ("racemate A" from "racemate B") under reversed-phase mode. After this preliminary achiral separation step, two parallel SMB runs must be carried out using a chiral stationary phase to achieve the complete separation of all the four nadolol stereoisomers. Extensive experimental and simulation results will be presented including solvent screening, measurement of equilibrium and kinetic data, and both fixed-bed and SMB preparative separations.

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OC-05

An expanded bed chromatography approach for improving human mesenchymal stem cells purification

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In the last decade, it has been observed an increasing interest on using human mesenchymal stem cells (hMSC) for clinical applications. Their immunomodulatory characteristics, as well as capacity in secreting bioactive molecules with anti-inflammatory and regenerative features, have been making them attractive candidates for autologous and allogeneic therapies. However, to be applied in a clinical setting hMSC need to comply with specific requirements in terms of identity, potency and purity.

The reported work aims at improving the established tangential flow filtration (TFF)-based washing strategies, using negative mode expanded bed adsorption (EBA) chromatography with a new multimodal prototype matrix based on core-shell bead technology. The proposed approach enabled an efficient protein clearance (>70%) with high cell recovery yields (78%), compatible with stem cell manufacturing.

Moreover, we also show that EBA chromatography can be efficiently integrated on the already established downstream processing train for hMSC, where it improved the washing efficiency more than 10-fold, recovering approximately 70% of cells after total processing. This strategy did not impact cell viability (> 95%), neither hMSC's characteristics in terms of morphology, immunophenotype, proliferation and adhesion capacity and multipotent differentiation potential.

Overall, negative mode chromatography represents the beginning of a promising platform for cell therapy applications, where new adsorbents can be designed to have affinity with target impurities (e.g. BSA) and not to the final product itself, the cells. This means that the methodologies herein developed can be adopted to other type of cell products relevant for cell therapy applications.

OC-06

Chromatographic analysis of biological samples using monolithic columns

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Analysis of biological samples poses complex challenges in chromatography. Starting with the low levels in which most analytes are present, requiring sample preconcentration, the presence of interfering species also precludes direct sample introduction in chromatographic analysis. Most of the times, extensive sample treatment is required in order to remove molecules that can cause clogging in particle packed columns, which demands precipitation and centrifugation of samples prior to analysis. In this context, monolithic columns present a sound alternative to particulate columns regarding chromatographic analysis of small molecules present in complex samples, namely those rich in macromolecules from biological systems. Monolithic columns are porous rod structures characterized by mesopores and macropores that provide monoliths with high permeability, a large number of channels, and a high surface area available for molecular interaction. In opposition to particle packed columns, low back pressures are attained, even at higher flow rates (> 3 mL min-1).

The aim of this communication is to show the relevance of monolithic columns in bioanalysis. Several examples will be examined, regarding the quantification of nanoencapsulated compounds in permeation studies [1, 2] and the evaluation of pharmacokinetics and drug distribution in mice using nanodelivery strategies [3]. For these case studies, minimal sample treatment was implemented, requiring extraction with acetonitrile and resuspension in mobile phase [3], or only dilution with acetonitrile for nanoparticle disassembly [2]. Finally, the potential of monoliths for in-line low pressure chromatography was exploited for direct measurement of caffeine loaded into lipid nanoparticles, requiring no sample treatment before analysis as possible interfering species were separated from caffeine using a short C18 monolith. Due to the real time automated sampling and direct chromatographic analysis, transdermal permeation profiles of nanoformulations were established within a time frame not observed by conventional techniques [1].

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OC-07

Avaliação da qualidade do ar em espaços de um edifício de ciências

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Durante as últimas duas décadas tem havido crescente preocupação na comunidade científica sobre os efeitos da qualidade do ar interior (QAI) na saúde, pelo facto de, nas sociedades atuais, as pessoas passarem a maior parte do tempo em ambientes interiores, nomeadamente, nas suas casas, nos locais de trabalho, em zonas comerciais, estabelecimentos de ensino e de lazer, etc.
O presente trabalho teve como principal objetivo, a verificação da QAI no 5º piso do edifício C8, da Faculdade de Ciências da Universidade de Lisboa, particularmente em dois laboratórios de química com diferentes atividades experimentais e ainda no corredor do mesmo piso, junto às escadas e ao elevador.
De entre os parâmetros presentes na lei para a verificação da qualidade do ar no interior dos edifícios, a presente contribuição visou a matéria particulada (PM), mais especificamente as $PM_{10-2.5}$ e as $PM_{2.5}$ tanto no que se refere à sua composição inorgânica como à presença de compostos orgânicos voláteis - VOCs. Para além da análise gravimétrica foram ainda utilizadas a cromatografia iónica (IC) para determinação dos iões presentes nas amostras e a cromatografia em fase gasosa acoplada a espetrometria de massa (GC-MS) para a identificação dos VOCs.
Os resultados mostraram que as concentrações das partículas $PM_{10-2.5}$ e $PM_{2.5}$, não ultrapassaram os valores legalmente estabelecidos. Na análise qualitativa dos VOCs, verificou-se a presença acentuada de alcanos e alcenos, e ainda de outros compostos orgânicos, em todas as amostras estudadas.
A análise da PM é bastante importante pois estima-se que aproximadamente 3 % das mortes por doença cardiopulmonar e 5% das mortes por cancro de pulmão são atribuíveis globalmente a este parâmetro.

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OC-08

Engineered polymer particles for the valorization of phenolic compounds present in mixtures obtained through supercritical extraction

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Molecularly imprinted polymer (MIP) particles are here developed to target phenolic compounds present in mixtures resulting from supercritical extraction processes. Engineered MIP particles are synthetized considering different polymerization processes to tailor products morphology (e.g. precipitation or inverse-suspension polymerization to obtain micro-particles [1]) and also diverse functional monomers to explore preferential interactions with the template polyphenols (e.g. polydatin, resveratrol, etc). Moreover, MIP particles with surface grafted functional polymer chains (e.g. using RAFT polymerization [2,3]) are produced to assess the improvement of the selectivity of MIPs towards the target polyphenols, namely through the tuning of the hydrophilic/hydrophobic effects (amphiphilic materials are generated). The produced MIP particles are applied for the identification, separation and concentration of phenolic compounds present in vegetable extracts. Different plants abundant in the Trás-os-Montes and Alto Douro region (e.g. vineyard, chestnut tree, olive tree, cherry tree, etc) are considered as potential sources of phenolic compounds. Supercritical extraction with CO_2 is used to obtain the vegetable extracts (see Figure 1) and the effects of the operation conditions (temperature, pressure, vegetable used, etc) on extract composition is also assessed.
Molecular recognition capabilities of the MIPs synthetized towards the polyphenols are evidenced (e.g. packing the particles for chromatography) but hydrophilic/hydrophobic interactions are unavoidable and a solvent gradient is needed.

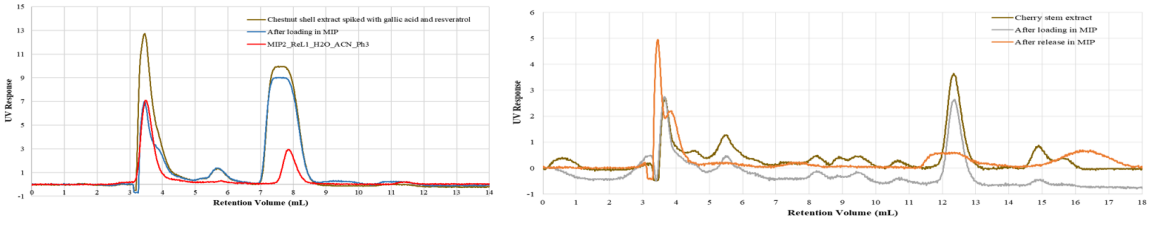

Figure 1. *HPLC analysis for chestnut shell and cherry stem SCCO2 extracts and correspondent upload/release fractions obtained with MIP particles.*

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OC-09

Thermostability studies of oil-soluble cyanidin-3-glucoside dyes

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Anthocyanins are natural water-soluble polyphenols present in many foods [1] and beverages such as red wine and red fruit juices. They are attractive pigments because of their appealing colors and health-promoting properties widely reported such as biological and antioxidant activity [2-4]. However, the technological applications of these natural compounds in the cosmetic, pharmaceutical and food industry are limited due to factors such as low lipophilicity, temperature, light and chemical equilibrium to pH variation.

It has been reported that adding hydrophobic molecules to polyphenols, their stability is raised as well as their bioavailability [5,6]. In previous works, the synthesis of malvidin-3-glucoside-fatty acid conjugates [7,8] was well succeed and have confirmed the enhancement of antioxidant properties of the anthocyanin derivatives as well as their solubility in organic solvents by simply adding fatty acid residues to the anthocyanins skeleton [9].

This work focuses on the improvement of anthocyanins stabilization, namely cyanidin-3-glucoside (Cy3glc), by enzymatic esterification with different saturated chain length fatty acids. All cyanidin-3-glucoside-lipophilic derivatives were structurally characterized and preliminary assays of thermostability at pH 3 showed that lipophilic derivatives are more stable towards higher temperatures than non-modified cyanidin-3-glucoside. They results are promising for a potential application of these anthocyanin derivatives in lipid-based food matrixes.

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OC-10

Adsorption equilibrium and kinetics of CO_2, CH_4 and N_2 on zeolite BETA with different cations and SiO_2/Al_2O_3 ratio

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Biogas is a gas mixture obtained by the anaerobic decomposition of organic matter, being mainly composed by methane, carbon dioxide and other compounds in minor amounts, such as nitrogen, hydrogen sulfide, water vapor and others. Due to the high quantity of methane (higher than 50%), the biogas can be used as a fuel, but, it's necessary to go through a process of purification, to remove CO2 and transform it in biomethane (around 95%), a fuel similar to natural gas [1,2].

In this way, gas adsorption equilibrium of CO_2, CH_4 and N_2 were investigated in three different types of zeolite BETA materials namely H-BETA-25, Na-BETA-25 and H-BETA-150 at temperatures 313, 373 and 423 K under partial pressures between 0.33 and 4.16 bar. For this experimental analysis a gas chromatographic system was utilized. The isotherm data has been satisfactorily demonstrated with the Langmuir model and the amount adsorbed follows the decreasing order as CO_2 > CH_4 > N_2 for all materials used. Considering experimental temperature, all materials show better absorption at the lowest temperature (313 K) for obvious reason.

First, when we compare two zeolites containing the same SiO_2/Al_2O_3 molar ratio but different compensation cations (H-BEA-25 and Na-BEA-25), interesting results are obtained. It is found that only due to presence of Na+ cationic center, zeolite Na-BETA-25 adsorbed quite higher amount of gases than H-BETA-25 (2.84, 1.59 and 0.97 mol/kg, and 2.28, 1.31 and 0.83 mol/kg respectively of CO_2, CH_4 and N_2 gases at 313 K).

In second comparison between two different SiO_2/Al_2O_3 molar ratio (H-BETA-25 and H-BETA-150), zeolite H-BETA-25 adsorbed a little bit higher amount of gases than H-BETA-150 (2.23, 0.98 and 0.58 mol/kg). But when we consider selectivity of gases the zeolite H-BETA-150 (adsorbs lowest amount of gases) showed to be the best over other two materials with decreasing value in pair order CO_2/N_2 > CO_2/CH_4 > CH_4/N_2 (6.65, 3.24 and 2.05 at 313 K). The selectivity order is the same for all three zeolites at mentioned temperatures.

The mass transfer studies were made using the Zero Length Column (ZLC) technique, at 313 K. But it is very difficult to determine the mechanism that controls the diffusion onto three zeolites, as experiments are so fast that ZLC studies were made in equilibrium conditions. Accordingly, it was not possible to obtain kinetic information for the adsorbents. However, it can be said that there are no resistances to the mass transfer in the studied conditions.

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OC-11

Seawater degradation studies of gallic acid persulfate, a promising synthetic antifouling agent

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Marine sulfated secondary metabolites, namely zosteric acid (ZA), are recognized as potential antifoulants with low or nontoxic effects to the environment [1]. In our research group, some nature-inspired sulfated compounds were synthesized and interesting antifouling (AF) properties were disclosed with no toxicity observed against several organisms [2]. In this work, seawater (SW) degradation of one of the most promising synthetic sulfated compounds, gallic acid persulfate (AGS), was evaluated. Only AF agents with rapid degradation in seawater will survive the close regulatory scrutiny to which they are being increasingly subjected to be approved.

The quantitative analysis of AGS was accomplished by ion-pairing reverse-phase high performance liquid chromatography with diode array detector (IP-RP-HPLC-DAD) using a C18 column (Fortis BIOC18 column) and an aqueous solution containing 25 mM of tetrabutylammonium bromide (TBAB) and acetonitrile (38:62 *v/v*) at a flow rate of 1 mL/min and an injection volume of 20 µL. Test solutions of the compound in SW were diluted with TBAB before injecting to allow a complete ion pairing and a desirable retention time within 10 min. The method was shown to be linear (r> 0.999) over the concentration range of 10-500 µM.

After several months in different stress conditions (4°C, 18°C, and 25°C in the dark; 25°C in natural light), the highest degradation rate was observed after exposure to natural light at 25°C. The high-SW solubility of AGS (higher than 1000 mg/mL) and the low Log P (-7.02) [2], allow us to predict that AGS will not accumulate into soil and fatty tissues. Taking into account all the environmental fate parameters, it is possible to conclude that AGS is a good candidate to be used in marine AF paints.

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OC-12

BeerOmics: how can advanced gas chromatography help to understand beer aroma properties?

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Beer is one of the most popular alcoholic beverages worldwide, being taste and flavor the main factors which contribute for consumers' acceptance. Its volatile components represent the major contributors for beer global and peculiar aroma properties, which result from a network of biochemical processes. An emergent concept called food metabolomics has been applied to the study of food system processes and it may be useful to understand the nutritional and sensory food properties, through foods metabolites profiling. This work intends to establish a comprehensive study of the beer small metabolites, mainly those associated to raw materials' and yeasts' metabolism: acids, alcohols, esters, monoterpenic compounds, norisoprenoids, sesquiterpenic compounds, sulfur compounds, and volatile phenols. A high throughput and high sensitive methodology combining the direct analysis of beer by headspace solid-phase microextraction (HS-SPME) with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-ToFMS) was used [1]. Indeed, GC×GC-ToFMS orthogonal mechanism (combination of non-polar/polar GC columns, connected in series through a cryomodulator), and also the ToF analyzer increases the chromatographic and spectral resolution and sensibility. These features are important for the simultaneous analysis of major and trace analytes, improvement of the detection limits, separation of chemical components from background, and/or deconvolution of co-eluted peaks. Several beers produced at different countries and breweries were analysed. A wide set of small molecules were identified, including 329 putative metabolites from the 8 targeted chemical families, which was defined as BeerOmics. They may be useful to have deeper and simultaneous information about beer volatile composition and its related factors (e.g. raw materials composition, brewing steps, off-flavors, beer aroma, beer typing). Moreover, considering the literature aroma notes of the identified metabolites, it will be possible to add a new information level to the flavor wheel (currently with 3 different levels, with exiguous chemical information), thus allowing to understand their contribution to the beer flavor wheel. Therefore, the BeerOmics, achieved through advanced gas chromatography, may potentially help in the understanding of the distinctive beer styles or beer typing.

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OC-13

Phenolic composition, antioxidant and biological activities of Portuguese vine shoot from *Touriga Nacional* and *Tinta Roriz* varieties

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Vine-shoots are an important waste in all viticulture areas that should be re-used with innovative applications [1,2]. The aim of the present study was to characterize the vine shoots from two important Portuguese grape varieties (*Touriga Nacional* - TN and *Tinta Roriz* - TR) from Dão region (North Center of Portugal) for further potential use as a source of phenolic compounds. For this purpose, three techniques, namely microwave-assisted extraction (MAE), subcritical-water extraction (SWE) and conventional extraction (CE), were tested and compared. The phenolic composition, antioxidant and biological activities from the obtained extracts were quantified by spectrophotometric and chromatographic techniques. The highest concentrated extracts were obtained by the MAE and SWE techniques. Concerning the differences in the vine shoot varieties, TR had the highest amount of total phenolic (32.1±0.9 mg GAE/g dry sample) and total flavonoids compounds (18.7±1.2 mg EE/g dry sample), as well as the highest antioxidant activity. The biological activity showed that all the obtained extracts had antimicrobial potential against different bacteria and yeasts, and the ability of inhibiting α-amylase and acetylcholinesterase enzymes, with MAE TR extracts being the most efficient. HPLC analysis enabled the identification of phenolic compounds belonging to different families, with gallic acid, and the flavonoids catechin, myricetin and kaempferol-3-*O*-rutinoside being the main contributors to the demonstrated antioxidant and biological activities of the vine shoot extracts.

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OC-14

Caracterização química e bioatividades de *Hibiscus sabdariffa* L.

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A grande diversidade na composição química das plantas é responsável pelas suas múltiplas aplicações. Para além das propriedades bioativas, as plantas são também fonte de nutrientes e metabolitos secundários nomeadamente, pigmentos naturais que podem ser utilizados em alternativa aos corantes artificiais [1,2]. Neste trabalho, foi determinada a composição de *Hibiscus sabdariffa* L. em açúcares, ácidos gordos, ácidos orgânicos e tocoferóis, tendo sido também avaliado o potencial bioativo e a composição em fenólicos dos seus extratos aquoso e hidroetanólico. O perfil individual de açúcares foi determinado por HPLC-RI, os ácidos gordos por GC-FID, os ácidos orgânicos por HPLC-DAD e os tocoferóis por HPLC-fluorescência. A análise de compostos fenólicos foi efetuada por HPLC-DAD-ESI/MS, enquanto que as propriedades bioativas foram avaliadas através de ensaios de atividade antioxidante e antimicrobiana. A hepatotoxicidade dos extratos foi também testada através de uma cultura primária de células de fígado de porco. A glucose (açúcar), o ácido málico (ácido orgânico), o α-tocoferol (tocoferol) e o ácido linoleico (ácido gordo) foram os principais constituintes nas classes correspondentes. 5-(Hidroximetil)furfural foi o composto não-antociânico mais abundante, enquanto delfinidina-3-*O*-sambobiósido foi a antocianina presente em maior concentração em ambos os extratos testados. Apesar de ambos os extratos de *H. sabdariffa* terem demonstrado atividade antioxidante e antimicrobiana, destaca-se o extrato hidroetanólico com uma maior capacidade de inibição da peroxidação lipídica em homogeneizados de células cerebrais de porco e maiores efeitos bactericidas e fungicidas. Foi também evidente que nenhum dos extratos analisados revelou hepatotoxicidade. Deste modo, a espécie *H. sabdariffa* revelou ser interessante não só como fonte de nutrientes, mas também de compostos bioativas e pigmentos, com enorme interesse para as indústrias alimentar, cosmética e farmacêutica.

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OC-15

Selective capillary coatings for in-tube SPME off-line or on-line with LC-MS/MS for bioanalysis

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In-tube solid-phase microextraction (in-tube SPME) off-line or on-line with high-performance liquid chromatography (LC) uses a capillary column as extraction device. The in-tube SPME-LC approach enables continuous extraction, concentration, desorption, and injection by means of an LC autosampler. This work describes the recent development of selective in-tube SPME capillary columns by our research group for bioanalysis, as follows. (1) Development of an organic-inorganic monolithic capillary column functionalized with cyanopropyl groups for in-tube SPME-LC-MS/MS to determine sixteen psychotropic drugs, at pg mL−1 levels, in plasma samples obtained from schizophrenic patients for therapeutic drug monitoring [1]. The capillary microsized macropores ensure fast dynamic transport and low backpressure, leading to high flow-rate and analytical speed. (2) Modification of a new molecularly imprinted polymer with restricted access material (a hydrophilic external layer), which affords an MIP-RAM phase via in situ polymerization in an open fused silica capillary [2]. This stationary phase can be used as sorbent for in-tube solid-phase microextraction (in-tube SPME) to determine parabens in breast milk samples by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The MIP-RAM capillary establishes specific interactions with parabens present in the milk samples and excludes most endogenous components by the hydrophilic external layer (non-adsorptive network). (3) Development of an in-tube SPME-LC-MS/MS method with polymeric ionic liquids (PIL) to determine endocannabinoids [anandamide (AEA) and 2-arachidonoylglycerol (2-AG)] in plasma samples. Different proportions of the monomers [C16VIM][Br], [C6VIM][Cl], and [(VIM)2C10]2[Br] are combined for *in-situ* polymerization in a fused silica capillary. The PIL coatings are chemically attached to the capillary, to afford a uniform film with thickness of approximately 1.3 µm. The capillary synthesized with [C16VIM][Br] and [(VIM)2C10]2[Br] presents adequate extraction efficiency for AEA and 2-AG. By employing the optimized in-tube SPME variables (sample volume, flow rate, sorption and desorption conditions, and cleanup conditions), the in-tube SPME/LC-MS/MS method provides adequate linearity to determine AEA and 2-AG in Alzheimer patients. This work emphasizes that in-tube SPME-LC methods are powerful alternatives to determine low drug and biomarker levels in various biological samples.

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OC-16

Preparative separation of nadolol racemates by fixed-bed liquid chromatography using C18 columns

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Nadolol is a non-selective beta-adrenergic antagonist pharmaceutical drug. This class of pharmaceutical drugs is prescribed mainly, to treat arrhythmias, angina pectoris, hypertension, migraine disorders and for tremor. Nadolol drug is still marketed as a mixture of equal amounts of four stereoisomers. Some authors refer that this fact could be related to some severe risks, such as depression, insomnia, cardiovascular failure among others [1].

An extensive set of experimental results for the separation of nadolol racemates using different achiral C18 columns, such as, XBridge, XBridge Shield RP18, XSelect CSH will be presented: Screening of mobile phase composition, solubility of nadolol racemates using different pure solvents and solvent mixtures, pulses under analytical and preparative conditions, equilibrium adsorption isotherms and breakthrough measurements. Additionally, experimental results will include the preparative separation by fixed-bed chromatography using an Azura Prep LC unit equipped with two 250 mL/min pump heads and a XBridge Prep OBD C18 10 μm (250x30 mm) column with a 10 μm particle size diameter [2].

Experimental results presented in this work will stress the advantage of using an intermediate step based on achiral reversed-phase liquid chromatography to perform the separation of the two racemates of nadolol. After this preparative pseudo-binary separation, two binary separations of the two racemates must be performed using a chiral stationary phase, such as Chiralpak IA to achieve the complete separation of all the four stereoisomers of nadolol. These two final preparative separations can be carried out using both fixed-bed or simulated moving bed technologies.

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OC-17

Hollow fiber microextraction (HF*μ*E) - A new hybrid microextraction technique for trace analysis

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The microextraction techniques have played an important role as modern sample enrichment approaches for trace analysis of complex matrices. In the last decades, passive microextraction techniques presented several advantages over the active ones, such as the use of miniaturized devices, simplification, easy manipulation, reduction or absence of the use of toxic organic solvents, selectivity and sensitivity enhancement, as well as low sample volume. Good examples of sorption-based techniques that have been successfully applied for sample preparation are solid phase microextraction, stir bar sorptive extraction and bar adsorptive microextraction (BAμE) [1,2]. On the other hand, liquid-based microextraction approaches, such as dispersive liquid-liquid microextraction, single-drop microextraction (SDME) and hollow fiber liquid-phase microextraction (HF-LPME) also demonstrated large applicability in the sample preparation field.

As all the techniques mentioned above show advantages and limitations, in the present contribution a novel hybrid microextraction approach is proposed, mixing the best features of some of them. In this new technique, hollow fiber microextraction (HFμE), analytical devices constituted by polypropylene membranes (10 mm in length and 0.6 mm in internal diameter; very cheap and easy to manipulate like in LPME), embedded with organic solvents (< 25 μL; due the high partitioning capacity and fast kinetics they exhibit in SDME) are used under the 'floating sampling technology' (showing high effectiveness and reproducibility as in BAμE) during the microextraction stage. Due the miniaturization, great flexibility and the low cost of the devices involved, a very simple strategy could be implemented for both microextraction and back-extraction stages, as well as, compatibility with the current autosampler systems.

To evaluate the performance of this technique, seventeen organochlorine pesticides (OCPs) were used as model compounds followed by gas chromatography-mass spectrometry analysis. The proposed methodology was successfully applied to monitor OCPs in environmental and food matrices and proved to be very user-friendly, eco-friendly, cost-effective and competitive for the routine work. In short, the novel HFμE proved to be a remarkable alternative for ultra-trace analysis of emerging compounds in real matrices over other well-stablished microextraction approaches.

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OC-18

Tape Adsorptive Microextraction - A new analytical approach for sample enrichment

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Modern sample enrichment techniques run towards the great simplification, miniaturization, strong reduction or absence of organic toxic solvents, as well as, low sample volume requirements in agreement with the green analytical chemistry principles [1]. For instance, an effective static microextraction technique was introduced in 2010, i.e. bar adsorptive microextraction (BAμE) that uses a bar-shaped geometrical support coated with convenient sorbents. This technique has proved to be a remarkable alternative for trace analysis of medium-polar to polar compounds in aqueous media, being already successfully applied to several classes of emerging or priority compounds in aqueous media. This analytical approach operates under the floating sampling technology and present several advantages such as the possibility to choose the most favorable sorbent phase (e.g. activated carbons, polymers, etc.) according to each type of application, when compared with other well-established microextraction techniques [2,3].

In this contribution, we propose 'tape adsorptive microextraction' (TAμE) as a green, and convenient sample preparation tool, which compared to BAμE devices, provides a larger sorbent volume with an increased surface area-to-volume ratio, resulting in faster extraction rates and enhanced sensitivity. This new approach consists in the use of tape as a simple support for coating with sorbent phases (fig. 1). Tape presents several advantages, e.g. low price, easy to obtain, robust, flexible, variable size and thickness, that make it attractive for enrichment purposes. By using TAμE, it allows the elimination of the solvent switch step, often cumbersome and time consuming, making possible the back-extraction stage in only single step with reduced volume of organic solvent, making the manipulation much more simple, effective, user-friendly and environmentally-friendly. To demonstrate the effectiveness of the proposed analytical tool, several assays were performed using different classes of organic pollutants in water matrices at the trace level.

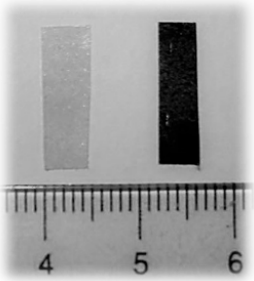

Figure 1. *Tape coated with two different sorbent phases.*

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OC-19

Multidimensional chromatographic techniques applied to chemical ecology

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The characterization of biological matrices is an unavoidable demand in chemical ecology. Notwithstanding the continuous development of gas chromatography (GC) and mass spectrometry (MS) instrumentation techniques and analytical methodologies, the total separation of all compounds and their unequivocal full or partial identification, in complex biological samples, is generally impractical or unachievable. Classical one dimensional chromatographic (1D) approaches for characterization of biological complex matrices in spite of achieving valid results, may not always give satisfactory results due to a potential considerable amount of information that remains unexploited or hidden, and thus demanding an alternative strategy to the use of a single column separation, if an increased resolution is needed.

The emergence of comprehensive two dimensional chromatography (GC×GC) in the last decade, and the resurgence, of a new generation of heart-cutting devices (MDGC) capable of delivering multidimensional gas chromatography (MDGC) with high accuracy (e.g. capillary flow Deans switch) opens a new door to allow the characterization of complex mixtures by enabling the separation of analytes in complex mixtures that cannot be otherwise achieved.

In this work, samples of extracts and volatiles emitted by trees and insects were analyzed using one-dimensional gas chromatography (1D-GC), comprehensive two-dimensional gas chromatography (GCxGC), heart-cut MDGC and by GC-MS/EAD. The increased resolution and sensitivity achieved proved to be an advantage, which is beneficial for the needs of trace analysis and the complex sample characterization usually essential in chemical ecology research

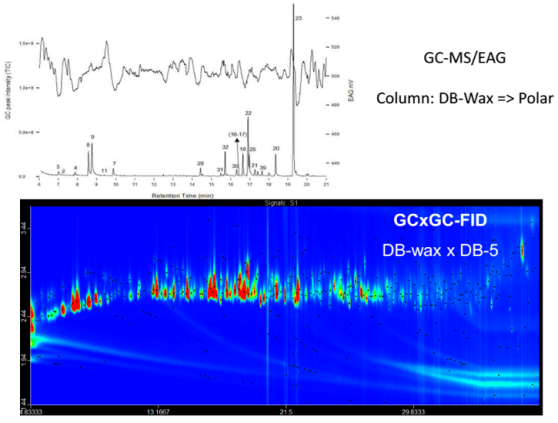

Figure 1. *GC-MS/EAD and GCxGC chromatograms*

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OC-20

Síntese de fase estacionária monolítica e posterior imobilização térmica de polidimetilsiloxano (PDMS) sobre a superfície porosa para aplicação em cromatografia líquida capilar

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Nos últimos anos vem crescendo o interesse nos materiais monolíticos como suporte de fases estacionárias em cromatografia líquida capilar, devido à sua elevada permeabilidade aliada à alta eficiência de separação, porém, ainda existem poucas opções disponíveis comercialmente. Neste contexto, o objetivo do trabalho foi estudar diversas variáveis no processo de síntese *in situ* deste tipo de fase estacionária dentro dos capilares para uso em cromatografia líquida capilar.

Na primeira etapa do trabalho avaliou-se: o tipo de suporte monolítico (sílica pura ou híbrido orgânico-inorgânico), as massas molares (2000 ou 6000 g/mol) do polidimetilsiloxano (PDMS) que recobre o monolito e as proporções do PDMS no solvente hexano (10, 30 e 50% *v/v*) na etapa de recobrimento. As fases passaram por um processo de autoimobilização durante 1 dia. Em seguida, foi feito um tratamento térmico de 100 °C por 24 horas seguido de 120 °C por 4 horas. A coluna que apresentou melhor eficiência cromatográfica foi a que empregou o monolito de sílica pura como suporte monolítico, PDMS com massa molar 6000 g/mol e na proporção 50% *v/v* em hexano.

Na segunda parte do trabalho, empregando as condições otimizadas na etapa anterior, avaliou-se o tempo e a temperatura do tratamento térmico para imobilização do PDMS: 100 °C por 24 horas na 1ª etapa e 120 ou 150 °C, por 4 ou 16 horas, na 2ª etapa. Nesta parte do trabalho, o tempo de autoimobilização foi aumentado para 4 dias. A coluna que apresentou a melhor eficiência foi a que empregou na 2ª etapa do tratamento térmico 150 °C por 16 horas, com uma eficiência de 68400 pratos/m, para o composto mais retido, conforme cromatograma da figura 1.

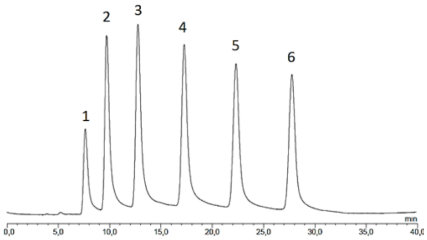

Figure 1. *Cromatograma da fase estacionária monolítica. Analitos: 1) Benzeno, 2) Tolueno, 3) Etilbenzeno, 4) Propilbenzeno, 5) Butilbenzeno e 6) Pentilbenzeno. Fase Móvel: Gradiente ACN 40-65% em meio aquoso, vazão de 1,2 µL/min, detecção com cela de 3 nL em 215 nm e volume de injeção de 0,05 µL. Coluna capilar: 15 cm x 200 m d.i.*

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OC-21

Permeation of caffeine, CQA and HMF from Coffee silverskin extracts on EpiSkin™ 3D model

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Coffee silverskin (CS) is a main by-product of the coffee roasting procedure and has no commercial value, being discarded as a solid waste [1]. Some work has been performed on the properties of CS, in particular its antioxidant behavior and caffeine content [2,3]. These compounds are believed to provide *in vivo* protection against free radical damage, being of huge interest for cosmetic industry. Nevertheless, according to the European regulation the evaluation of cosmetic ingredients on animals is forbidden. A number of *in vitro* tests to assess permeation have been developed such as the reconstructed human epidermis EpiSkin™. The aim of this study is to evaluate the permeation of CS extracts on an EpiskinTM model. Extracts (aqueous, hydro-alcoholic and alcoholic) were prepared according to the procedure described by Rodrigues *et al.* [2]. After the extract contact with the model, the content of caffeine, chlorogenic acid (CQA) and hydroxymethylfurfural (HMF) that pass through the model was evaluated by HPLC. Quantitative chromatographic determinations revealed the permeation of caffeine and HMF for the three extracts prepared, but there were no traces of CQA (Table 1). The hydro-alcoholic extract presented the best results in what concerns caffeine permeation and no statistical differences were observed for HMF. The absence of CGA permeation is justified by the retention effect of the epidermal model, which prevents the compounds passage.

Table 1. *Quantification of HMF, CGA and CAF in aqueous (W), hydro-alcoholic (WA) and alcoholic (A) extracts of CS medium after EpiSkin™ assay.*

Extract	HMF (μg/mL extract)	CGA (μg/mL extract)	Caffeine (μg/mL extract)
W	2.28 ± 0.18	-	1.26 ± 0.06
WA	2.39 ± 0.27	-	1.54 ± 0.14*
A	2.26 ± 0.09	-	1.40 ± 0.07

Values are expressed as mean ± SD (n = 3). *Significant difference ($p < 0.05$).

To the best of our knowledge, this is the first study that reports the permeation of caffeine, CQA and HMF after an EpiSkin™ assay using CS extracts.

Acknowledgements:
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OC-22

NTME/GC-qMS: a powerful strategy for selection sets of cancer-specific VOMs with potential for cancer differentiation

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Nowadays cancer related mortality remains unacceptably high (12 million in 2012, WHO) being the leading cause of death in many countries, surpassed only by heart disease. The worldwide high incidence and mortality associated with cancer justify the urgent need for the search of reliable diagnostic set of biomarkers using non-invasive techniques to screen and identify asymptomatic cancer patients.
Since the early applications, chromatographic-based analysis of biological samples has become increasingly popular as a result of perceived advantages such as compatibility with biological samples, high sensitivity, good spectral information content allowing analyte characterization (including identification or confirmation of identity). Therefore, an enormous expansion in the development and application of chromatographic analysis was observed in recent years. In this study, GC-qMS combined with an innovative extraction technique, Needle Trap MicroExtraction (NTME), was evaluated for the isolation and identification of volatile organic metabolites (VOMs) from urine samples of different groups of oncologic patients - colon, lung and breast cancer patients, in addition to healthy individuals, as a powerful strategy to select a set of cancer-specific VOMs with potential for its differentiation. In this context, and using DVB/Car1000/CarX as sorbent, more than 80 VOMs belonging to different chemical families, including ketones, sulfur, furan and terpene compounds, were identified. The data matrix was subjected to advanced multivariate statistical analysis (partial least squares - PLS-DA, ROC curves).
As far as we know, this is the first time that NTME/GC-qMS methodology was used to establish the volatomic pattern of urine samples for cancer patient discrimination. The results are very promising since the expression of volatile profiles showed higher complexity when compared with reference extraction methodologies.

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OC-23

Validation of a dSPE-HPLC methodology for the determination of biogenic amines in wines

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Biogenic amines are organic bases compounds found in several foods. Lactic acid bacteria produce them during malolatic fermentation by aminoacid decarboxylation. The main requirements for the formation of biogenic amines are free aminoacids available, presence of decarboxylase-positive microorganisms and conditions that allow bacterial growth and decarboxylase synthesis and activity [1]. In low concentration, biogenic amines contribute for physiological functions like regulation of body temperature, stomach pH or brain activity. However, the consumption of foods containing high amounts of biogenic amines, several toxicological effects may occur such as headaches, renal intoxication, nausea, hypotension, hypertension and in severe situations intracerebral haemorrhage or even death [2]. The monitoring of the presence of these compounds in food is very important, not only from the toxicological point of view, but can also be used as an indicator of spoilage [3]. In this work a dispersive solid phase extraction (dSPE) was developed for sample clean-up and pre-concentration of biogenic amines in wines. The derivatisation with benzoyl chloride and then the extraction with diethyl ether steps were optimized. High performance liquid chromatography with diode array detector (HPLC/DAD) was used as analytical technique and this method was validated for twelve biogenic amines – ethylamine, propylamine, butylamine, putrescine, cadaverine, tryptamine, β-phenylethylamine, amylamine, spermidine, hexylamine, spermine and histamine. The results indicate that this method is suitable for the intended purpose with a good recovery, precision and detection and quantification limits, and with a suitable range for the amount of biogenic amines present in wines.

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OC-24

Looking for new contributions in asthma biomarkers - a chromatographic-based approach

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Asthma is a heterogeneous disease caused by an alteration of genetic and environmental factors, described as a chronic inflammation and long term irreversible remodeling of the airways associated to an inappropriate inflammatory response. Depending on its nature, asthma can be essentially classified in two phenotypes: atopic and non-atopic asthma. Lipid peroxidation, is believed to contribute to the pathophysiology of asthma and involves all oxidation of fatty acids by enzymatic and non-enzymatic reactions. Oxidation of arachidonic acid is a fundamental enzymatic lipid peroxidation that leads to the formation of bioactive metabolites such as prostaglandins and leukotrienes with an important role on asthma. These metabolites can be used as asthma biomarkers, in evaluation of disease progression and follow of the therapy efficiency.

Taking into consideration the most recent trends in liquid chromatography, UHPLC has been widely spread because of its increased sensitivity, ultra resolution and ultrafast analysis time. Therefore, the present work aimed to develop an improved and highly sensitive UHPLC-based approach - to identify and quantify lipid peroxidation biomarkers related with asthma - leukotrienes E_4 and B_4 and 11β-prostaglandin $F_{2\alpha}$ – present in urine (non-invasive sampling procedure) of asthmatic individuals. In order to achieve the best performance the instrumental parameters, with influence on the chromatographic resolution, were optimized. A semi-automatic extraction procedure using an eVol-MEPS was used to isolate the target analytes. Several experimental parameters with influence on the efficiency of the extraction procedure including nature of the sorbent, number of loading cycles, elution solvent system and pH, were evaluated and optimized. The developed method was also fully validated in terms of selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), intra- and inter-day precision, accuracy, extraction efficiency, extracts stability and matrix effect.

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OC-25

Gas-chromatography mass spectrometry analysis of ^{13C}-labeled fatty acids revealed new information about the ruminal biohydrogenation of α-linolenic acid

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α-Linolenic acid (18:3n-3) is a polyunsaturated fatty acid (FA) that is available to ruminants through the intake of herbage, forage or linseeds. In ruminants stomach the action of the microbial population on the 18:3n-3 generates many intermediates, mostly *trans* FA, which are produced through hydrogenation and/or isomerization of the double bonds, i.e. biohydrogenation (BH). These intermediates can be absorbed and deposited in tissues, affecting the nutritional quality of ruminants' meat and milk. We used isotope labeled 18:3n-3, to follow the products formed under the conditions of "*t*10-shift" in the rumen, which is associated to animals produced under intensive feeding and that promotes the accumulation of *t*10-18:1. The importance t10-18:1 containing products is relevant as it has been associated with increased risk of cardiovascular disease in humans.

In vitro batch incubations using 0.5 mg/mL of labeled U^{13}C-18:3n-3 and unlabeled 18:3n-3 at five incubations times (0, 2, 4, 10 and 24 h) were performed. Rumen fluid was collected at a commercial abattoir, diluted into Goering and Van Soest's medium and dispensed anaerobically to Hungate tubes containing a concentrate diet. Tubes were sealed under CO_2 and incubated at 39ºC. Fatty acids were prepared according to Alves *et al.* [1]. Samples were analyzed by GC-MS with a Shimadzu 2010 Plus (Kyoto, Japan) using selected ion monitoring (SIM) for determination of abundances of the ion (M) and (M+18) ions. These abundances were used to calculate the enrichment of each FA with ^{13}C. Our results demonstrate that t10-shifted BH pathway of 18:3n-3 does not produce *t*10-18:1 as the main isomer but leaves preferentially the double bond at position 15. These results are very promising for defining feeding strategies to prevent the accumulation of the *t*10-18:1 in animals produced under intensive feeding conditions.

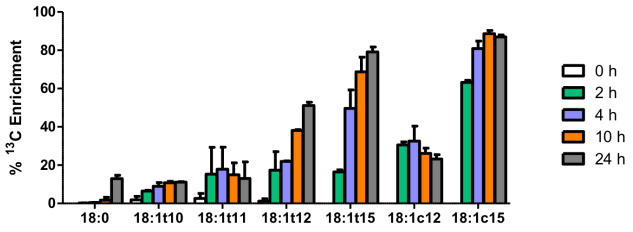

Figure 1. *The percentage of ^{13}C enrichment of 18:0 and 18:1 isomers over time when labeled and unlabeled 18:3n-3 was added to mixed rumen microbes.*

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OC-26

Multidetection of antibiotics in edible tissues: evolution of analytical strategies

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In the recent years Food Safety became one of the most important concerns for many international organizations, including the European Commission that defined this subject as top priority. For the protection of consumers and to prevent health risks associated with the presence of residues of veterinary drugs in food products, European regulatory agencies established rules to perform the control of this situation in foodstuff from animal origin. In the particular case of antibiotics, which use are widely implemented in food producing animals for prevention and treatment of different types of diseases, their abusive administration as growth promotes as to be monitored. To guarantee an efficient control, sensitive and specific analytical methodologies are requested for the determination of antibiotic residues in different products of animal origin, destined to human consumption. Although microbiological and immunoassays tests are still often used as screening methods to detect the presence of these compounds in edible tissues, their lack of specificity for multi-residue methods is a huge restriction. The most recent improvements are in the physic-chemical methods that allow the development of reliable, specific and sensitive methodologies allowing the determination of a huge number of compounds at once guaranteeing the accurate identification and fulfilling the requirements of European guidelines 2002/657/EC[1] for official residues control.

INIAV, as National Reference Laboratory in this field, has recently developed two analytical strategies, for antibiotics determination, to achieve the desirable performance: screening, by UHPLC-ToF-MS, and confirmation/quantification by UHPLC-MS/MS. Representative compounds from the major classes of antibiotics used in veterinary medicine (tetracyclines, beta-lactams, quinolones, macrolides and sulfonamides) can be detected in a single run in complex biological matrixes samples (milk, meat and fish).

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OC-27

Chromatographic analysis of nutritional and bioactive compounds in vegetative parts of *Fragaria vesca* L. obtained by *in vitro* culture

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Fragaria vesca L. is mainly consumed and appreciated by its sweet small fruits. However, the vegetative parts have been described as important sources of macro and micronutrients as also phenolic compounds (procyanidins and hydroxycinnamoyl and ellagic acid derivatives) [1,2]. The growing demand for natural products with nutritional value and, simultaneously, bioactive properties requires innovation in how to obtain these products, namely protecting the wild populations from where they are collected avoiding direct competition with human food stuff cultures [3]. Cell and tissue culture emerges as viable technique to produce secondary metabolites, being a sustainable and ecological alternative to obtain bioactive compounds that can be applied in pharmaceutical/medical and/or food industry (endorsed by FAO) [5]. Herein, the vegetative parts of *F. vesca* were obtained by *in vitro* culture and characterized in terms of macronutrients (AOAC methods), fatty acids (GC-FID), sugars (HPLC-RI), organic acids (HPLC-DAD), tocopherols (HPLC-Fluorescence), and phenolic compounds (HPLC-DAD-ESI/MS). The antioxidant activity was also evaluated. The studies were carried out with lyophilized material and hydromethanolic and aqueous (infusions and decoctions) extracts. In order to establish the in vitro culture, a four week interval was followed for micropropagation In each one, the vegetative parts were collected and characterized, presenting higher contents of proteins, fatty acids, sugars, organic acids (including ascorbic acid) and tocopherols (mainly α-tocopherol) than the samples obtained from wild vegetative parts collected in field and previously studied by our research group [2]. Furthermore, the hydromethanolic extracts of the obtained *in vitro* samples also revealed higher antioxidant activity that the *F. vesca* samples collected in field [4]. On the contrary and despite the similarity between the phenolic profile of both samples, lower concentrations were detected in the *in vitro* cultured *F. vesca*. Further studies are required to understand how to increase the concentration of phenolic compounds by elicitation of the *in vitro* culture. Within this work, it was intended to explore the *in vitro* culture as a biotechnological tool for the obtention of high value phytochemicals with application in different industrial sectors.

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OC-28

Liquid by-products from canned fish industry as sources of omega-3 polyunsaturated fatty acids

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Canning fish processing consists in a set of sequential operations (fish reception, washing, brining and cutting, cooking, canning, sealing and sterilization), most of which involve the use of water. Therefore, liquid effluents are extremely variable in qualitative and quantitative terms, being usually discarded, after a proper treatment. However, such treatment processes may also target the recovery of specific valuable products, thus becoming a relevant source of revenue to companies [1].

Studies concerning valorization of liquid wastes from fish processing are scarce, and more focused on the wastewater treatment for reuse or recycling into the industrial unit [2]. Therefore, the present study intended to evaluate green and economically sustainable methodologies for the extraction of ω-3 rich lipids from fish-canning liquid by-products.

Physical-chemical (pressurized extraction processes) and biological-chemical methods were selected for the extraction of ω-3 rich lipids, as they link the efficient utilization of (food grade) organic solvents with temperature, pressure or enzymes, in an improved and more environmentally friendly solution. The fatty acid profile of the obtained extracts was evaluated by GC-FID.

Results indicated extraction with high hydrostatic pressure as the most suitable for ω-3 lipid recovery. The studied procedures allow to obtain alternative (and traceable) sources of ω3 lipids, able to be used as functional ingredients and, simultaneously, decrease environmental concerns related to the discharge of large volumes of liquid effluents from fish canning industries.

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OC-29

Fingerprinting of volatomic profile of lemon (*Citrus limonum*) based on a new analytical approach based on NTME/GC-MS analysis

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Lemon (*Citrus limonum*) is a well known citrus fruit widely used throughout the world. It is an important source of secondary metabolites for nutrition, health, and industrial applications. In addition different lemon varieties, and also lemons from the same viriety but from different geographic regions, have different organoleptic and nutraceutical characteristics.
In this context, this study was conducted in order to gain deep insights on the volatile composition of lemon from eureka variety, cultivated at different geographic regions, in order to identify the volatile organic metabolites (VOMS) responsible for its peculiar aroma. Eighty VOMs, belonging to different chemical classes, namely terpenes, sesquiterpenes, higher alcohols and carbonyl compounds, were identified in the targeted citrus fruit using a new analytical approach based on Needle Trap Micro-Extraction (NTME) followed by GC-qMS analysis. In order to achieve the best extraction performance, several extraction-influencing parameters, namely sample amount, extraction volume, sample temperature and equilibration time, were optimized. As far as we know, is the first time that this extraction technique is used in food research.
A comparison with the volatomic profile obtained through SPME/GC-MS analysis , shown a better peak resolution with the NTME/GC-MS strategy, although the most complete volatomic fingerprinting when using HS-SPME/GC-MS. In addition, since some of the identified volatiles, limonene, γ-terpinene, β-pinene, α-pinene, β-myrcene or sabinene, exert effective health benefits, the investigated fruits can be used as a potential source of bioactive compounds for several industrial applications (food, cosmetics, …). The obtained data matrices, submitted to principal component analysis (PCA) revealed that the VOM profiles were able to differentiate lemon fruits according to geographic region.
The chromatographic data combined with advanced multivariate statistical analysis could be used to define potential “markers compounds” of lemons providing a useful tool for its authentication according to farming regions contributing with the compliance os current regulations about geopgraphical protection of food.

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OC-30

Perfil fenólico e bioatividades de maçã portuguesa da variedade "Bravo de Esmolfe"

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'Bravo de Esmolfe' é uma variedade de maçã portuguesa com um aroma intenso e, por isso, muito apreciada pelos consumidores. Esta variedade de maçã tem Denominação de Origem Protegida (DOP), o que valorizou enormemente o produto e conduziu a um elevado impacto na economia local e nacional [1]. Esta variedade é produzida no norte de Portugal numa pequena região do interior, correspondendo a uma produção de 200 ton por ano. Devido às suas características sensoriais, nomeadamente doçura e sabor, o seu consumo tem vindo a aumentar consideravelmente [2]. Neste trabalho a maçã "Bravo de Esmolfe" foi caracterizada em termos de compostos fenólicos, determinados por cromatografia líquida de alta eficiência acoplada a um detetor de díodos e a um espectrómetro de massa (HPLC-DAD-ESI/MS). A identificação foi realizada usando compostos padrão, quando disponíveis, comparando os seus tempos de retenção, os espetros de massa e os espetros UV-Vis. Na ausência de padrões, a identificação foi efetuada pelo perfil de fragmentação e comparação da informação obtida com dados da literatura. A quantificação foi realizada a partir das áreas dos picos registados nos respetivos comprimentos de onda (utilizando 280, 330 e 370 nm, preferencialmente), em comparação com as curvas de calibração dos padrões. Foram também avaliadas as suas propriedades bioativas (atividades antioxidante e antimicrobiana) e correlacionadas com a sua composição em compostos fenólicos. Foram identificados 15 compostos fenólicos, 7 derivados de flavan-3-óis (epicatequina e procianidinas do tipo B), 4 ácidos hidroxicinamoilquínicos, duas di-hidrochalconas (derivado de floretina) e dois compostos desconhecidos. Os compostos mais abundantes foram o ácido 5-*O*-cafeoilquínico (52 mg/100 g matéria seca) seguido da procianidina B2 (35 mg/100 g). Os extratos metanol/água (80:20, *v/v*) preparados a partir de maçã 'Bravo de Esmolfe' também demonstraram atividade antioxidante (atividade captadora de radicais livres e inibição da peroxidação lipídica) e efeitos antibacterianos contra bactérias Gram-positivo (*Listeria monocytogenes* e MRSA) e Gram-negativo (*Escherichia coli* e *Morganella morganii*). Existe pouca informação sobre a maçã 'Bravo de Esmolfe', o que torna este estudo inovador e essencial uma vez que esta variedade é uma fonte de compostos fenólicos com propriedades bioativas.

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OC-31

The volatile profile for discrimination of lavender and heather honey, using solid phase microextraction and gas chromatography-mass spectrometry

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Honey is a natural product produced by *Apis mellifera* bees from the nectar or secretions of plants, which has a long history of human consumption. It is also used in various foods and beverages as a sweetener and flavouring. The main parameters of honey quality, which also influence its price, are derived from its botanical origin. Honey volatiles have been used as markers for its authenticity. They may arise from the nectar source, from the transformation of plant compounds by the honeybee, directly generated by honeybee, from heating or handling during honey processing and storage or from microbial or environmental contamination [1].

The aim of this work is the discrimination of monofloral *Lavandula* spp. and *Erica* spp. honeys through its volatile profile. For that, eighteen samples from both lavender and heather honey, were analyzed. Volatiles were sampled by headspace solid phase microextraction (HS-SPME) using a 65 µm polydimethylsiloxane divinylbenzene (PDMS/DVB) fiber. The chemical identification was performed by gas chromatography-mass spectrometry (GC-MS). A complex total ion chromatogram was obtained, with nearly seventy compounds identified and quantified. The aldehydes and terpenic derivatives were the most likely to relate honey to its floral origin, being phenylacetaldehyde and nonanal the most representative in lavender honey while hotrienol was the most abundant in the heather honeys. The above methodology was suitable for the isolation of low-molecular-weight aroma compounds, particularly for the short-chain aliphatic compounds that are important for authentication of lavender and heather honey.

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OC-32

Geranium robertianum L. phenolic compounds: individual characterization of stems and leaves profile

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Geranium robertianum L., commonly known as Herb Robert, is a widely distributed herbaceous plant, commonly used in traditional medicine for the treatment several ailments and currently commercialized in the form of tea sachets or even food supplements for therapeutic purposes [1]. Plants from this genus have been described as a good source of phenolic compounds, particularly flavonoids and tannins [2]. In this context, this study aimed to perform a detailed phenolic characterization for two different *G. robertianum* extracts obtained from its two most representative organs, i.e., stems and leaves.

After the decoction of the plant material, the extracts were analyzed through high performance liquid chromatography with diode array detector coupled to an electrospray ionization mass spectrometer operating in negative mode.

Overall, both *G. robertianum* aqueous extracts revealed very similar phenolic profiles, with more than 50% of the phenolic compounds quantified corresponding to ellagic acid and its derivatives. Ellagic acid (λ_{max} at 253 nm, $[M–H]^-$ at *m/z* 301229) and brevifolin carboxylic acid (λ_{max} at 277 nm, $[M–H]^-$ at *m/z* 291247) were the two most prominent compounds found in both extracts, although the former was more abundant in leaves (249.6 ± 0.5 against 156.5 ± 0.9 mg.g extract^{-1}) and the latter in stems (177.7 ± 0.05 against 153.4 ± 0.3 mg.g extract^{-1}). Relevant amounts of corilagin (λ_{max} at 269 nm, $[M–H]^-$ at *m/z* 633301, 463, 275) were detected in the two extracts as well (40.7 ± 0.3 and 57.1 ± 0.5 mg.g extract^{-1}, for leaves and stems respectively), while geraniin (λ_{max} at 274 nm, $[M–H]^-$ at *m/z* 951933, 301) was only present in leaves. Other compounds found in these extracts include the tannins tris-galloyl-HHDP-hexose (MW=952 Da), repandusidic acid A (MW=970 Da), phyllanthusiin C (MW= 926 Da) and phyllanthusiin B (MW=970 Da), together with other ellagic and gallic acid derivatives such as ellagic acid-(*p*-coumaroyl)-hexose (MW=610 Da), ellagic acid pentoside (MW=434 Da), galloyl-hexoside (MW=332 Da), galloylquinic acid (MW=344 Da) and digalloyl-hexose (MW=484 Da). Chlorogenic acid and rutin were also detected in trace amounts as the only hydroxycinnamic acid and flavonoid compounds identified in these *G. robertianum* extracts.

With this study, it was possible to conclude that the phenolic profile of *G. robertianum* aqueous extracts were particularly abundant in ellagic acid, brevifolin carboxylic acid and several hydrolysable tannins, which have been previously described for their promising bioactivities, and therefore might be key contributors for the claimed health benefits attributed to this species.

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OC-33

Ácido 4-hidrazinobenzoico como agente derivatizante para a determinação de aldeídos por HPLC-UV e LC-MS

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Os compostos carbonílicos constituem um dos grupos mais importante de poluentes ambientais e, dentro deste grupo, os aldeídos de baixa massa molecular como o formaldeído ou o acetaldeído são ainda mais relevantes, visto que representam sérios riscos para a saúde humana. Estes compostos encontram-se presentes em produtos de caraterísticas tão distintas, como alimentos, produtos de higiene pessoal e materiais de construção, sendo necessários métodos versáteis e robustos para permitir a sua determinação em matrizes tão díspares. Habitualmente recorrem-se a reações de derivatização para permitir a deteção dos compostos carbonílicos através de métodos cromatográficos, sendo a 2,4-dinitrofenilhidrazina (DNPH) o derivatizante mais utilizado. Contudo, a DNPH apresenta um conjunto de desvantagens, tais como a baixa solubilidade em água, necessidade de purificação e interferências com outros compostos químicos como o ozono e os óxidos de azoto.

Neste trabalho o ácido 4-hidrazinobenzóico (HBA) [1] foi utilizado pela primeira vez como agente derivatizante para a determinação analítica de aldeídos de baixa massa molecular por cromatografia líquida (HPLC-UV). A reação de derivatização foi realizada em simultâneo com o processo de extração, através da microextração por difusão gasosa (GDME) [2].

Simultaneamente, estudos de espetrometria de massa (LC-MS) foram também realizados, de forma a demonstrar a possibilidade de identificação de cada um dos compostos derivatizados. No geral, o HBA mostrou ser um reagente derivatizante bastante vantajoso, em particular devido ao seu número reduzido de impurezas, à sua estabilidade e solubilidade relativamente alta em água e outros solventes, alta seletividade e sensibilidade, assim como aplicabilidade a diferentes técnicas de deteção.

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OC-34

Effects of natural colourants on the fatty acids profile of different ice cream formulations

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Ice cream is a complex food colloid formed of air bubbles, fat globules, ice crystals and a serum unfrozen phase [1]. Although presenting a complex composition, including milk, sweeteners, emulsifiers, stabilizers, colourants and flavouring agents, ice cream is very poor in some essential components such as natural antioxidants, dietary fibres, minerals and vitamins, all representing features desired by consumers [2]. Nevertheless, the addition of a natural food colourant to ice cream, besides fulfilling its main colouring function, might be an effective way to functionalize it, due to the antioxidant activity of most natural colourants (*e.g.*, anthocyanins, carotenoids or betacyanins). *Gomphrena globosa* L. is a good example of an alternative source of colouring compounds of natural origin, namely betacyanins, which present a threefold higher colouring capacity than its commercial analogue (anthocyanins), additionally possessing antioxidant, antiviral, and antimicrobial properties [3]. Therefore, betacyanins extracted from the purple flowers of *G. globosa* were evaluated as colouring/functionalizing agents in ice-cream formulations, aiming to verify potential improvements on fatty acids profile, analysed by gas chromatography (GC) coupled to a flame ionization detector (FID). In order to acquire comprehensive conclusions, other ice-cream formulations (IF) were prepared and analysed in 5 storage times (ST). Besides the ice cream containing *G. globosa* extract, three formulations were prepared: control ice cream (without colourants), ice cream with commercial betalain and ice cream with *Beta vulgaris* extract (E-162). The profiles in fatty acids were especially sensitive to ST, which induced a significant effect in all cases except C18:1 (p=0.271) and MUFA (p=0.108), in contrast with IF, which was only significant in the cases of C14:0, C16:1, C18:1 and MUFA. In general, the new colouring approach allowed maintain the most relevant fatty acids unchanged.

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OC-35

High Throughput Bar Adsorptive Microextraction (HT-BAμE): A novel cost-effective tool for monitoring psychotropic drugs in biological matrices

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In the last decades public concern has emerged in regards to the chronic and/or acute consumption of psychotropic drugs for medical and/or non-medical purposes. Of these, benzodiazepines are prominent. These compounds are the most commonly consumed psychotropic pharmaceuticals in Portugal and in the European Union, being mostly prescribed for their anxiolytic, sedative and hypnotic effects [1]. However, many symptoms of toxicity due to their exacerbated consumption have been reported, including death [2]. For these reasons, there is a need for innovative analytical approaches that allow a robust and effective monitoring of these substances in biological matrices such as blood, plasma, serum or urine. However, these psychotropic drugs are usually detected in these complex matrices at trace or ultra-trace level [3]. Therefore, any instrumental analysis will require a previous sample preparation stage, in order to enhance the limits of detection. Additionally, there is also the need for techniques and methodologies that allow the routine analysis of large number of samples without compromising performance.

In this contribution we present the development and optimization of high throughput bar adsorptive microextraction (HT-BAμE) as a new cost-effective tool for monitoring psychoactive drugs in biological matrices. This novel approach was combined with micro-liquid desorption (μLD) and conventional high performance liquid chromatography with diode array detection (HPLC-DAD) for the analysis of diazepam, clorazepate dipotassium, prazepam, bromazepam, oxazepam, lorazepam, alprazolam, temazepam and loflazepate in blood, plasma, serum and urine matrices. The device has the possibility of accommodating 100 sampling vials for simultaneous microextraction and subsequent back-extraction. Preliminary data shows that average sample preparation time of around 2 min per sample was achieved (recoveries > 80 % for all target compounds), demonstrating that the proposed approach has great potential for further advancements and applications.

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OC-36

Caracterização do perfil fenólico de agrião por HPLC-DAD-ESI/MS e otimização da extração por alta pressão hidrostática utilizando a metodologia de superfície de resposta

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A tecnologia de alta pressão (AP) é cada vez mais utilizada na indústria alimentar como método de pasteurização a frio. A sua utilização para extração de compostos de elevado valor acrescentado a partir de matrizes vegetais é relativamente recente e tem sido indicada como uma boa alternativa aos métodos convencionais, pois evita a degradação de moléculas termossensíveis e pode melhorar a eficiência da extração em termos de rendimento, qualidade e seletividade [1-4]. Neste sentido, este trabalho teve como objetivos caracterizar o perfil fenólico de agrião (*Nasturtium officinale* R. Br.), uma planta semiaquática de crescimento rápido com propriedades medicinais [5], e otimizar a extração destes compostos por AP utilizando a metodologia de superfície de resposta. As amostras de agrião liofilizadas foram processadas por AP de acordo com um desenho fatorial completo de cinco níveis, combinando as variáveis tempo de processamento (*t*, 1.5-33.5 min), pressão (*P*, 0.1-600 MPa) e solvente (*S*, 0-100 % de etanol, v/v). Os compostos fenólicos foram analisados por HPLC-DAD-ESI/MS e os resultados da quantificação foram usados como variáveis de resposta. Os flavonoides predominaram sobre os ácidos fenólicos e, em geral, foram quantificados mais derivados glicosídeos de quercetina e de isoramnetina do que ácidos fenólicos. Quatro derivados glicosídeos de kaempferol foram identificados pela primeira vez nesta espécie. Os modelos teóricos obtidos foram ajustados com sucesso aos dados experimentais, validados estatisticamente e utilizados nas etapas de otimização e predição. As condições ótimas de AP para extração foram as seguintes: *t*= 3,1 min, *P*= 600 MPa e *S*= 100% e originaram 64,68±2,97 mg/g de extrato [4]. Este estudo destacou a AP como uma tecnologia promissora para extrair compostos fenólicos (ácidos fenólicos e flavonoides) de agrião de forma seletiva usando um solvente verde e tempos de extração reduzidos.

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OC-37

Polyols based solvents for the extraction of phenolic compounds from *Juglans regia* L. leaves

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In this work, a set of polyols was selected to be used as solvents for the extraction of phenolic compounds from *Juglans regia* L. (walnut) leaves. Their biocompatibility and low toxicity makes them an interesting option with potential industrial applications in the cosmetic, pharmaceutical or food areas. In fact, some of them are already used as cosmetic ingredients and/or can be obtained from renewable raw materials [1, 2].

Walnut leaves are an important source of bioactive molecules, such as phenolic compounds, suggesting that their extracts (or pure components) could be used as natural antioxidants in different applications, e.g. to replace synthetic antioxidants such as BHT (2,6-di-*tert*-butyl-4-methylphenol) or in dermatological bases preventing oxidative damage [3,4].

Hence, the extraction performance of glycerol and a series of diols (1,2-etanodiol, 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, 1,2-pentanediol, 1,5-pentanediol, 1,2-hexanediol, 1,6-hexanediol) was screened and the phenolic profile, characterized by HPLC-DAD. To evaluate the response, only the main bioactive compounds present in the extract (acid 3-*O*-caffeyloquinic acid, quercetin 3-*O*-glucoside and quercetin *O*-pentoside) were considered. In a second phase, for comparison purposes, the most promising polyols were selected to prepare betaine based deep eutectic mixtures. After selecting the best solvent, the remaining extraction conditions (time, temperature and water content) were further optimized. These results provide relevant information for the design of an extraction process of phenolic compounds from *J. regia* leaves using alternative solvents that could also play a role as a formulation medium.

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OC-38

Oncolytic virus purification using multi-column chromatography

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With the growing interest on complex biopharmaceutical products such as virus particles for advanced therapies and medicinal products, downstream processing (DSP) is becoming ever more relevant. Therefore, the biopharmaceutical industry is looking for alternative downstream strategies capable of improving purification yields whilst improving product quality and lowering costs [1]. One of the most promising improvements to DSP is to replace single-column batch operation by continuous, or semi-continuous, multi-column chromatography [2].

We report on the development of a multi-column chromatographic process aimed at the purification of oncolytic adenovirus. The process described herein is based on direct product capture using an anion exchange chromatographic media and subsequent elution with the modulation of ionic strength. By using a multi-column approach, one is able to overcome the limits of dynamic binding capacity characteristic of single-column batch processes, thus increasing the media capacity utilization [3]. The volumetric productivity of this process step depends not only on the optimal scheduling of the referred steps, but also upon factors such as media capacity for the product and related impurities, operational flow-rates, and mechanical limitations of the systems used.

The proposed process will be accessed in terms of volumetric productivity, resin capacity utilization, equipment footprint, skid complexity and connectivity with the surrounding unitary operations of the purification train.

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OC-39

Effects of e-beam irradiation on bioactive content of cherry tomatoes

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Cherry tomato is a fresh fruit present in diets worldwide, rich in antioxidants, vitamins A and C and minerals that can promote beneficial health effects when ingested. Tomatoes constitute the predominant source of lycopene, a bright red carotenoid pigment that has been associated with the prevention of cardiovascular diseases and cancer of the prostate, breast, lung, bladder, ovaries, colon and pancreas [1]. One of the main demands of consumers is for minimally processed, high-nutrition/low-energy natural foods with none or minimal chemical preservatives. The shelf stability of this fruit ranges from five to seven days depending on the time of harvest, thus showing some limitations regarding fresh market utilization [2]. Extending the shelf life, while improving the food safety, will have a positive impact on both the agroindustry and the consumers. Considering the above and knowing the importance of consuming fruit with quality, the aim of this study is to evaluate the effects of electron beam radiation on bioactive content (lycopene content and antioxidant activity) of cherry tomatoes, to assess the potential of irradiation post-harvest treatment for fruit shelf-life extension.

The fresh tomatoes were irradiated in a linear electron-beam accelerator (LINAC) at two doses: 1.5 and 3 kGy (dose rate: 0.5 kGy/min). Lycopene was extracted from the lyophilized tomatoes by conventional method with hexane-acetone and its quantification was performed by High Performance Liquid Chromatography (HPLC). The antioxidant activity of lycopene extracts was measured by DPPH technique.

The results suggested that 3 kGy ionizing radiation treatment could preserve the lycopene content immediately after irradiation (time 0), although an increase of the antioxidant activity of the extract was verified. The studies of storage time extension are ongoing. These preliminary results are part of a wider project that aims to evaluate fresh fruits post-harvest treatment with high-energy electron beam, which includes the evaluation of microbiological parameters, quality attributes and cytotoxicity. The data from this comprehensive study is highlighting the feasibility of e-beam irradiation as post-harvest treatment of cherry tomatoes.

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OC-40

Otimização da extração de antocianinas de cereja madura através da metodologia de superfície de resposta

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A cereja (*Prunus avium* L.) é um fruto muito apreciado pelo seu sabor doce e aparência apelativa. A sua cor varia de acordo com o ponto de maturação, sendo um excelente indicador da melhor altura de colheita. No pico da maturação, adquire uma cor avermelhada mais escura que é maioritariamente influenciada pela concentração de antocianinas presentes na pele e na polpa do fruto [1]. Estes compostos estão presentes em muitas matrizes naturais e a sua cor pode variar de azul a violeta e vermelho dependendo das condições do meio onde se encontram (pH, temperatura, humidade, salinidade, condições de *stress* e armazenamento, etc.) [2]. Por serem compostos com elevada capacidade corante, para além das suas reconhecidas propriedades bioativas, têm vindo a ser cada vez mais explorados para aplicação na indústria alimentar.

Assim, o presente estudo teve como objetivo a otimização da extração destes compostos da cereja através do estudo das condições que maximizam o rendimento de extração por maceração. Para tal, aplicou-se uma metodologia de superfície de resposta usando o desenho composto central circunscrito com cinco níveis em cada uma das variáveis independentes estudadas (tempo, temperatura e proporção de água-metanol como solvente). A quantificação de antocianinas presentes nos diferentes extratos obtidos foi efetuada por cromatografia líquida de alta eficiência acoplada a um detetor de díodos (HPLC-DAD). A concentração de antocianinas e o rendimento de extração do resíduo foram as respostas utilizadas para o modelo.

Através deste método de extração foi possível obter um rendimento de extração de antocianinas de 1,86±0,41 mg/g de massa seca de cereja, nas condições ótimas de extração (63,0±3,2 min; 61,7±1,3ºC e 53,1±1,4% de etanol). O resíduo obtido representou 87% da massa seca total da cereja e o teor de antocianinas foi de 3,05±0,41 mg/g de resíduo seco. Os resultados obtidos neste estudo demonstram a potencial utilização da cereja como fonte de antocianinas.

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OC-41

Efeito da radiação gama e feixe de eletrões na concentração de ergosterol em *Agaricus bisporus* (J.E. Lange) Imbach

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O cogumelo *Agaricus bisporus* (J.E. Lange) Imbach é dos mais consumidos e cultivados em todo o mundo, devido não só às suas características sensoriais únicas, mas também devido às suas propriedades bioativas, nomeadamente antioxidante, antimicrobiana e anti-inflamatória. Estas bioatividades são devidas a várias moléculas incluindo polissacáridos, glucoproteínas, compostos fenólicos, esteróis, entre outras. Em particular, o ergosterol é um componente da membrana celular dos fungos que tem atraído muita atenção devido às suas propriedades hipocolesterolémicas [1].

A irradiação é uma técnica de conservação de alimentos cuja utilização está regulamentada na U.E. para vários produtos alimentares através da Diretiva 1999/3/CE [2]. Neste trabalho, avaliou-se o efeito da radiação gama e feixe de eletrões como tecnologias de processamento pós-colheita na concentração de ergosterol de *A. bisporus*. Para cada tipo de radiação, as amostras foram divididas em quatro grupos, um controlo (não-irradiado) e os restantes irradiados com as doses 1, 2 e 5 kGy. O ergosterol foi determinado por cromatografia líquida de alta eficiência com deteção por ultravioleta (HPLC-UV). A sua separação foi efetuada numa coluna C18 utilizando uma fase móvel de acetonitrilo/metanol (70:30, *v/v*) em modo isocrático, com um fluxo de 1 mL/min.

Nas amostras submetidas à radiação gama, a concentração de ergosterol mais elevada foi detectada para as amostra irradiadas a 1 kGy (383 mg/100 g de massa seca). No que se refere à irradiação com feixe de eletrões, as amostras irradiadas às doses de 2 e 5 kGy, apresentaram maiores concentrações de ergosterol (237 e 333 mg/100 g de massa seca, respetivamente). Estes resultados indicam o potencial de utilização de radiação gama e feixe de eletrões como tecnologias de processamento que permitem aumentar a extração de ergosterol a partir de corpos frutíferos irradiados de cogumelos *A. bisporus*.

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OC-42

Optimization of the extraction of triterpenes from *Ganoderma lucidum*

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Ganoderma lucidum is a mycological source rich in several bioactive compounds. Among their constituents, triterpenes have been widely reported to be responsible for some of *G. lucidum* bioactive properties [1]. It has been used, since ancestral times, due to their medicinal and nutraceutical properties. In this context, the achievement of extracts enriched in target compounds, and obtained at optimized extraction conditions, is a relevant field of research where the use of statistical tools, such as response surface methodology (RSM), is becoming increasingly important.

In the present study, the extraction of triterpenes from *G. lucidum* was optimized by using maceration (ME) and ultrasound assisted extraction (UAE), and compared with Soxhlet extraction (SE). The SE technique was considered effective by applying 7 cycles while to optimize ME and UAE, RSM was applied using a circumscribed central composite design with three independent variables (time, ethanol content, temperature (ME) or power (UAE)). The extraction yield and content in triterpenes were maximized and the extracts obtained under the optimal conditions characterized in terms of individual triterpenoids by HPLC-DAD-ESI/MS analysis.

With the SE technique, a positive linear dependency was achieved for the extraction yield while an asymptotic-decreasing behavior was observed for triterpenes' content. The conditions maximizing responses were: 78.9 min, 90ºC and 62.5% ethanol and 40 min, 100 W and 89.5 % ethanol for ME and UAE, respectively. The later was the most effective, yielding 4.9±0.6% and 435.6±21.1 mg equiv. ursolic acid/g extract whereas the first yielded 5.2±0.6% and 285.7±31.2 mg equiv. ursolic acid/g extract. Under the optimum conditions, HPLC-DAD-ESI/MSn analysis confirmed the presence of a total of 24, 26 and 28 triterpenes in the extracts obtained by ME, UAE and SE, respectively. These results show the potential of *G. lucidum* as a source of bioactive compounds pointing out for their use in several industrial applications.

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OC-43

Unveiling the chemical composition of willow added-value lipophilic extractives by gas chromatography-mass spectrometry

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Willow (*Salix* spp.) biomass has aroused particular attention, due to its energy applicability, representing a renewable and sustainable alternative to fossil fuels [1]. Moreover, *Salix* shrubs are recognized for antipyretic, anti-inflammatory and analgesic actions [2]. In addition to salicin, other phenolic compounds are described in their bark, namely *p*-hydroxybenzoic acid, fragilin and vimalin [3,4]. The economic value of *Salix* spp. can be improved by exploiting lipophilic extracts potential, but the knowledge of their composition is scarce [5]. Therefore, the present study aims at the detailed chemical characterisation of lipophilic extractives, derived from *S. atrocinerea*, *S. viminalis* and *S. fragilis* bark and wood, by gas chromatography-mass spectrometry.

Aromatic compounds, pentacyclic triterpenes and sterols were found in *Salix* spp. dichloromethane extracts. In fact, *S. atrocinerea* bark exhibited the highest aromatic compounds content, whereas bark and wood of *S. fragilis* and *S. viminalis* were richer in pentacyclic triterpenes. Piceol, lupeol and β-sitosterol (Figure 1) were among the most abundant components. Taking these findings into account, novel food and nutraceutical perspectives may be traced towards *Salix* spp. valorisation.

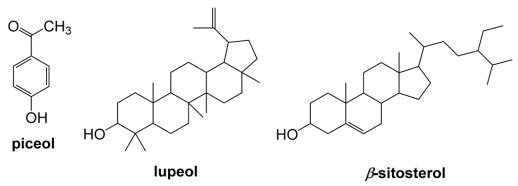

Figure 1. *Structures of the main lipophilic compounds identified in Salix spp. bark and wood.*

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OC-44

Application of anti-hail net in apple orchards: effects on fruits chemical characteristics

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Apple production in Portugal, over the last 15 years, has been subjected to increasing crop damage due to hailstorms and related meteorological extreme conditions. This problem has led farmers and researchers to find practical solutions to protect the orchards, such is the case of netting devices. The application of anti-hail nets in apple tree orchards looks to provide protection against the roughest meteorological events (such as hail, strong winds, and sunburn), while avoiding to upset the development of the plant and or, if possible, helping to improve the plant's productivity.

During 2016, this work was carried out in Carrazeda de Ansiães, a northeast Portuguese plateau zone that is a primary location for apple production, and the application of a grey anti-hail net, which reduces photosynthetically active radiation by 12 %, was tested in an orchard with the cultivars Golden delicious and Fuji of apple tree (*Malus domestica* Borkh). Control without screen net applied was also used. In order to understand the effects of the net, apples were collected and analysed for their chemical characteristics (total phenols, *ortho*-diphenols and flavonoids content, ABTS and polyphenolic profile).

The obtained results in apple peel revealed, in both cultivars, a decrease in total phenols, ortho-diphenols and flavonoids concentration, whereas in the Fuji cultivar the antioxidant activity, determined by the ABTS assay, was maintained. Pulp results showed little difference with lower ($P<0.001$) *ortho*-diphenols content in Fuji cultivar and slightly lower ($P<0.05$) ABTS activity in Golden delicious cultivar. Peel and pulp methanolic extracts were analysed by HPLC-DAD. The phenolic profile was similar for both cultivars, with the exception of anthocyanins, being identified chlorogenic acid and derivatives of quercetin. The anti-hail net did not affect the phenolic profile, only decreased the polyphenols concentration.

The use of a grey anti-hail net on apple orchards is a suitable alternative for the protection of apple trees against hail ensuring the production of the crop without compromising fruit quality.

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OC-45

Characterization of the volatile composition of encapsuled coffee

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The coffee capsules have allowed to facilitate and democratize the consumption of coffee, simplifying the extraction process for home consumption. The quality and consistency of the encapsulated coffee extraction process allows obtaining the exact amount of ground coffee and the desired arabica/robusta blend, simplifying the extraction process for consumption.
For this purpose, the volatile composition of the various mixtures of encapsulated coffee was studied. Based on the study of the volatile composition of the 222 volatile compounds detected (tentatively identified compounds), the possibility of discriminating different mixtures in capsules based on the Arabica / Robusta mixture ratio, geographic origin and capsule sets is proposed.
The study was performed using headspace analysis by solid phase microextraction (HS-SPME) and subsequent gas chromatography coupled to mass spectrometry (GC/MS) analysis, the multivariable and hierarchical clusters analysis was then used for the determination of volatile compounds that characterized each of the blends.
In this work it was observed that between blends, there are differences based on the geographical origin, mixture of arabica/robusta and temporal degradation. These differences were detected based on PCA and HCA, and it was verified that with the normalization of the total area it is possible to distinguish arabica/robusta mixture (87.22% of total variance) and with normalization of base 10 logarithm a separation occurs By lot type, geographical origin and arabica/robusta mixture (57.82% of total variance).

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OC-46

Increased productivity in impurity profile characterization of innovative pharmaceuticals

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The quantification of an Active Pharmaceutical Ingredient (API) and the determination of its impurity profile is commonly performed using High Performance Liquid Chromatography (HPLC) methods [1]. The majority of related impurities have a similar chemical structure to the API itself. However, in some cases, they may be conjugated with additional chemical entities that confers a significantly different polarity to these impurities. Therefore, these may have a higher affinity to the stationary phase, leading to time consuming methods.

The impurity profiles characterization is described in several pharmacopeias for a higher number of APIs. In the specific case of the API covered in this work, five impurities are described in the correspondent European Pharmacopeia monograph. One of the impurities has a relative retention time that is about 23 times higher in comparison to the API. For this reason, the method described in Eu.Phr.monograph has a total run time of 75 minutes. During method assessment, it was noticed that all impurities were eluting at the beginning of the run and only one impurity was observed at the end, with a gap of more than 50 minutes without any impurity elution. This extended run time turns it into a very time consuming method.

As the method was going to be used as a quality control tool in the analytical laboratories, method re-development was conducted with the goal of reducing the total running time. HPLC conditions were adjusted to an Ultra Pressure Liquid Chromatography (UPLC) system. Different gradient programs, mobile phase flows and column temperatures were tested. Selection of adequate conditions led to a total running period of 20 minutes. Additionally, apart from the characterization of the API impurity profile, same method conditions were successfully applied to drug product characterization, namely blend homogeneity, drug product impurity profile and drug substance load in the final drug product.

In addition to a reduction in the running time of about 73%, the new developed method is more sustainable and cost effective, as the amount of organic solvents used is significantly lower.

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OC-47

Characterization of phospholipids, including plasmalogens, in bivalves of the Portuguese coast using solid-phase extraction followed by gas-liquid chromatography

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Despite the existence of research involving the lipid characterization of bivalves, there are no much studies about the bivalves of the Portuguese Coast, particularly regarding the lipid characterization of plasmalogens. Plasmalogens are phospholipids existing in cell membranes, which seem to be relevant to human's health and sickness due to their biological functions. The goal of this study was to determine the lipid profile, especially plasmalogens, and characterize the fatty acids (FA) and dimetyl acetals (DMA) of several bivalves of the Portuguese coast, namely Japanese carpet shell (*Ruditapes phillippinarum*), peppery furrow shell (*Scrobicularia plana*), cockle (*Cerastoderma* spp.), mussel (*Mytillus* spp.), oyster (*Crassostera* spp.) and razor shell (*Ensis* spp.) in order to verify if bivalves are a good nutritional source of plasmalogens.

Lipids from bivalves were extracted using dichloromethane and methanol (2:1), and then plasmalogens were isolated by hydrolysis using phospholipase A1 from *Aspergillus orizae* according to Mawatari *et al.* [1]. Next, lipid classes were fractionated into cholesteryl esters (CE), triacylglycerols (TAG), free fatty acids (FFA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) by solid-phase extraction (SPE) using NH_2-aminopropyl cartridges. Fatty acid methyl esters and DMA were prepared from each lipid fraction by basic followed by acid methylation [2]. Finally, FA methyl esters and DMA were identified by gas-chromatography mass spectrometry (GC-MS) and quantified by gas-chromatography with flame ionization detection (GC-FID).

The results showed that the FA composition on the plasmalogens is variable among bivalves. However, all of them presented an large abundance of 16:0 and 18:0, as well as, the presence of very long chain polyunsaturated FA, such as EPA and DHA, and a variable amount of non-methylene interrupted polyunsaturated FA. Regarding the DMA, the most common was the 18:0. The separation of lipid classes allowed the determination of lipid conversion factors for bivalves. Our results also showed that mussels was the bivalve with the highest content of plasmalogens per edible fraction (58,6 mg/100g).

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OC-48

Characterization and Identification of Four Essential Oils by GC-MS

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An essential oil is a volatile aromatic compound found in plants, such as seeds, bark, stems, roots, flowers and other parts. The nature of the essential oil varies from plant to plant within botanical families and from species to species. Essential oils have been used for thousands of years in various cultures for medicinal to health purposes. For example, aromatherapy, household cleaning products, personal beauty care and natural medicine treatments. Essential oils are several important properties like anticancer, antiviral, antibacterial, antimicrobial, anti-inflammatory and antioxidant [1].
Gas chromatography coupled to mass spectrometry (GC-MS) is an important analytical technique to study and analyze complex mixtures as essential oils. In general, essential oils are complex mixtures of organic compounds constituted by terpenes (monoterpenes and sesquiterpenes) and terpenoids (monoterpenoids and sesquiterpenoids) [2]. The difference between the two type of organic compounds is that terpenes are hydrocarbons and terpenoids are oxygen containing hydrocarbons.
In this work, we characterized and identified by GC-MS the components of several essential oils: *Cedrus Atlantica*, *Cupressus Funebris*, *Corymbia Citriodora* and *Eucalyptus Radiata*. The equipment used was a Trace GC Ultra coupled to an ITQ 900 mass spectrometer with an automatic injector Triplus Rsh from Thermo Scientific. The results were acquired and processed by Xcalibur (version 1.2) from Thermo Scientific). All the peaks were identified by comparison with literature and multiple libraries, such as NIST, Mainlib and Wiley 6.

Figure 1. *Plants of: Cedrus Atlantica, Cupressus Funebris, Corymbia Citriodora and Eucalyptus Radiata (left to right).*

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J. Sá Morais

PC
Panel Communications
Comunicações em poster

PC-01

Optimization of an HPLC analysis to study the interactions between a *Saccharomyces cerevisiae* protein-rich extract and wine procyanidins

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One of the main goals of the wine sector is to improve wine quality, elaborating wines that satisfy consumer demands and expanding the offer of quality wines. Wine stabilization and limpidity is obtained after winemaking through the precipitation of unstable compounds and the sedimentation of the clouding particles. Stabilization and clarification processes occur naturally by several physical and chemical phenomena but they are slow and may not be enough for proper clarity and stability of the wine [1]. Therefore, these processes are often improved by using different agents that will interact with wine components [1]. Clarification using fining agents can reach a better limpidity in less time and also improve the stability of wines. Additionally, protein based fining agents could induce some organoleptic changes. The fining process occurs directly from the precipitation of proanthocyanidins induced by the proteins. Gelatin, β-Lactoglobulin, ovoalbumin and casein are the proteins from animal origin most used as fining agents but most of them have been widely related with the incidence of food allergies [1]. Hence, alternatives to traditional wine fining agents are being developed and among these, enological yeast protein extracts (YPE) are very promising. Indeed, natural yeast proteins have recently attracted the attention of enologists, not only for their well-known effect on wine stability but also for their positive influence on a number of technological and quality properties of red wines.

We are working on the characterization of a new YPE to be used as a fining agent. Our major goal is to fully characterize this product and study its effect on wines' clarification and improvement of organoleptic properties. So far, a High-Performance Liquid Chromatography (HPLC) method was optimized to characterize the proteins present in the YPE, as well as to study the protein-procyanidins interactions. Proteins from YPE have been precipitated after binding with wine procyanidins and the chromatographic profile of both YPE proteins and wine procyanidins have been highlighted. Indeed, qualitative and quantitative changes have been observed resulting on protein-procyanidins interactions. Furthermore, the effect of lyophilization and purification of the extract has also been analyzed. Results derived from this study mimic the phenolic changes induced in wine after fining by YPE.

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PC-02

Phenolic compounds from *Annona muricata* L.: HPLC-DAD analysis of the aqueous extract and nanoformulations

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Annona muricata L. is a species native to South America. Decoctions and infusions prepared from its leaves are used worldwide to treat several ailments, including brain disorders (e.g. seizures, anxiety, depression and insomnia) [1]. Under a screening study of medicinal plants with claimed antidepressant-like activity, *A. muricata* decoction stood out as a moderate monoamine oxidase A inhibitor [2] and strong reactive oxygen species scavenger. In order to protect this extract from gastrointestinal biotransformation and to improve its permeability across the blood-brain barrier, four mApoE modified phospholipid nanoformulations were produced, namely, liposomes and phytosomes with and without cholesterol. Aqueous extract and nanoformulations were characterized by HPLC-DAD, revealing the presence of catechin, 5-*O*-caffeoylquinic acid, epicatechin, epicatechin-3-*O*-gallate, *p*-coumaric acid, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside. Four other compounds with UV/vis spectra characteristic of flavonol derivatives were detected but not fully identified. Although some alkaloids and acetogenins are reported to be present in this species, such compounds were not detected in our extract by neither chromatographic procedures nor precipitation methods.

These results reinforce the idea of the neuroprotective effect of dietary phenolic compounds, and give further insights about a multitarget approach for the treatment of depression based on plant extracts.

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PC-03

Fatty acid profile of seaweeds from the North Portuguese Coast

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Seaweeds have been consumed worldwide for centuries due to their nutritional content in proteins, peptides, vitamins, fibre, trace minerals and specific lipids, among others [1]. Although seaweed consumption in Portugal is traditionally low, its inclusion in the diet has been increasing.

When compared with other food products, seaweeds usually exhibit a relatively low percentage of lipids (1-5% per dry weight) [2]. However, their polyunsaturated fatty acid (PUFA) content, usually associated with a lower occurrence of chronic diseases such as diabetes, obesity and heart diseases, can be higher than in terrestrial plants [3, 4].

In this work, the FA profile of ten seaweed species (*Ascophyllum nodosum* (AN), *Chondrus crispus*, *Fucus spiralis*, *Gracilaria* sp (GR), *Laminaria ochroleuca*, *Osmundea pinnatifida*, *Porphyra* sp, *Saccorhiza polyschides* (SP), *Ulva* sp (UL) and *Undaria pinnatifida* (UP)), collected along 4 beaches in the Portuguese North Coast, was evaluated. Lipids were extracted with Soxhlet and subsequently derivatized and analysed by GC-FID.

The FA profile within each species presents variations with the geographical location and harvest season, as well as storage time [5]. The saturated, monounsaturated and polyunsaturated fatty acids showed higher values for GR in spring/summer, AN in both seasons, and UL in autumn/winter, respectively. The omega-3 and omega-6 PUFA were higher for UP and SP, respectively, both in autumn/winter.

The lipid quality index (LQI) varied among the species and, within the same species, according to season. From the species studied, *Ascophyllum nodosum* presents the best LQI, thus supporting its inclusion in a healthy balanced diet.

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PC-04

GC-MS identification of oligosaccharides produced by nonenzymatic transglycosylation reactions

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Carbohydrates are the main constituents of honey (95% of honey dry weight), from which 5 to 10 % are oligosaccharides [1]. It is known that honey maturation conditions, such as high sugar concentrations in acidic media, induce condensation of carbohydrates [2]. Therefore, nonenzymatic transglycosylation reactions could occur in honey, as well as in solutions mimicking these conditions, resulting in the formation of oligosaccharides. In order to validate this hypothesis, six aqueous model solutions (moisture content of 20 %) containing sucrose plus glucose, and sucrose plus fructose were prepared using water, diluted citric acid at pH 4.0 and at pH 2.0; and were kept at 35 ^{0}C. Electrospray ionization mass spectrometry (ESI-MS) analysis revealed the occurrence of oligosaccharide synthesis with a degree of polymerization (DP) up to 6 after 5 months. Ligand-exchange/size-exclusion chromatography (LEX-SEC) performed the separation of the oligosaccharides with different DP. The identification of the carbohydrates was accessed with gas-chromatography coupled to mass-spectrometry (GC-MS), due to its sensitivity for this type of compounds. Thus, oligosaccharides were derivatized to alditol acetates to produce volatile molecules amenable to GC-MS analysis. Derivatives such as 1,3,4,6-tetra-*O*-acetyl-β-D-fructofuranosyl-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (sucrose octaacetate), 1,2,3,5,6-penta-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranose (maltose nonaacetate) and 1,3,4,6-tetra-*O*-acetyl-β-D-fructofuranosyl-3,4,6-tri-*O*-acetyl-β-D-fructofuranosyl-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (1-kestose undecacetate) were found in the majority of model solutions, showing the presence of sucrose, maltose, and 1-kestose. Furthermore, glycosidic-linkage composition was also determined from partially methylated alditol acetates (PMAA). The analysis of PMAA by GC-qMS showed a greater proportion of terminally-linked glucose (Glc) and fructose (Fru) residues, observed as 1,5-di-*O*-acetyl-(1-deuterio)-2,3,4,6-tetra-*O*-methyl-D-glucitol and 2,5-di-*O*-acetyl-(2-deuterio)-1,3,4,6-tetra-*O*-methyl-D-mannitol/glucitol, respectively. Other PMAA were found in lower amounts, which were diagnosed as (1→2)-, (1→4)-, (1→6)-Glc and (2→1)-, (2→6)-Fru. This data was the key to infer about the possible oligosaccharides produced in the designated conditions. In conclusion, synthesis of oligosaccharides occurs in solutions undergoing similar conditions as those of honey during ripening.

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PC-05

Chemical characterization of three *Thymus* species: *T. herba-barona*, *T. pseudolanuginosus* and *T. caespititius*

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The genus *Thymus*, belonging to *Lamiaceae* family, is rich in medicinal and aromatic species and well-known by several health promoting activities [1,2]. Despite this genus has been extensively studied, some species remain unexploited. In this study, *Thymus herba-barona*, *Thymus pseudolanuginosus* and *Thymus caespititius* decoctions were screened for their phenolic constituents by ultra-high performance liquid chromatography coupled to diode array detector and an electrospray mass spectrometer (UHPLC-DAD-ESI-MSn) operating in negative mode.

The three aqueous extracts were rich in caffeic acid derivatives, mainly rosmarinic acid (MW 359) and its structural isomers, that accounted for 55.8 ± 2.8 mg/g in T. herba-barona and 40.2 ± 0.9 and 43.2 ± 3.2 mg/g in *T. pseudolanuginosus* and *T. caespititius*, respectively. In turn, other depsides were differently distributed in the three Thyme extracts: while dihydro-salvianolic acid B (MW 716 Da) and caffeoyl rosmarinic acid were particularly representative in *T. herba-barona*, salvianolic acids K ([M-H]⁻ at *m/z* 555 493 359) and B ([M-H]⁻ at *m/z* 717 519 475) were found in moderate amounts in *T. caespititius* extract. On the other hand, *T. pseudolanuginosus* was clearly distinguished by its richness in the flavone luteolin-O-glucuronide ([M – H]⁻ at *m/z* 461→285).

Overall, this work is an important contribution for the phytochemical characterization of these three *Thymus* species, which are poorly explored.

Table 1. *Identification and quantification of main UHPLC eluting fractions by UHPLC-DAD-MSn of T. herba-barona, T. pseudolanuginosus, and T. caespititius aqueous extracts.*

	[M-H]⁻	T. h-b	T. p	T. c
Rosmarinic acid	359	55.8 ± 2.8	40.2 ± 0.9	43.2 ± 3.2
Luteolin-O-glcuronide	461	10.5 ± 0.2*	54.1 ± 0.6	17.3 ± 1.1
Dedihydro-Salvianolic Acid B	715	10.8 ± 0.1	-	-
Salvianolic Acid B	717	-	-	6.9 ± 0.5
Salvianolic Acid K	555	D	-	10.5 ± 0.1
Caffeoyl Rosmarinic acid	537	10.5 ± 0.06	D	D

**Structural isomer; D: detected*

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PC-06

Phytochemicals of *Salvia africana* and *Salvia elegans* and *Salvia officinalis 'Icterina'*

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Salvia species are used worldwide for medicine purposes. In general, these medicinal plants have high amounts of flavonoids and phenolic acids that are thought to be closely related to their health properties [1,2]. Albeit that, the phenolic composition of many *Salvia* species remains unclear. In this work, aqueous extracts from *S. africana*, *S. elegans* and *S. officinalis 'Icterina'* were prepared in order to elucidate the phenolic composition by high performance liquid chromatography with diode array detector coupled to an electrospray ionization mass spectrometer (HPLC-DAD-MSn).

Similarly to other *Salvia* species [2], these extracts were rich in rosmarinic acid (UV max 290, 328 nm) as well the flavone luteolin-O-glucuronide (MW 462) (UV max 250, 267, 345 nm). In addition, the extracts of *S. africana* and *S. officinalis 'Icterina'* contain moderate amounts of danshensu ([M-H]$^-$ at *m/z* 395→197→179, UV max 280 nm) while *S. africana* have also present yunnaneic acid D ([M-H]$^-$ at *m/z* 539→297, 359, 179, UV max 270 nm). The extract of *S. elegans* is composed by the caffeic acid derivatives 3′-*O*-(8″-Z-caffeoyl) rosmarinic acid and lithospermic acid B ([M-H]$^-$ at *m/z* 717→, 519, 475, 537, UV max 290, 337 nm). This latter compound that is also present in *S. officinalis 'Icterina'* extracts together other depside called salvianolic acid K (MW 556) detected in moderate quantities.

This work is an important contribution for the phytochemical characterization of these three *Salvia* species.

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PC-07

Applying an API HPLC Related Substances Monograph Method to an Inhalation Drug Product

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Pulmonary drug delivery has been widely studied according to the potential to produce maximum therapeutic benefit to patients by directly targeting drug to the site of pathology in the lungs [1]. Due to this fact, the use of a low dosage of Active Pharmaceutical Ingredient (API) is enough to achieve the therapeutic effect, which classifies the product as highly potent. In terms of manufacturing and analysis, the use of potent products is a challenge in what concerns Safety. Thus, it is occasionally necessary to use model molecules (instead of the highly potent API), in order to create safe analysis procedures which will be later applied to the potent product. Additionally, inhalation products are composed of a low dosage of API in a large amount of excipient and, therefore, the analytical methods must be equally suitable for the API determination and for the general formulation (which includes taking into consideration all the manufacturing steps).

The aim of this work was the development of a new High Performance Liquid Chromatography (HPLC) method for the determination of the Acetylsalicylic Acid, as a model molecule, in an inhalation drug product. The official European Pharmacopoeia (Ph. Eur.) suggests the determination of the Related Substances of this drug by HPLC with ultraviolet detection. As Salicylic Acid is a degradation product of Acetylsalicylic Acid, the method must be able to distinguish and quantify this component too. The different components of the inhalation product were taken into consideration: powder (composed of API and excipients), capsules (where the powder is filled into) and device (used for the product administration to the patient). The method must be suitable, capable of quantifying API dosages as low as 0.050 µg/mL and selective in relation to excipients and other formulation/administration components.

Considering the above requirements, based on the method presented for the API on the Ph. Eur. Monograph, a new HPLC method was developed to quantify the API and its major degradation product, salicylic acid, as well as to identify the excipients. The main differences between the Ph. Eur. monograph and the developed method were the diluent and the run time. The Ph. Eur. Method describes the use of acetonitrile 100% as diluent and a mix of acetonitrile and water (50:50) was applied in this work, to allow dissolution of all drug product components. As an advantage, the run time was reduced and the selectivity of the method was achieved for all formulation components. Additionally, a limit of quantitation (LOQ) was determined and method's linearity was proven during the study. The purpose of this study was achieved and it is demonstrated that it is possible to adapt an API monograph method to a formulation, by changing only a few parameters.

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PC-08

Perfil cromatográfico em ácidos gordos de seis genótipos de *Portulaca olerace* L.: uma fonte alternativa de ómega-3

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Portulaca oleracea L. é conhecida vulgarmente como beldroega comum e é uma planta de folhas suculentas e flores coloridas pertencente à família das Portulacaceae. Apesar de ser considerada uma erva daninha, é bastante apreciada e consumida crua, em saladas ou cozinhada; as suas folhas têm um sabor ligeiramente ácido e salgado [1]. Contém vários compostos bioativos, nomeadamente ácidos gordos ómega-3, que são considerados benéficos nos distúrbios cardíacos [2].

Neste trabalho, estudou-se o perfil em ácidos gordos de seis genótipos de beldroegas: três ecótipos silvestres do mar Cáspio, região do Irão (genótipo A, B e C das cidades de Sari, Gorgan e Aliabad, respetivamente), uma variedade local proveniente da região de "Domokos" na Grécia central (genótipo D) e duas cultivares comerciais de beldroega comum de Gemma S.A. (genótipo E) e beldroega verde escura (genótipo F). O perfil individual em ácidos gordos foi determinado por cromatografia gasosa acoplada a um detetor de ionização de chama (GC-FID).

Os ácidos gordos mais abundantes nas amostras estudadas foram o ácido palmítico (C16:0), o ácido oleico (C18:1), o ácido linoleico (C18:2n6) e o ácido alfa-linolénico (C18:3n3), com diferenças significativas nos teores detetados em cada um dos diferentes genótipos avaliados. O genótipo D apresentou uma maior percentagem relativa de ácido alfa-linolénico. O genótipo E revelou possuir uma composição equilibrada de ácidos gordos ómega-3 e ómega-6. As variedades comerciais (genótipos E e F) revelaram perfis de ácidos gordos bastante similares aos dos genótipos B e C. Assim, o uso da diversidade genética para o desenvolvimento de cultivares de qualidade e alto rendimento em ácidos gordos ómega-3 deve ser considerado com vista à sua potencial utilização como alimento funcional.

Acknowledgements:
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PC-09

Fatty acids profile contribution for the discrimination of olive oil production year

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Olive oil is a highly appreciated food product mainly due to its nutritional and healthy properties. Olive oils (cv. Arbequina) produced during 4 consecutive crop years were evaluated regarding some quality attributes, fatty acids (GC-FID), and tocopherol (HPLC-FLD) compositions, total phenols contents (Folin–Ciocalteu) and radical scavenging activities (DPPH and ABTS). The results showed that, based on all parameters it was possible to split the olive oil according to the production year, using Principal Component Analysis (PCA). Also, Linear Discriminant Analysis (LDA) together with the simulated annealing (SA) variable selection meta-heuristic algorithm showed that the contents of C16:0, C16:1, C17:0, C18:1, C18:2, C18:3, C20:0, C20:1, SFA and PUFA allowed discriminating the olive oil according to the production year (Figure 2), pointing out that fatty acids composition was greatly affected by the production year. Indeed, a predictive overall mean sensitivity of 99.6% was achieved using a repeated K-fold cross-validation procedure (4 folds × 10 repeats).

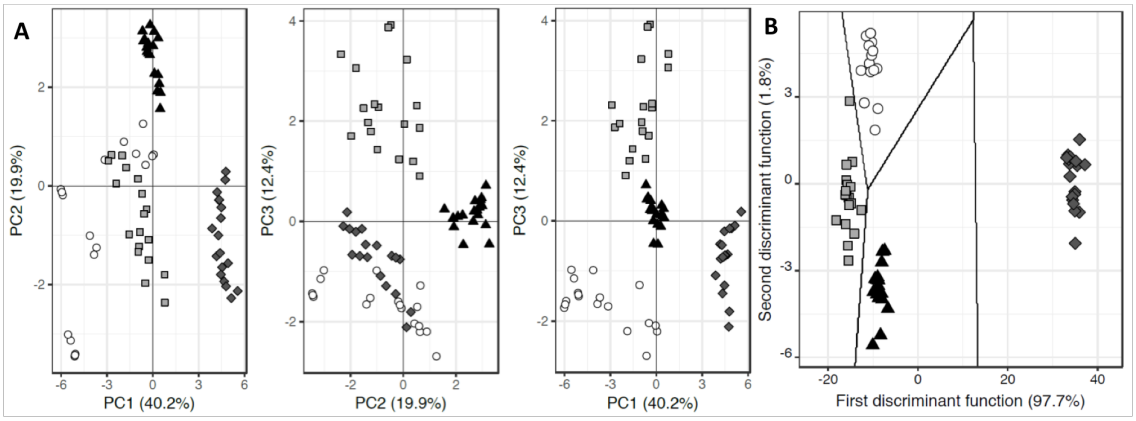

Figure 1. *(A) PCA showing the olive oil split according to the production year, using all variables evaluated. (B) LDA: olive oil grouped by production year based on the fatty acids profile (C16:0, C16:1, C17:0, C18:1, C18:2, C18:3, C20:0, C20:1, SFA and PUFA)*

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PC-10

Monitoring fructooligossacharides production using *Aspergillus aculeatus* by HPLC-ELSD

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Fructooligosaccharides (FOS) are present in plants and fruits at low concentrations, thus their extraction from natural sources may not be economically viable for a large scale industrial application. Therefore, FOS production by fermentation using fungi can be an alternative. In this work, FOS were produced using *Aspergillus aculeatus* at different sucrose initial concentrations (88 to 265 g/L) and at temperatures from 22 to 32ºC. FOS production was monitored by HPLC-ELSD, allowing to confirm that the initial sucrose concentration significantly influenced biomass growth (a maximum value of 16 ± 2 g was achieved) although it did not significantly affect the maximum FOS yield (amount of FOS produced per initial sucrose) obtained, which varied from 51 to 59 g/g) obtained, which varied from 51 to 59 g/g. Finally, the preliminary results enabled verifying that depending on the fermentation conditions, slightly different FOS production profiles were obtained (Figure 1), revealing differences in the individual FOS concentrations (i.e., 1-kestose, nystose and fructofuranosylnystose), which could be of interest since it has been reported that the beneficial health effects of FOS may depend on the relative FOS composition.

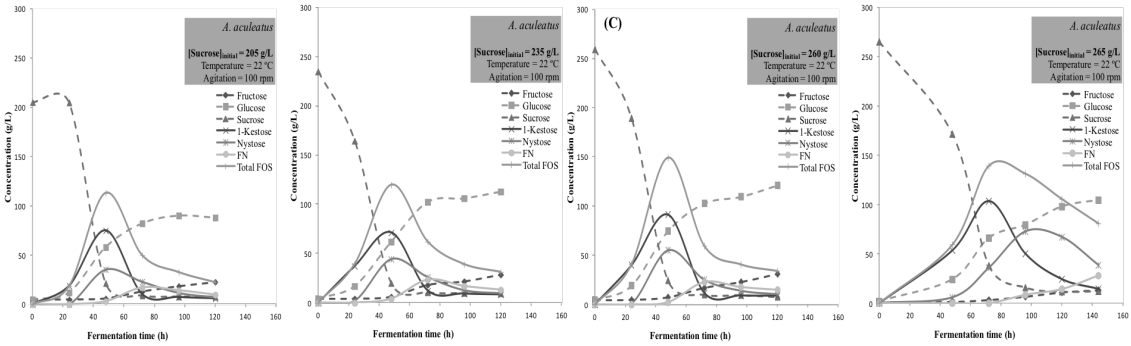

Figure 1. *Concentration profiles of substrate (sucrose) and fermentation products (glucose, fructose, 1-kestose, nystose, fructofuranosyl nystose and total FOS) determined by HPLC-ELSD.*

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PC-11

Selection of spme fiber for the identification of the pheromone rhynchophorol by GC/MS

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The use of pheromones is gaining ground together with integrated pest management techniques, increasing the efficiency of control methods with the capturing of insects employing funnel traps[1]. The aggregation pheromone rhynchophorol (6-methyl-2(E)hepten-4-ol) is used to attract the insect *Rhynchophorus palmarum* L. (South American palm weevil), a pest present in palm cultures and the main vector of the nematode *Bursaphelenchus cocophilus*[2]. Since this compound is an 8-carbon aliphatic alcohol its evaporation rate is high and, as a result, it is easily dispersed in the environment, hindering its identification and additional techniques for its concentration may be required. The objective of this study was to seek an analytical technique for the identification of the pheromone rhynchophorol at low concentrations in its volatized form. Samples (1 µL; ±0.78 mg) were placed in 20 mL headspace vials and analyzed on a PerkinElmer gas chromatograph (model Clarus 680) coupled to a PerkinElmer quadrupole mass spectrometer (model Clarus 600C). Injections were carried out using four SPME fibers (PDMS/DVB, DVB/CAR/PDMS, CAR/PDMS and PA) with the pheromone adsorbed, employing a CombiPal automatic injector (model CTC CombiPal). The results obtained show that the SPME fiber coated with DVB/CAR/PDMS had a better affinity with the pheromone rhynchophorol, as verified through the larger peak area, when the four fibers were compared. Through the carrying out of this study, it was possible to identify the best SMPE fiber (DVB/CAR/PDMS) for use in future analytical techniques for the determination of the pheromone rhynchophorol, particularly for concentration stages.

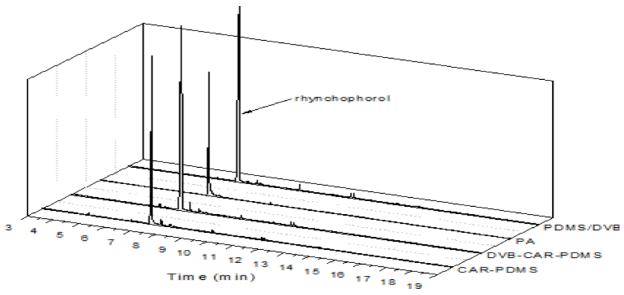

Figure 1. *Chromatograms between SPME fibers evaluated*

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PC-12

Similaridade da farinha da casca do maracujá amarelo (*Passiflora edulis flavicarpa*) com pectina e ácido galacturônico comerciais por CLAE/IR

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O Brasil é considerado o maior produtor mundial de maracujá amarelo (*Passiflora edulis flavicarpa*) [1]. A industrialização do fruto do maracujá gera por ano aproximadamente 54 mil toneladas de resíduos, tais como sementes e cascas [2]. As cascas são ricas principalmente em pectina e fibras [3,1]. A pectina apresenta uma complexa cadeia constituída sobretudo pelo ácido galacturônico, e seu emprego na indústria alimentícia está associado especialmente às suas propriedades espessante e geleificante [4]. O objetivo deste trabalho foi determinar a similaridade da pectina presente na farinha do maracujá visando posterior aplicação industrial, por técnica de exclusão molécular/CLAE – IR, comparando com padrões de ácido galacturônico e pectina cítrica. Para avaliação, foi utilizado um sistema de cromatografia por exclusão de tamanho CPC-CLAE (PerkElmer), utilizando colunas Shodex SB 803, 804, 805 e 806 conectadas em série e acopladas a um detector de IR. Solução de $NaNO_3$ a 0,05 M a fluxo de 1,0 $mL.min^{-1}$ foi utilizada como fase móvel do sistema. As amostras da farinha e da pectina sigma foram diluídas em água miliq à 80°C. Em seguida centrifugada 4,0 rpm por 10 minutos a 20°C. A parte sobrenadante das amostras foi injetada para análise. Na farinha da casca do maracujá, foi possível verificar semelhança com os padrões de ácido galacturônico e pectina (Figura 1). Em análises complementares foi possível obter 16,42% de pectina na farinha do maracujá, confirmando assim, que a farinha na forma bruta é rica em tal material. Através do presente trabalho, é possível concluir que a farinha da casca do maracujá amarelo, mesmo sem procedimento para extração da pectina, possui elevado teor de pectina e ácido galacturônico, tornando este rejeito uma nova matéria-prima de baixo custo a ser utilizada na indústria alimentícia como aditivo natural.

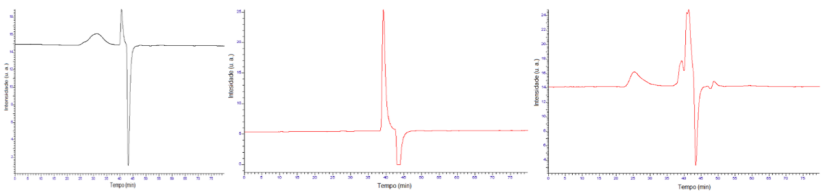

Figura 1. *Cromagramas de Exclusão da farinha de maracujá e padrão – (a) Pectina sigma; (b) Ácido Galacturônico Sigma; (c) Farinha diluída a 80°C.*

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PC-13

Optimization of an analytical method for the determination of underivatized triclosan and related compounds by gas chromatography-triple quadrupole mass spectrometry

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Triclosan (TCS), an antimicrobial agent, has been ubiquitously found in wastewater and sewage sludge. TCS may suffer transformation or degradation during the wastewater treatment. Some of the resulted products such as 2,4-dichlorophenol and methyl-triclosan are presumed toxic/persistent compounds.
Liquid chromatography has become one of the most widely used techniques for identify and quantify PPCPs in water samples, due to its high sensitivity, specificity and not limited by the non-volatility of compounds. However, gas chromatography (GC) offers better sensitivity and lower detection limits for complex sample investigations.
An effective and non-use of derivatization method for determination of TCS and related compounds in effluent from urban wastewater treatment plant was developed. The optimization of the method complies the different GC/MS/MS and MD-GC/MS/MS conditions: injector temperature, transfer line, source, collision gas pressure and energy, injection modes: split, splitless, pulse and liners. Full scan was also investigated, SIM and MRM mode. The range of concentration tests were: 1ppm to 1ppt.
This method will allow the fast and efficient environmental monitoring of TCS and related compounds in aqueous matrices.

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Project "Development of Nanostrutures for Detection of Triclosan Traces on Aquatic Environments" (PTDC/FIS-NAN/0909/2014). CENSE-Center for Environmental and Sustainability Research which is financed by national funds from FCT/MEC (UID/AMB/04085/2013). C. Magro acknowledges Fundação para a Ciência e a Tecnologia for her PhD fellowship (SFRH/BD/114674/2016).

PC-14

Development and validation of an HPLC method for quantification of the biocide Econea®

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Econea® has been suggested as a metal-free biocide for the antifouling (AF) market, due to its fast degradation and slow release mechanisms [1]. In order to quantify Econea® in artificial seawater (aSW), a method using solid phase extraction (SPE) followed by reverse-phase high performance liquid chromatography with diode array detector (RP-HPLC-DAD) was properly developed and validated according the ICH Guidance for Industry Q2 (R1) through several parameters, namely specificity/ selectivity, linearity, precision, accuracy, range, limits of detection (LOD) and quantification (LOQ) [2].
Artificial seawater (aSW) was spiked with Econea® in methanol and passed through SPE cartridges (OASIS® HLB 6cc) to concentrate the analyte. Following, the analyte was eluted with methanol, dried, and reconstituted with 200 µL of methanol. Econea® was quantified by RP-HPLC-DAD with a C18 column and an aqueous solution with 0.1 % of TFA and acetonitrile (45:55 *v/v*). Recoveries rate of 99 % were obtained and validation parameters obtained are depicted in Table 1.

Table 1. *Validation parameters of RP-HPLC-DAD method for determination of Econea® in aSW*

Linear regression	r^2	Accuracy (%)	RSD (%)	LOD (μM)	LOQ (μM)
y = 122742x – 148336	0.9999	105 ± 0.05	2.4 ± 2.2	0.2	1

RSD = relative standard deviation

Taking into account the several parameters, it was possible to conclude that the developed method was successfully validated for future studies concerning Econea®.

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PC-15

Efeito do processamento no perfil lipídico do feijão mangalô (*Phaseolus lunatus*) germinado

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As leguminosas têm um importante papel na alimentação humana devido ao seu valor nutritivo, especialmente quando associada aos cereais. A técnica da germinação, associada à elaboração de farinhas, tem sido aplicada visando melhorar o valor nutricional de cereais e leguminosas. Este trabalho teve como objetivo avaliar o efeito do processamento no perfil de ácidos gordos de feijão mangalô germinado. O processo de germinação foi realizado segundo Berni e Canniatti-Brazaca (2011) [1], com pequenas adaptações. Após a germinação, uma parte da amostra foi liofilizada e a outra submetida a secagem em estufa de ar forçado, à temperatura de 55 ºC, durante 5 horas. O perfil de ácidos gordos foi determinado por cromatografia gasosa com deteção por ionização de chama [2]. Para as amostras liofilizadas (MGL) e na forma de farinha (MGF), os ácidos gordos maioritários foram os ácidos linoleico (53,77 e 42,09%), palmítico (11,65 e 20,13%), ácido oleico (10,55 e 17,86%) e α-linolénico (14,25 e 8,76%), respetivamente. O teor total de ácidos gordos monoinsaturados foi de 11% (MGL) e 19% (MGF) e o dos ácidos gordos polinsaturados foi de 68% (MGL) e 51% (MGF). No MGF foi observada uma redução do ácido gordos linoleico e α-linolénico e um aumento da percentagem relativa dos ácidos palmítico e oleico quando comparado com o MGL. Esta diferença deve-se, possivelmente, à exposição dos ácidos gordos polinsaturados ao oxigénio durante o processo de elaboração da farinha. Com efeito, é possível concluir que o processamento da farinha provoca uma redução dos ácidos gordos polinsaturados no mangalô germinado.

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PC-16

Vitamin E profile of green (in natura) seeds from different species of legumes

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Legumes are an important protein source all over the world, especially in the poorest countries, and have attracted a lot of interest, since they are environmentally friendly crops [1]. They are also a good source of other nutrients and bioactive compounds, such as fiber, minerals and vitamins.

Vitamin E, an antioxidant fat-soluble vitamin, embraces four tocopherols (α-, β-, γ- and δ-) and the respective tocotrienols. Their consumption has been associated to the prevention of cardiovascular diseases and some eye pathologies [1, 2].

The aim of this work was to compare the vitamin E profile of green seeds (*in natura*) from four species of legumes, namely *Vigna unguiculata*, Vicia faba, *Cajanus cajan* and *Phaseolus lunatus*., all from Brazilian origin.

Seed lipids were obtained by Soxhlet using n-hexane as extraction solvent (4 h). The vitamin E profile was analysed by normal phase-high perfomance liquid chromatography with diode-array and fluorescence detection (Jasco, Japan). The chromatographic separation was achieved on a normal phase Supelcosil™ LC-SI (3 µm; 75 x 3.0 mm; Supelco, Bellefonte, PA, USA) according to Alves *et al.* [3]. The fluorescence detector was programmed for excitation and emission at 290 and 330 nm, respectively. The compounds identity was confirmed by comparison with individual standards and by their UV spectra. Tocol was used as internal standard and butylated hydroxytoluene was employed as antioxidant.

Only four vitamers were identified in the samples, namely α-tocopherol, α-tocotrienol, γ-tocopherol, and δ-tocopherol. Total vitamin E contents ranged from 1.3 to 5.3 mg/ 100 g (in a dry weight basis), with *C. cajan* and *V. unguiculata* presenting the lowest and highest values, respectively. γ-Tocopherol was the predominant vitamer found in *V. faba*, *C. cajan* and *P. lunatus*, while δ-tocopherol was dominant in *Vigna unguiculata* seeds. The seeds of *P. lunatus* contained significantly higher amounts ($p<0.05$) of α- and γ-tocopherol, compared to the other species. The minor vitamer present in the samples was α-tocotrienol (2-6% of total vitamin E).

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PC-17

RP-HPLC analysis of 21 amino acids in edible seaweeds from the Portuguese coast after OPA/FMOC derivatization

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The total and free amino acid profile of 10 edible seaweeds species (n=60) collected in the North Portuguese coast was evaluated. For this purpose, a reversed-phase high-performance liquid-chromatographic (RP-HPLC) method was optimized and validated. Samples (free and total hydrolysates) were derivatized with OPA-3MPA and FMOC-HCl, according to the protocol described by Heems *et al.* [1] with some modifications; norvalin was used as internal standard.

The combined OPA-3MPA/FMOC-HCl derivatization step was completed after 3 min and the RP-HPPLC analysis enabled a good separation of 21 amino acids within 35 min. This method had a wide working range from 0.0073-30 mg/L for each individual amino acid, and good linearity with regression coefficients greater than 0.987. Precision measured in terms of repeatability and reproducibility (% RSD) was below 8% for all the amino acids analyzed. The recoveries obtained after fortification at three concentration levels were in the range 75-102% and 78-103%, respectively for free and total amino acids. The amino acids threonine, histidine and serine showed the lowest values of recovery. Hence, this method was found to be suited for routine analysis of amino acid composition in the harvested seaweeds.

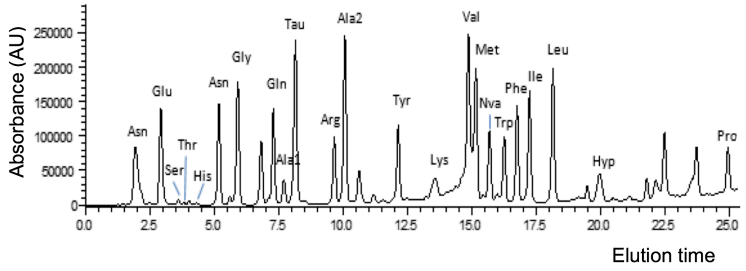

Figure 1. *Example of the RP-HPLC profile of amino acids of a brown seaweed (Fucus spiralis).*

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PC-18

Ion source-MS parameters optimization for pharmaceuticals compounds

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Pharmaceutical residues in the environment have been recognized as one of the emerging research areas in environmental chemistry [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics drugs are two of the most commonly used group of drugs worldwide [2].

The present work presents the application of the experimental design to improve the mass spectrometry (MS) signal of thirteen pharmaceuticals from the group of NSAIDs and analgesics drugs (Figure 1). Ion source factors (interface voltage, drying gas flow rate, nebulizing gas flow rate, heat block temperature, and desolvation line temperature) with significant effect on each compound MS response were identified through the Plackett-Burman design and then, complete experimental designs were applied to the significant factors. Full factorial and central composite face-centered designs were implemented to obtain the best ion source conditions that maximize the MS/MS signal [3].

It was observed that the maximum signal occurs when both interface voltage and nebulizing gas flow rate were set at level +1 for all pharmaceuticals.

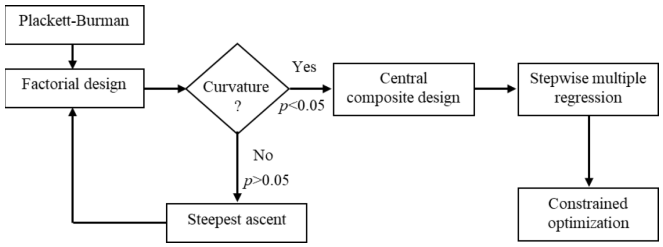

Figure 1. *Flow chart of the algorithm designed for mass spectrometry ion source parameters optimization.*

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PC-19

Perfil cromatográfico de ácidos gordos e açúcares em *cupcakes* funcionalizados com um extrato rico em acido rosmarínico

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Atualmente, a indústria alimentar interessa-se pela substituição de aditivos artificiais por ingredientes naturais. Alguns extratos vegetais têm surgido como possíveis alternativas aos conservantes artificiais, nomeadamente antioxidantes [1]. De facto, têm sido desenvolvidos produtos lácteos, cárneos e de panificação, incorporando extratos de plantas aromáticas, especiarias ou frutos, que apresentam propriedades antioxidantes [2-4]. Neste trabalho, pretendeu-se comprovar a eficácia de um extrato rico em ácido rosmarínico como conservante natural de *cupcakes*, comparativamente a um aditivo artificial (sorbato de potássio, E202). O extrato foi obtido a partir de *Melissa officinalis* L. (cidreira) pela técnica de ultrassons utilizando uma mistura de etanol:água como solvente de extração. Após comprovar as suas propriedades antioxidantes (efeito captador de radicais livres, EC_{50} = 79 ± 2 µg/mL; poder redutor EC_{50} = 49 ± 1 µg/mL), antimicrobianas (contra 8 bactérias e 8 fungos contaminantes alimentares) e ausência de toxicidade (em linhas celulares), procedeu-se à sua incorporação nos *cupcakes*. O seu efeito foi comparado com o E202, imediatamente após incorporação e ao longo de 3 e 5 dias de armazenamento no escuro à temperatura ambiente. Todas as amostras foram analisados cromatograficamente em termos de ácidos gordos (GC-FID) e açúcares livres (HPLC-RI).

Num total de 21 ácidos gordos identificados, os saturados predominaram sobre os insaturados em todas as amostras de *cupcakes*, sendo o ácido palmítico e o ácido oleico os maioritários. Relativamente aos açúcares, a sacarose e a glucose foram as moléculas identificadas nas amostras, sendo a quantidade de sacarose muito mais elevada. Os resultados obtidos demonstram que o extrato rico em ácido rosmarínico tem potencial para ser utilizado como aditivo natural em produtos de pastelaria, indo de encontro à atual tendência de procura dos consumidores.

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PC-20

Monitorização cromatográfica de um extrato de *Melissa officinalis* L. obtido com diferentes técnicas

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Devido à grande procura dos consumidores por uma alimentação cada vez mais saudável, a indústria alimentar tem-se interessado por alternativas de origem natural, nomeadamente provenientes de extratos vegetais, com vista à substituição de aditivos artificiais (1,2). No entanto, o desenvolvimento destes ingredientes naturais exige estudos preliminares que avaliem a melhor metodologia e condições de extração dos compostos de interesse (3,4).

Neste trabalho, pretendeu-se comparar três técnicas de extração de ácido rosmarínico a partir de *Melissa officinalis* L. (vulgarmente designada cidreira): extração por maceração, extração assistida por micro-ondas e extração assistida por ultrassons. Utilizou-se a metodologia de superfície de resposta para obter as condições que maximizam a extração do ácido rosmarínico (otimização do processo de extração). Como respostas utilizaram-se a quantidade de ácido rosmarínico (obtida por HPLC-DAD) e o rendimento de extração.

A extração por ultrassons mostrou ser a técnica mais eficaz, conduzindo a 86 ± 4 mg de ácido rosmarínico/g de massa seca de planta nas condições de extração ótimas (33 ± 3 min, 372 ± 19 W e 40 ± 1 % de etanol).

Os resultados obtidos destacam a espécie *M. officinalis* como uma fonte de ácido rosmarínico, bem como as melhores condições para a extração desta molécula, com um grande interesse de aplicação em matrizes alimentares.

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PC-21

Biogenic amine formation during smoking process of traditional Portuguese meat sausages *chouriças* and *alheiras*

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Smoked products are considered safe foods due to their reduced water activity and pH. However, food processing and preservation can also lead to the formation of chemical compounds that may have an impact on consumer's health [1]. Biogenic amines (ABs) can appear in foods by microbial decarboxylation of amino acids. Interest in these compounds arises not only because of their ability to exert toxicity and induce allergic reactions, but also because they are important markers of food deterioration. Thus, they become of great interest in food industry in order to evaluate the quality of the raw material and the impact of the production processes on the quality of the final product [2, 3].

This work evaluated the ABs content in smoked and non-smoked typical Portuguese meat sausages (*chouriça* and *alheira*). The aim of this study was to evaluate the impact of the smoking process on the final amine content and consequent overall quality of the mentioned products. The determination of ABs was performed after one-step salting-out assisted liquid-liquid extraction with derivatization and subsequent analysis by HPLC-DAD-ESI/ MS.

In general, the concentration of ABs increased during the smoking process. The most relevant ABs identified in smoked *chouriças* and *alheiras* were putrescine, cadaverine and tyramine, with maximum values of 1371 ± 70 µg/g and 196 ± 29 µg/g for putrescine in *chouriças* and *alheiras*, respectively; and minimum values of 213 ± 87 µg/g and 17 ± 3 µg/g for tyramine in *chouriças* and *alheiras*, respectively. Moreover, a higher amount of ABs was formed in *chouriças* than in *alheiras*, which may be related to the fact that the duration of the smoking process is three times longer for *chouriça* samples (15 days) and may lead to food degradation due to microbial activity. Though, the content of ABs found in smoked samples is below the toxicity values documented for these compounds (2000 µg/g) and does not represent a hazard. In conclusion, the salting out assisted method developed for ABs extraction from smoked sausages may help to control some critical parameters of their production.

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PC-22

No dilute" just shoot LC-ESI-MS/MS : feasibility and robustness of a maintenance-free source and interface for applications in low level pesticide residue analysis

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Os Benefícios e Consequências do use de pesticidas na agricultura industrial têm sido bem documentados nos últimos anos com um interesse concertado na biomonitorização de populações com riscos de exposição permanente. Por este motivo, o esforço contínuo na melhoria dos métodos analíticos é uma prática recorrente nos meios académicos, governamentais e institucionais. Neste estudo foi utilizado um equipamento de remoção de solvent por fonte de calor (HSID) para avaliar a possibilidade de eliminação de procedimentos de preparação de amostras na análise de pesticidas em sumos de frutas e vinhos. Com este pressuposto pretende-se uma maior produtividade e diminuição dos custos de implementação de métodos analíticos em laboratórios de controlo e segurança alimentar. Este estudo inclui um estudo de reprodutibilidade para a análise de amostras não preparadas para demonstrar a robustez do equipamento.

The benefits and consequences of pesticide use in the agriculture industry have been well reported in recent years with a concerted interest in biomonitoring populations for persistent exposure assessment. For this reason, efforts towards continuous improvement in analytical method development are standard practice in academic, government and industrial labs. In this study, a hot source induced desolvation (HSID) apparatus was employed to evaluate the potential of eliminating sample preparation in a pesticide analytical workflow for finished product fruit juices and wine samples. The anticipated impact would include higher throughput / lower cost method implementation in modern environmental surveillance /food safety laboratories. This work includes a repeatability study of no prep applications to demonstrate the robustness of this instrument platform.

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PC-23

Development and application of a fast HPLC method for dissolution evaluation of amorphous pharmaceuticals materials

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In the pharmaceutical industry, dissolution testing is widely used in formulation screening, for monitoring the manufacturing process during development stages, and as a routine quality control test for product release and stability studies of oral solid dosage forms.

In order to evaluate the dissolution performance of Amorphous Spray Dried Dispersion (ASDD) powders as well as of final dosage forms, such as granules, capsules and tablets, there is the need to have simple, fast and accurate analytical quantitative methods with broad application.

The UV-vis spectroscopy has been reported as the direct detection technique mostly applied to evaluate the dissolution performance because it is cheap, simple and somehow fast [1]. However, HPLC offers many advantages, such as better specificity, greater linear dynamic range and increased versatility, especially for early drug development when different formulations and strengths are screened. The goal is to create fast elution programs for dissolution testing, making the speed of HPLC competitive with UV analysis, and assists the implementation of automated on-line HPLC-based systems, to improve productivity and effectivity.

A simple, stepwise procedure that allows a fast elution, ideal for dissolution testing, was developed at Hovione. The proposed method was proven to be precise, accurate and selective for Active Drug Substance (API) quantitation during dissolution testing of an ASDD powder.

The composition of the mobile phase was a mixture of 0.05% TFA in water and 0.05% TFA in acetonitrile (20:80 *v/v*), at a flow rate of 1.0 mL/min, and a Waters XBridge C18 3.5µm 4.6 x 150mm column was used as stationary phase. The retention time of the API was 2 minutes for a total run time of 3 minutes. Linearity was obtained within an API concentration range of 2-200µg/ml and a %RSD of less than 2% was obtained during accuracy and precision evaluation.

Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method is successfully applied for dissolution performance evaluation of ASDD powders and also to the correspondent final dosage forms.

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PC-24

The effect of storage in HMF of Portuguese honey samples: a 4-year study

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Honey is a natural and sweet substance produced by honey bees, being quite appreciated and sought by consumers due its association to health benefits. It is essentially composed by different sugars (mainly fructose and glucose), water, and several bioactive compounds. The composition and properties of honey are dependent on several factors such as botanical and geographical origins, honey bee's species involved in its production, and environmental factors [1, 2]. Additionally, the processing, manipulation, packaging and storage time also contributes to honey properties [2].

One of the most important parameters for honey quality evaluation is the hydroxymethylfurfural (HMF) content. The presence of this compound in honey above the limit indicated by legislation (40 mg/kg) can be related to an improper processing or an inappropriate storage [3, 4]. Moreover, when present at high levels, this parameter can be related to adulteration with commercial sugar [5].

Aiming the evaluation of the storage effect on the HMF content of honey, fifteen samples were acquired directly from producers (in 2012) in labeled glass containers (500 g) in the North and Centre of Portugal. After purchase, the HMF was immediately analysed by HPLC-DAD. The influence of storage time on HMF was evaluated after 4 years of storage at 25ºC in the dark (in 2016). This period was selected considering the maximum storage validity mentioned on the labels of the analysed samples.

The HMF content in 2012 were typical of unprocessed and fresh honeys, being in agreement with the legislation for all samples. After 4 years of storage, the HMF content increased for all samples, although below the maximum limit of legislation, with exception of four samples. In general, the results showed that a storage time of 4 years, at 25ºC and protected from light, contributes to an increase of the HMF content, however not enough to discard honey in respect to this parameter.

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PC-25

Dairy products fortified with *Pleurotus ostreatus* beta-glucans

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Dairy products belong to widely used and beneficial foods in the world. Yogurt is a functional food product containing probiotic cultures and amino acids. Many manufacturers enrich yogurts with various vitamins, minerals and natural flavor additives. Yogurt is the popular base for functional products. Useful additives can be simply added into yogurt, but they can also impact on process of milk fermentation by lactic acid bacteria: *Lactobacillus bulgaricus* and *Streptococcus thermophilus* [1].

Pleurotus ostreatus is well-known and commercially important edible mushroom. Polysaccharides, possessing immune modulating activity obtained from *P. ostreatus*, are suitable candidates for R&D of new functional foods and nutraceuticals [2]. β-glucans, isolated from basidiomycetes, are of considerable interest due to their various useful preventive and functional properties to widely used ordinary food products, such as immune stimulation, hypoglycemic, antitumour, anti-inflammatory and hypoglycemic properties [3].

From submerged biomass of *P. ostreatus* different preparations with combination of ethanol and water extractions were obtained. We have studied effect of various fractions which contain water-soluble and water-insoluble polysaccharides. In all fractions β-glucans were determined using the assay kit (Megazyme, USA).

The influence of *P. ostreatus* preparations on the fermentation of milk by individual cultures of lactic acid bacteria: *L. bulgaricus* and *S. thermophilus* was studied. Then we made the yogurt using both cultures with adding of the preparations of *P. ostreatus*. As for products with individual cultures, as for yogurt products with combination of cultures – for all samples dynamics of titratable acidity and water-holding capacity were determinated.

The addition of polysaccharides positively affects the process of lactic acid fermentation, carried out by the studied cultures of microorganisms. The addition of polysaccharide preparations before the lactic fermentation stage reduces the fermentation period and improves the physico-chemical properties of fermented dairy products. The results obtained suggest the possibility of using fungal polysaccharides containing β-glucans to create functional foods based on fermented milk products and in a near future we are planning to use chromatographic processes in order to purify beta-glucans.

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PC-26

Efeitos de radiação ionizante no perfil fenólico de *Melissa officinalis* L. e de *Melittis melissophyllum* L.

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As plantas medicinais e os seus extratos ou compostos isolados têm várias aplicações na industria alimentar e farmacêutica, nomeadamente como ingredientes alimentares e/ou bioativos (como nutracêuticos ou em formulações de alimentos funcionais) [1]. No entanto, é necessária a aplicação de técnicas de descontaminação e conservação adequadas, com a finalidade de manter a sua qualidade salvaguardando sempre a saúde do consumidor [2]. Entre as tecnologias disponíveis, a irradiação tem suscitado um crescente interesse como um método de descontaminação e preservação viável e seguro [3]. Assim, neste trabalho avaliaram-se os efeitos da radiação ionizante (gama e feixe de eletrões, aplicando doses de 1 e 10 kGy) no perfil fenólico de infusões preparadas a partir de duas espécies bastante apreciadas e amplamente utilizadas no quotidiano - *Melissa officinalis* L. e *Melittis melissophyllum* L.. A análise do perfil fenólico individual de cada espécie foi feita por HPLC-DAD-ESI/MS. Em geral, observou-se um aumento dos compostos fenólicos individuais como resultado da irradiação, especialmente com raios gama. O ácido litospérmico A, em *M. officinalis*, e o ácido 5-*O*-cafeoilquínico, em *M. melissophyllum*, foram os compostos onde se observou de forma mais acentuada um aumento de concentração nas infusões preparadas a partir de amostras irradiadas. Estes resultados representam bons indicadores para a aplicação de ambos os tipos de radiação ionizante nestas matrizes. Contribuem, também, para um conhecimento mais amplo dos efeitos da irradiação na extractabilidade dos vários compostos estudados, de forma a adequar sempre as doses e as fontes de radiação a aplicar.

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PC-27

Influência da origem geográfica no perfil fenólico de *Lavandula pedunculata* (Mill.) Cav

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Ao longo da história da humanidade, aproximadamente 7000 espécies de plantas têm sido utilizadas como alimento e também como um recurso essencial para o nosso bem-estar [1]. As espécies do género Lavandula são muito utilizadas, não só na medicina popular, como também na indústria alimentar, no fabrico de bebidas aromatizadas, gelados, doces e pastelaria. São também matéria-prima nas indústrias de perfumaria e farmacêutica, fazendo parte da formulação de sabonetes, perfumes, colónias, loções e outros cosméticos (Kim & Lee, 2002). Assim, neste trabalho pretendeu-se analisar o perfil fenólico de extratos aquosos (obtidos por infusão) e hidroetanólicos (etanol:água 80:20, *v/v*) de diferentes amostras de *Lavandula pedunculata* (Mill.) Cav., cultivadas no Banco Português de Germoplasma (BPGV) a partir de sementes recolhidas em exemplares silvestres de populações provenientes de diferentes regiões de Portugal, e conservadas *ex-situ* no BPGV. O objetivo final era estabelecer uma eventual relação entre a composição fenólica e a proveniência das diferentes populações/amostras. A análise do perfil fenólico individual de cada amostra foi efetuada por HPLC-DAD-ESI/MS. Todas as amostras apresentaram um perfil idêntico, revelando a presença de treze compostos fenólicos diferentes, destacando-se o ácido salvianólico B como o composto maioritário. A amostra proveniente de Ponte de Sôr (Portalegre), salientou-se das restantes por apresentar maior concentração de ácidos fenólicos, flavonoides e compostos fenólicos totais para ambos os extratos estudados. Comparando os diferentes tipos de extrato, em geral, os extratos aquosos apresentaram maior concentração em compostos fenólicos. Estes resultados evidenciam não só o perfil fenólico desta espécie, como também, a existência de variações entre amostras com diferentes origens geográficas, dando relevância aos fatores bióticos e abióticos na composição química das espécies.

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PC-28

Optimization of the method for determining the residual amounts of florasulam in crops by HPLC

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In this work, we optimized the method for determining florasulam in green mass, grain and straw of cereal crops. Florasulam refers to herbicides. Inhibits the enzyme acetolactate synthase and stops cell division at shoot and root growth points in sensitive weeds [1]. When analyzing florasulam by a known method, it was found that most of the material is lost during sample preparation. In this connection, all stages of sample preparation were tested and optimized.

For the analysis, a highly efficient liquid chromatograph "Alliance" (Waters) was used with a UV detector, a degasser, an automatic sampler and a column thermostat. Chromatographic column Sun Fire C-18, 250 mm long with an inner diameter of 4.6 mm and a phase graining of 5 µm (Waters).

Sufficient sensitivity of the detector and optimal chromatographic conditions allowed us to determine florasulam at a concentration of 0.25 µg / ml, which corresponds to a grain content of 0.025 mg / kg, straw and green mass of 0.05 mg / kg. The percentage yield of florasulam when using the optimized method exceeded 80%. The results obtained are presented in table 1.

Table 1. *Recovery of florasulam from plant matrixes using an optimized technique (n = 10, P = 0.95)*

Analyzed object	Range of determined concentrations, mg / kg	Recovery, ± SD, %
Green mass	0.05—0.5	83.2 ± 5.50
Grain	0.025—0.25	80.3 ± 3.85
Straw	0.05—0.5	85.2 ± 3.29

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PC-29

Caracterização fenólica da casca do fruto de *Ficus carica* L. por LC-DAD-ESI/MS

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Os efeitos adversos causados por alguns aditivos artificiais têm conduzido a um interesse crescente pela procura de alternativas naturais e sem toxicidade associada [1]. As antocianinas têm sido descritas como uma excelente fonte de corantes naturais para aplicação alimentar, aspeto impulsionado pela diversidade de cores que estas podem apresentar (vermelho, violeta, roxo e azul) e pelo facto de não existirem restrições quanto à sua utilização [2].

O processamento industrial de fruta gera grandes quantidades de resíduos (por exemplo frutos não conformes, restos de polpa, casca, caroços ou sementes), que poderão ser uma fonte importante de pigmentos e/ou moléculas bioativas, nomeadamente compostos fenólicos. Neste contexto, no presente trabalho a casca do fruto de *Ficus carica* L. (figo) foi estudada visando a sua valorização como fonte de corantes naturais, em particular como fonte de antocianinas, para aplicação na indústria alimentar. A sua caracterização foi efetuada por cromatografia líquida de alta eficiência acoplada a um detetor de díodos e a um espetrómetro de massa (LC-DAD-ESI/MS). A identificação dos compostos detetados foi realizada por comparação com os tempos de retenção, padrão de fragmentação e espectros UV-Vis de compostos padrão, ou recorrendo a dados existentes na literatura. Os cromatogramas foram adquiridos a 520 nm e a quantificação dos compostos identificados, através do uso de retas de calibração obtidas a partir de padrões comerciais.

A casca de figo apresentou apenas uma antocianina, a cianidina-3-rutinósido ($[M-H]^-$ a *m/z* 595), sendo esta a molécula responsável pela coloração apresentada. Em síntese, esta matriz pode ser valorizada como uma fonte de ingredientes corantes, constituindo uma alternativa aos corantes artificiais.

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PC-30

Determinação de antocianinas no epicarpo de frutos de *Prunus spinosa* L.

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A fim de otimizar processos e/ou desenvolver novos produtos, a indústria alimentar tem utilizado diversas substâncias, denominadas aditivos alimentares [1]. Estes aditivos são adicionados aos alimentos de forma a terem uma contribuição tecnológica e/ou fisiológica, no entanto, existe também uma preocupação por parte dos consumidores relativa aos efeitos adversos que alguns dos aditivos artificiais podem ter. Os corantes são uma das classes de aditivos, amplamente utilizados para conferir coloração aos alimentos. Atualmente, existe uma grande tendência de substituição de corantes artificiais por alternativas naturais. As antocianinas são um grupo de pigmentos que apresentam uma coloração vermelha, azul e/ou violeta, estando presentes em muitas flores e frutos, podendo ser consideradas alternativas aos corantes artificiais [2]. Os frutos de *Prunus spinosa* L. (abrunho) apresentam um epicarpo de coloração azul intensa evidenciando ser uma fonte promissora de corantes naturais. Neste trabalho, efetuou-se a caracterização do epicarpo do abrunho em termos de antocianinas, determinadas por cromatografia líquida de alta eficiência acoplada a um detetor de díodos e a um espetrómetro de massa (HPLC-DAD-ESI/MS). A identificação dos compostos foi realizada utilizando padrões, quando disponíveis, comparando os seus tempos de retenção, espetros UV-Vis e espetros de massa. Na ausência de padrões, a identificação foi efetuada pelo perfil de fragmentação e por comparação com a informação disponível na literatura. A quantificação foi realizada a partir das áreas dos picos registados ao comprimento de onda de 520 nm, por comparação com as curvas de calibração dos padrões. O epicarpo do abrunho apresentou dois compostos antociânicos, cianidina-3-rutinósido ($[M-H]^-$ a *m/z* 595) e peonidina-3-rutinósido ($[M-H]^-$ a *m/z* 609), estando a primeira molécula em maior concentração. Esta matriz poderá ser considerada uma fonte de pigmentos na gama de cores vermelho-roxo, tendo aplicabilidade tanto na indústria alimentar como farmacêutica.

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PC-31

Biodiesel production through esterification using ionic liquids as catalysts

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There is a growing interest in the development of alternative technologies to the oil economy, based on renewable energy sources. A possible solution is a biofuel usable in compression-ignition engines, produced from biomass rich in fats and oils. Biodiesel is an alternative fuel that can be produced from a wide range of raw materials such as vegetable oils and animal fats. Yet, the use of sources that do not compete with the food market, like waste cooking oils - which usually feature high levels of free fatty acids (FFA's) -, can lead to problems in the process of biodiesel production through alkaline transesterification. Ionic liquids (ILs) could be employed in the biodiesel production to partially overcome these problems; since they are able to catalyze the esterification reaction of FFA's to biodiesel. In this work, experimental results will be presented concerning the study of the influence of ILs in the catalysis of esterification reactions of organic acids to the corresponding methyl esters.

Different imidazolium-based ILs were tested for biodiesel production through an esterification reaction of oleic acid, using a previously optimized reaction methodology [1]: 1-butyl-3-methylimidazolium hydrogen sulfate ([BMIM][HSO_4]), 1-butyl-3-methylimidazolium methanesulfonate, 1-butyl-3-methylimidazolium methyl sulfate, 1-metylimidazolium hydrogen sulfate ([HMIM][HSO_4]) and tributylmethylammonium methylsulfate. The experimental values obtained for the conversion of the oleic acid through an esterification reaction showed that the ionic liquid ([BMIM][HSO_4]) would be one of the most promising catalysts.

The recovery of the selected [BMIM][HSO_4] ionic liquid was studied for different catalyst loading: 10, 15 and 20 wt% - relative to the mass of oleic acid. The reaction yield was determined by acidity using a titrimetric method (EN 14104). The composition characterization of the biodiesel samples (identification of fatty acid methyl esters) was evaluated by gas chromatography with FID detector (EN 14103) [2]. Table 1 displays the variation in the yield after several cycles for each catalyst loading. The obtained results confirm that it is possible to reuse [BMIM][HSO_4] ionic liquid in successive reactions without great loss of yield and, thereafter, to significantly reduce the costs associated with the use of ILs as catalysts. Moreover, the esterification reaction with the [HMIM][HSO_4] IL was also studied and further comparison of the methyl esters content obtained with each catalyst will be possible.

Table 1. *Reaction yield after several cycles*

Catalyst loading (wt%)	Number of cycles	1st reaction	Last reaction
10	4	76.6%	58.8%
15	5	83.3%	75.2%
20	5	84.8%	77.1%

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PC-32

Efeito do teor de etanol na composição de compostos fenólicos extraídos da casca de sementes de pinhão

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As sementes de *Araucaria angustifolia* (Bertol.) Kuntze, designadas por pinhão, são normalmente consumidas após cozedura, sendo as cascas descartadas. Contudo, estes bioresíduos podem ser aproveitados como fonte de compostos antioxidantes. Neste trabalho, procedeu-se à extração e caracterização de antioxidantes a partir da casca de pinhão, previamente submetida a cozedura. As cascas, secas e trituradas, foram submetidas a extração (8,75 g_{cascas}/100 $mL_{solvente}$) com misturas etanol:água (97% e 38%; *v/v* etanol) em homogeneizador Ultra-Turrax (a 12.000 rpm, 15 min e temperatura controlada num banho termostático a 42,5ºC). Os extratos foram posteriormente analisados quanto ao perfil fenólico (HPLC-DAD-ESI/MS), de acordo com um procedimento previamente otimizado por alguns dos autores [1]. Adicionalmente, foi avaliado o rendimento em volume de extrato obtido em relação ao volume inicial de solvente utilizado na extração, e analisada a microestrutura (Microscopia Eletrónica de Varrimento, MEV) das amostras após o processo de extração. Foram identificados treze compostos fenólicos, dez proantocianidinas (catequina e derivados de epicatequina), dois ácidos fenólicos (ácido protocatéquico e derivados de ácido ferúlico), um flavonol (quercetina-3-*O*-glucósido) e uma flavona (eriodictiol-*O*-hexósido). A extração usando um teor superior de etanol (97%) conduziu a uma concentração de compostos fenólicos extraídos superior (60,66±0,83 $mg/g_{extrato}$), comparativamente com a extração efetuada com 38% de etanol (22,28±0,13 $mg/g_{extrato}$). Quanto ao rendimento em volume foram obtidos os seguintes resultados: 44% e 62% para 97% e 38% de etanol, respetivamente. Com as imagens de MEV concluiu-se que um teor superior de água no solvente de extração favoreceu o inchamento e o dilaceramento das amostras. Em conclusão, a utilização de teores superiores em etanol favoreceu a extração de compostos fenólicos e conduziu à recuperação de um volume de extrato final superior.

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PC-33

Perfis cromatográficos de açúcares livres e ácidos gordos em amostras de iogurtes aditivadas com o corante natural curcumina

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A aplicação de corantes em alimentos visa tornar os produtos mais atrativos para o consumidor. A curcumina, um diaril-heptanoide extraído dos rizomas de *Curcuma longa* Linn., apresenta coloração laranja-amarelada e elevado potencial para substituir corantes artificiais, compostos associados muitas vezes a efeitos colaterais adversos. A encapsulação da curcumina configura uma alternativa para ultrapassar a baixa solubilidade em água e controlar a coloração (dependente do pH). Neste estudo, utilizaram-se diferentes formulações de curcumina: i) forma pura (PC); ii) nanoencapsulada em poli(vinil pirrolidona) (PVP) pela técnica de dispersão sólida (NC); iii) forma dispersável em água, comercialmente disponível (DC). Estas formulações foram posteriormente utilizadas como agentes funcionais/corantes numa matriz de iogurte natural que foi posteriormente comparada quanto ao teor de ácidos gordos (GC-FID) e açúcares livres (HPLC-RI) (controlo: formulação sem curcumina) para três tempo de armazenamento a 4ºC (TA: 0, 7 e 15 dias). No que concerne aos diferentes tipos de iogurte (TI: PC, NC e DC), e considerando os resultados obtidos para os três tempos, verificou-se que as diferenças foram apenas significativas para os teores de lactose (máxima para TI=PC), C10:0 (máximo para TI=DC), C12:0 (máximo para TI=DC) e MUFA (máximos nos iogurtes com PC). Igualmente, o efeito induzido por TA nos diferentes TI foi apenas significativo nos casos da lactose, C10:0 (máxima para T=15 dias), C12:0 (máxima para T=15 dias) e MUFA (máxima para T=0 dias). Não se registou qualquer diferença significativa, quer em TI quer em TA, para os teores de glucose, C6:0, C14:0, C16:0, C18:0, C18:1n9c, C18:2n6c, SFA e PUFA. A manutenção destes parâmetros valida o uso eficaz das nanocápsulas de PVP/curcumina na funcionalização de iogurte, cujo perfil em açúcares livres e ácidos gordos, com especial destaque para o C18:1n9c e C18:2n6c, se mantém.

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PC-34

Influence of roasting on the amino acid profile of defatted almond flour

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Almond (*Prunus dulcis*) has a high total lipid content (40-67%) and is a good source of protein (15-22%) [1]. Seeds can be consumed *in natura*, being also very used in pastry and confectionary products. When submitted to extraction processing, almond oil is the obtained product, with high added-value, mainly used in the cosmetics industry [2]. After oil removing, the resulting by-product is a defatted almond flour, about which little information is known. The aim of this work was to study their protein fraction (protein content and amino acids profile). Two samples, unroasted and roasted seeds (150 °C for 30 min) were used to obtain defatted flours for evaluation. The oil extraction was carried out by hydraulic pressing (Mecamaq DEVF 80, Vila-Sana, Lleida, Spain). The remaining by-product was then analyzed. The protein was quantified by Kjeldahl procedure, while the total amino acid profile was determined by HPLC/FLD, after submitting samples to an acid hydrolysis (HCl 6 M, 110 ºC, 24 h), followed by derivatization with dansyl chloride (110 ºC, 10 min) [3].

Protein accounted for about 52 and 45% (dry weight, dw) in unroasted and roasted defatted flours, respectively. In both samples the main essential amino acids found were threonine, leucine and phenylalanine, and non-essential amino acids were aspartic and glutamic acids, arginine and glycine.

Roasting promoted a reduction of almost half the total amino acid content (from 42 to 25 mg total amino acids/100 g protein in dw). This should probably be due to the involvement of the amino acids in the Maillard reactions. Regarding the individual compounds' behaviour, histidine was the most resistant to roast (-15%), while aspartic and glutamic acids were degraded in 44%. Nevertheless, both type of flours can be highlighted as a good source of protein.

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PC-35

Gracilaria vermiculophylla: effect of preservation methods on the fatty acids profile

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Regardless their origin (either from wild harvest or from controlled production), macroalgae's chemical composition makes them a very worthy bio-sustainable ingredient for a wide range of applications. Despite having a low fat content (0.3–5% dry weight), they provide long-chain polyunsaturated essential fatty acids [1,2]. Macroalgae's development and composition is affected by genetics and the surrounding growth conditions, namely light, temperature, pH, salinity and nutrient variations. Disparities in the lipid content and profile have been reported, due to the already mentioned conditions, but also to different sample treatments and extraction methods [3].

This study aimed to compare the fatty acid profiles of *G. vermiculophylla* preserved by two different methods (dehydration (25ºC) and freeze-drying). The samples, grown in an Integrated Multi-Trophic Aquaculture system, were gently provided by AlgaPlus.

After lipid extraction and derivatization, fatty acid methyl esters were analysed in a Shimadzu GC-2010 Plus gas chromatograph equipped with a flame ionization detector (Shimadzu, Japan). A CP-Sil 88 silica capillary column (50 x 0.25 mm i.d, 0.20 µm) from Varian (Middelburg, Netherlands) was used for compounds separation, using a temperature program gradient.

Unsaturated fatty acids were predominant in both processed samples, despite freeze-dried presented higher total relative percentage. Significant differences (p<0.05) were observed between samples. For instance, arachidonic acid (C20:*4n6*) was the main fatty acid in freeze-dried samples, whereas palmitic acid (C16:0) was predominant in the dehydrated sample. Overall, comparing both conservation methods, freeze-drying appears to better preserve polyunsaturated compounds, probably due to a lesser external factors exposure of the samples during the water removal process.

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PC-36

Wild mushrooms as a possible source of nutraceuticals – Use of chromatographic techniques to obtain the species chemical profile

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Although mushrooms use has been reported for thousands of years, it has only been in recent years that the consumption of mushrooms has increased, mainly due to the increasing awareness that a stable and balanced diet exerts a key role in normal body functioning and sustaining health [1]. Indeed, some authors consider mushrooms as "inherent functional foods" [2].

This work presents the profiles of fatty acids, tocopherols and phenolic acids of two wild species from the genus *Suillus*, namely *S. granulatus* and *S. luteus*. Fatty acids were determined by GC-FID, tocopherols were analysed by HPLC coupled to a fluorescence detector, and phenolic acids by HPLC-PDA.

Oleic and linoleic acids were the prevailing fatty acids detected in both species (31 – 57% of total fatty acids). Regarding the tocopherols profile, the main vitamers quantified in *S. granulatus* were the β- and δ-tocopherols (175 and 102 µg/100 g dw, respectively), while the main vitamer found in *S. luteus* was γ-tocopherol (337 µg/100 g dw). Gallic and p-hydroxybenzoic acids, as also the related compound cinnamic acid, were identified in *S. granulatus* (0.1 – 0.5 mg/100 g dw); protocatechuic acid was the only phenolic acid detected in *S. luteus* (0.5 mg/100 g dw), as well as the related compound cinnamic acid (0.4 mg/100 g dw).

With this work, we were able to confirm that mushrooms can be a source of nutraceuticals, such as unsaturated fatty acids, vitamins and phenolic compounds. We could also conclude that species from the same genus, can present a similar chemical profile, but since mushrooms are highly influenced by the environmental conditions, the samples may present some differences between them.

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PC-37

Olive oil volatile organic compounds: Single column *vs.* coupled columns for GC/MS identification purposes

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Olive oil is one of the most important food products in Mediterranean diet. Being the most aromatic of vegetable oils, olive oil gives the food a unique flavour and aroma, due to its chemical, biological and organoleptic properties.

The volatile profile of olive oil was studied in order to identify the compounds present in its flavour using gas chromatography coupled to mass spectrometry, HS-SPME-GC/MS. However, the complexity of the volatile profile of this matrix causes several coelutions issues when the analytical method involves only one column with a polar or apolar stationary phase. This fact precludes the identification of many compounds. In particular some compounds that present in smaller proportions can, in a significant way, have a determining role in flavour characteristics of olive oil.

In this work two methodological approaches were used for the chromatographic analysis: one system using a polar capillary column and another system where two columns with different stationary phases were coupled. A set of polar-apolar columns was tested. The use of two coupled columns avoids coelutions in several cases because while the polar column separates the compounds according to their polarity, the non-polar column allows to separate the compounds presenting coelutions in the previous polar column fraction, through their boiling temperatures.

In this study, more then 300 volatile organic compounds were identified from different chemical families.

PC-38

Ultrahigh-Pressure Liquid Chromatography with fluorescent detection (UPLC-FLD) method for the identification of anthocyanins from Purple Sweet Potato

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Anthocyanins represent the main flavonoid group of water soluble pigments and are responsible for the red to blue pigmentation of foods[1].

Besides their biological role, anthocyanins are used in the industry as natural food colorants. Nevertheless, they are highly reactive and co-adjuvants such as sugars are usually required to increase their stability in food matrices[2].

Purple sweet potato anthocyanins are complex acylated anthocyanic pigments derived from native anthocyanins such as paeonidin-3-glucoside and cyanidin-3-glucoside and are described as more resistant to the chemical and physical challenges imposed by the food industry[3]. Also, their inner chemical and biological properties may differ completely from basic anthocyanins.

The aim of this project was to characterize the anthocyanin content of a Purple Sweet Potato variety.

Anthocyanins were extracted from Purple Sweet Potato using Ultrasound-Assisted Extraction (70% Ethanol), followed by LLE extraction and Amberlite XAD-7HP purification.

The extract obtained was analysed by fluorescence spectroscopy and showed remarkable fluorescence properties. A method was developed, using Ultrahigh-Pressure Liquid Chromatography coupled with a fluorescence detector to characterize the origin of fluorescence.

Furthermore, the anthocyanin detected as being the main responsible for the fluorescence was isolated by preparative HPLC and the results were once again confirmed by the developed method.

Paeonidin-3-(6'-p-hydroxybenzoyl)-sophoroside-5-glucoside was characterized by UPLC-MS/MS and RMN.

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PC-39

Is thermal treatment a concern for the nutritional quality of flaxseed, chia and sunflower seeds?

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Oilseeds production has increased due to several food industry applications to answer consumers demand for foods with potential health benefits. Most of these benefits are related to the fatty acids profile, since oilseeds are particularly rich in polyunsaturated fatty acids that decrease the risk of several chronic diseases [1]. From the food industry perspective, their application in the enrichment of breads, cakes, cookies and cereal bars, is a challenge. All these products are submitted to different processing methods, including heat treatment, being therefore essential to evaluate their impact on the nutritional value, namely in the fatty acid profile and oxidative stability of oilseeds. In 2016, samples of flaxseeds (*Linum usitatissimum* L.), chia (*Salvia hispanica*) and sunflower (*Helianthus annuus* L.) seeds were obtained from supermarkets in the Lisbon region. The samples were subjected to heat treatment (180 °C) for 10, 20, 30 and 60 minutes. Oilseeds fat was extracted with petroleum ether and for the methylation of the fatty acids a cold transesterification was performed using n-heptane and a methanolic solution of potassium hydroxide (2 M) [1,2]. Chromatographic separation of fatty acid methyl esters was then performed using a gas chromatograph coupled to flame ionization detector. For all the analysed oilseeds, the major fatty acids were polyunsaturated. Nonetheless, for chia and flaxseeds the major polyunsaturated fatty acid was alpha-linolenic (omega 3) fatty acid, while for sunflower seeds the major fatty acid was linoleic acid (omega 6). Foods containing high levels of polyunsaturated fatty acids are more susceptible to lipid oxidation, and some of the conditions that can trigger the oxidation process are the presence of oxygen, exposure to light, and/or heat treatment. In this work, after applying heat treatment on the different types of seeds, it was possible to conclude that no considerable changes were observed in the fatty acid profile of chia, sunflower and flaxseeds. This could be due, in part, to the presence of antioxidant compounds, such as phytosterols and tocopherols, but also due to the temperature of the heat treatment.

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PC-40

Phenolic profile obtained by HPLC-DAD-ESI/MS and *in vitro* bioactivities of *Equisetum giganteum* L. and *Tilia platyphyllos* Scop.

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Medicinal plants are a source of a wide range of bioactive compounds, such as alkaloids, terpenes, steroids and phenolic compounds, which are responsible for multiple biological effects [1, 2]. In the present work, the phenolic composition and bioactive potential of the aqueous and hydroethanolic extracts of *Equisetum giganteum* L. and *Tilia platyphyllos* Scop. were evaluated. The phenolic compounds were determined using a Hewlett-Packard 1100 chromatograph, with a diode array detector coupled to a MS detector API 3200 Qtrap through an ESI source and a triple quadrupole-ion trap mass analyser, while the bioactive properties were evaluated in terms of antioxidant, anti-inflammatory, and cytotoxic activities. The hydroethanolic extracts revealed higher amounts of phenolic compounds than infusions, being the concentration of flavonoids (81% of the phenolic composition) remarkably higher than the phenolic acids content (19%), in both species and extracts. *T. platyphyllos* presented a higher phenolic content (50.4 ± 0.4 mg/g of hydroethanolic extract and 11.65 ± 0.05 mg/g of lyophilized infusion), than *E. giganteum* (21.7 ± 0.4 mg/g and 4.98 ± 0.03 mg/g, respectively). Moreover, kaempferol-*O*-glucoside-*O*-rutinoside was the most abundant flavonoid in *E. giganteum* extract, while protocatechuic acid and (−)-epicatechin were the most abundant phenolic acid and flavonoid, respectively, in *T. platyphyllos* extract. Regarding the bioactive assays, both extracts obtained from *T. platyphyllos* showed the highest potential and none of the extracts showed toxicity in non-tumor liver cells. These biological properties were highly correlated with its content and composition in phenolic compounds. Thus, it would be interesting to evaluate the in vivo efficacy of both plant extracts to unveil the involved modes of action and to establish effective therapeutic doses.

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PC-41

Profiling the volatile fraction of ruminal content from Holstein dry-cows fed different diets

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Volatile compounds produced during the ruminal fermentation can be used to evaluate the efficiency of feed fermentation and diagnose digestive and metabolic disorders. Typically, ruminal fermentation is evaluated through determination of volatile markers from the degradation of carbohydrates and proteins, such as volatile fatty acids, H_2S and NH_3 [1,2]. Although other volatile compounds are produced in smaller amounts ($(CH_3)_2S$, $(CH_3)_2S_2$, acetaldehyde) [3] during feed fermentation in the rumen, their determination is poorly used in the nutritional evaluation of diets. Still, these volatile compounds can be important to the study of the ruminal fermentation.

This work presents an insight over the characterization of the volatile compounds extracted from ruminal content of Holstein dry-cows, aiming the identification of potential digestive and metabolic markers. The samples (ruminal contents) were collected from fistulated animals fed hay-silage and straw-based diets. The extraction was performed by headspace solid-phase microextraction (HS-SPME) and analysis was carried out by gas-chromatography with mass spectrometry detection (GC-MS). Volatile phenols, indole derivatives and ketones were identified as the major volatile components in the ruminal content samples of both diets. The main differences between samples were observed for terpenes and ketones. The monoterpenes limonene, eucalyptol and pinene, and the ketones 2-nonadecanone and 2-heptadecanone were identified only in the sample from hay-silage-based diet, whilst the sesquiterpene selinene was detected in the sample from the cow fed straw.

This first approach on the volatile composition of ruminal content allowed to create a base for the work in progress, by identifying the group of compounds with potential relevance to be used as digestive and metabolic markers in lactating dairy cows.

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PC-42

The impact of pH on the impurity profile of a model drug

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Robustness can be described as the ability to reproduce the analytical method under different circumstances without the occurrence of unexpected differences in the obtained result(s) [1]. The pH of a mobile phase is one of the many parameters that is commonly tested during method robustness assessment. pH has a very important role in the retention time (RT) and selectivity of charged compounds by being able to cause significant changes when slightly altered. This is mainly caused by the interactions between the stationary phase and the compounds of interest as the ionization state changes. Therefore, this influence needs to be carefully evaluated in the development stage of a HPLC method in order to select the mobile phase pH that provides the most reproducible results. The aim of this work was to test the pH robustness of an assay and impurity method, in order to establish the ideal pH range that allows an accurate quantification of both impurities and main peak. This study was made on a UPLC Acquity H class equipment with a PDA detector, using a gradient program. The nominal pH of mobile phase A is 7.4. In this work, the pH of the mobile phase A was modified to the following levels: 7.2, 7.3, 7.5 and 7.6. The impurity peak areas and resolution of impurities were assessed and compared with pH 7.4 chromatographic profiles, in order to understand the impact of the pH on method's performance. The results showed that the variation of the pH of the mobile phase A has a strong impact on the impurity profile, namely on the RT of impurity A (Figure 1). At pH 7.5 and 7.6, impurity A showed to co-elute with a second impurity – Impurity B (Figure 1) with an impact on the accurate quantification of both impurities.
In conclusion, impurity A proved to be highly sensitive to pH, which is probably related with an ionization of this impurity, leading to a change of its affinity to the stationary phase. The results proved that method´s pH has to be tightly controlled to allow a correct quantification of these 2 drug impurities, and therefore a correct drug quality assessment. This control can be made by the implementation of a resolution criterion between both impurities.

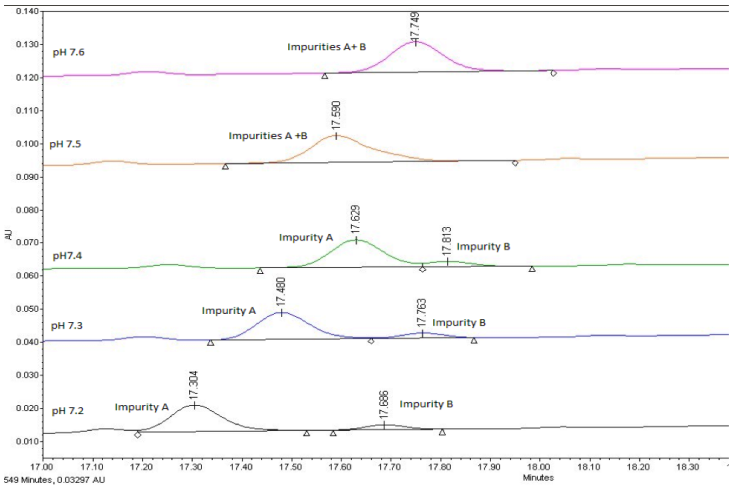

Figure 1. *Chromatographic profile of impurities A and B at different pH of mobile phase*

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PC-43

In-tube SPME-MS/MS with hybrid silica monolith as sorbent phase to determine amino acids and neurotransmitters in plasma samples

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Amino acid and neurotransmitter monitoring is an essential tool to elucidate normal and pathological neural system functions: changes in amino acid and neurotransmitter levels have been associated with various diseases and disorders such as schizophrenia [1]. Newly developed methods in the ambient mass spectrometry field include sample preparation devices, such as solid-phase microextraction (SPME) with biocompatible phases, which are directly coupled to the MS instrumentation [2]. Here, we evaluate direct coupling of the *in-tube* SPME technique to the MS/MS system to determine amino acids and neurotransmitters (alanine, serine, leucine, tryptophan, methionine, tyrosine, serotonin, and isoleucine) in plasma samples obtained from schizophrenic patients. This system uses a hybrid silica monolith capillary bearing aminopropyl and cyanopropyl groups to pre-concentrate the analytes selectively while excluding macromolecules from the plasma samples. After this step, the target analytes are quantitatively transferred to the tandem quadruple mass spectrometer system (multiple reaction monitoring mode). Monolith preparation is based on the sol–gel process via one-step catalysis, which involves reacting tetraethoxysilane (TEOS), as precursor; 3-cyanopropyltriethoxysilane (CN-TEOS) and 3-(aminopropyl)triethoxysilane (APTES), as hybrid monomers; and cetyltrimethylammonium bromide, as porogen. The resulting hybrid silica monolith is highly selective for the target analytes in the plasma samples and displays excellent mechanical strength. The capillary can be reused over 30 times without significant sensitivity loss. The *in-tube* SPME-MS/MS system is fast to operate and easy to automate, and it requires small volumes of the plasma sample (500 L) and organic solvents. The selectivity of both the monolithic capillary and the MS/MS system provides the method with analytical sensitivity and low limits of quantification. The proposed method successfully determines amino acids and neurotransmitters in plasma samples obtained from schizophrenic patients.

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PC-44

Design and optimization of a simulated moving bed unit for the separation of betulinic, oleanolic and ursolic acids mixtures: experimental and modeling studies

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Natural products emerged in the last few years as an important source of bioactive compounds for the nutraceutical and pharmaceutical industries due to their rich structural diversity [1]. However, most of these target products occur in small concentrations making difficult their separation/purification [2], as it is the case of betulinic, oleanolic and ursolic acids. These triterpenic acids (TTAs) are high value compounds with unique pharmacological properties (e.g., anti-inflammatory, antimicrobial, antitumor) and can be extracted from several natural sources like *Eucalyptus globulus* [3]. Nonetheless, their simultaneous occurrence and similar structures (figure 1) make their separation challenging.
Accordingly, a simulated moving bed (SMB) unit was designed for the separation of betulinic, oleanolic and ursolic acids in a two-stage process. Firstly, betulinic acid was isolated from oleanolic and ursolic acids, and then the oleanolic and ursolic acids were separated. HPLC pulse experiments were conducted to select suitable mobile and stationary phases, and the best results were found using an Apollo C-18 column with 95/5 (%, *v/v*) methanol/water as mobile phase. Breakthrough experiments of pure components were conducted to determine equilibrium and mass transport parameters. Afterwards they were successfully applied in the simulation of a ternary mixture breakthrough assay, validating their extension to multicomponent mixtures of the TTAs under study. Finally, a Design of Experiments (Response Surface Methodology) approach [4] was used to optimize the desired SMB unit. Rigorous phenomenological simulation results showed that the designed SMB can produce betulinic, oleanolic and ursolic acids with purities of at least 99.4, 99.1 and 99.4 wt.%, from a representative natural extract of *Eucalyptus globulus* bark containing 20, 25 and 55 wt.% of each acid, respectively.

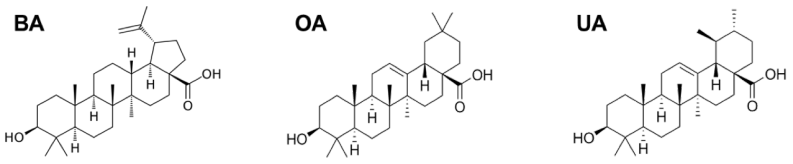

Figure 1. *Molecular structures of betulinic acid (BA), oleanolic acid (OA) and ursolic acid (UA).*

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PC-45

Chromatographic measurement of eucalyptol diffusivities in compressed fluids

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Eucalyptol is a monocyclic monoterpene used in food, cosmetic and pharmaceutical industries [1], which possesses anti-inflammatory and antibacterial activities [2,3]. It occurs abundantly in *Eucalyptus* essential oils (concentrations up to 90 %) making this biomass an excellent route to obtain Eucalyptol rich extracts. The compound can be isolated from other terpenes by conventional solvent extraction, two-step distillation process in the presence of phenols and cold treatment with a strong acid [1].

Supercritical fluid extraction (SFE) is a *green* alternative to conventional liquid-liquid and solid-liquid extractions using organic solvents. Supercritical carbon dioxide (SC-CO_2) is the most common supercritical solvent due to low cost, mild critical point, and high availability and recyclability [4]. The knowledge of transport properties, such as the tracer diffusivity (D_{12}), is very important for the design and optimization of industrial equipment and processes such as the above mentioned SFE. However despite the increasing scientific and industrial interest on supercritical processes, experimental data regarding D_{12} of bioactive compounds in SC-CO_2 (pure and modified with a cosolvent) are still scarce [5].

In this work, tracer diffusivities of Eucalyptol were measured in SC-CO_2 modified with 8.0 wt.% ethanol (ternary system) and in compressed liquid ethanol (binary system) over 303.15–333.15 K using the Chromatographic Peak Broadening technique [6]. The experimental method was accurately validated, and the obtained diffusivities ranged from 0.912×10^{-5} to 1.578×10^{-5} $cm^2\ s^{-1}$ and 0.547×10^{-4} to 1.042×10^{-4} $cm^2\ s^{-1}$ for the binary system (pressure range 1–100 bar) and for the ternary system (pressure range 150–275 bar) respectively.

The experimental data were modeled using predictive and correlation models from the literature, and relative deviations from 1.20 – 8.36 % were achieved.

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PC-46

A rapid UPLC method development for *in vitro* dissolution of supersaturation drug delivery systems

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In current pharmaceutical drug discovery, most compounds are poorly soluble in water, which can result in poor bioavailability. To overcome this challenge, formulation development continues to be a target on study, leading to the appearance of several strategies that create supersaturation of the drug.
This approach involves formulating the drug's amorphous form leading to the inhibition of crystallization, maintaining drug supersaturation and holding back the decline in the free drug concentration [1].
In the early stages of this study, the evaluation of the formulation's physical stability is key, as well as the choice of the polymeric excipient which directly influences the rate of drug release.
The ability of the polymers to inhibit Active Pharmaceutical Ingredient (API) crystallization and maintain supersaturation in solution was assessed using a dissolution methodology. To investigate the precipitation-inhibiting capacity of the polymers, experiments were conducted using a Dissolution Apparatus USP 2 with 500 mL round vessels at 37°C and a stirring speed of 200 rpm. Several polymers, such as HPMC-AS and PVP, were pre-dissolved in the dissolution medium (*e.g.* Fasted State Simulated Intestinal Fluid) and spiked with API at the target concentration. Within predetermined intervals, 2 mL was withdrawn from the supersaturated solution and centrifugation was applied at 14000 rpm for 5 minutes.
The resulting supernatant was analyzed by a simple and sensitive ultra-performance liquid chromatography method (RP-UPLC) allowing the estimation of the API concentration in the supersaturated samples.
Chromatographic separation was achieved on Acquity CHS C18 column using 10mM ammonium acetate (pH 5.0): ACN, as mobile phases at a flow rate of 0.7 ml/min and PDA detection at 237 nm.
With a 3 min run, a well resolved peak was obtained and supersaturation experiments can then be performed at a much faster rate, as these systems allow shorter analysis time (up to nine times, when compared with other systems) [2], high-resolution peaks and an automated process.
This UPLC/UV method has demonstrated a great potential to assist dissolution methodologies in early stages of pre-formulation for the characterization of supersaturated systems and to evaluate precipitation inhibitor effects.

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PC-47

Avaliação da composição em ácidos gordos de folhas de urtiga (*Urtica dioica*)

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A urtiga (*Urtica dioica* L.) é uma planta selvagem cujos benefícios dietéticos e terapêuticos são conhecidos desde tempos ancestrais. O seu uso na alimentação humana tem uma longa tradição, tendo sido muito utilizada pelos índios Americanos [1], estando igualmente descrita a sua utilização em diversos países Europeus [2]. Apesar do uso da urtiga na gastronomia tradicional ter caído em desuso, esta planta foi antigamente usada na confeção de sopas e outros pratos tais como omeletes, risotos, tartes e consumida como vegetal cozido [2,3]. Atualmente, em Portugal, o seu uso tem sido promovido pela Confraria da urtiga, criada em 2009, em Fornos-de-Algodres.

Neste trabalho procedeu-se à determinação da composição em ácidos gordos da gordura das folhas obtida por extração em Soxhlet. Foram analisadas duas amostras colhidas em Viseu em meses diferentes (Março e Junho) e uma colhida em Março em Vila Real, em 2017. As análises dos esteres metílicos de ácidos gordos (FAMEs) foram realizadas num sistema de cromatografia gasosa com deteção de ionização em chama (GC-FID Scion 436-GC, Bruker) usando uma coluna CP-Sil 88 (50m x 0.25mm i.d, 0.20µm, Agilent J&W). A temperatura do injetor e detetor foi de 260°C e 270 ºC, respetivamente. O forno foi inicialmente colocado a 160°C por 3 min, aumentando seguidamente a 3°C/min até 229 °C e mantendo-se durante dois minutos. Os compostos foram identificados por comparação dos tempos de retenção com uma mistura padrão de 37 FAMEs (CRM47885, Supelco).

No total, foram identificados 21 ácidos gordos, sendo os compostos maioritários os ácidos α-linolénico (41,9-51,3%), linoleico (19,9-30,2%) e palmítico (9,3-14,1%). De uma forma geral, o perfil qualitativo das três amostras foi similar entre si, apresentando contudo diferenças quantitativas. Quando comparadas as amostras colhidas no mês de Março, mas em regiões geográficas distintas, verifica-se que a amostra proveniente de Viseu apresentou um teor muito superior em ácido α-linoleico e menor em ácido linoleico. Quando comparadas as amostras colhidas no mesmo local, mas em meses diferentes (Março e Junho), verifica-se a diminuição do teor de ácido α-linolénico (51.3% para 41.9%) e um aumento dos ácidos palmítico, esteárico, oleico e linoleico. Os resultados deste trabalho preliminar sugerem a influência da localização geográfica e da época de colheita, sendo contudo necessária a realização de mais estudos. Considerando os resultados obtidos, a urtiga apresenta uma gordura com um perfil interessante dado o seu elevado teor em ácido α-linolénico e cujo consumo pode ser potencialmente benéfico para a saúde.

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PC-48

Preparation of a new chiral stationary phase for liquid chromatography based on a small molecule

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The development of chiral stationary phases (CSPs) for liquid chromatography (LC) revolutionized the enantioseparation and, nowadays, different types of CSPs are commercially available [1]. CSPs comprising small molecules as chiral selectors proved to be an excellent option for enantioresolution of several classes of analytes, including drugs [2].

Herein, the preparation of a CSP based on an enantiomerically pure small molecule (Figure 1) was described. The structure elucidation of the synthesized compound was established by spectroscopic methods (^{1}H and ^{13}C NMR and IR), and HRMS.

Column packing, selector loading and LC enantioresolution evaluation using diverse racemates were also described. The best enantioselectivity and resolution achieved showed α and R_S values of 1.78 and 7.79, respectively, using n-hexane/ethanol (8:2 *v/v*) as mobile phase.

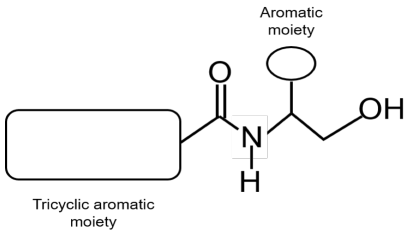

Figure 1. *Schematic representation of the small molecule.*

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PC-49

Liquid chromatography enantioseparation of xanthone derivatives on a human serum albumin stationary phase

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Chiral derivatives of xanthones (CDXs) have important biological activities being, in some cases, dependent on the stereochemistry [1,2]. Therefore, accurate methodology to enantioseparate and evaluate their enantiomeric purity plays a very important role.

In our group, liquid chromatography (LC) methods using different type of chiral stationary phases (CSPs), specifically macrocyclic glycopeptide antibiotic-based [3], Pirkle-type [4,5], and polysaccharide-based [6], have demonstrated to be efficient for enantioresolution of xanthone derivatives.

In order to expand the systematic investigation on enantioseparation of this important class of compounds using different types of CSPs, herein we report the development of enantioselective LC method for the resolution of enantiomeric mixtures of a series of CDXs by using a human serum albumin CSP. The enantioseparation was explored using different mobile phases, under reversed-phase elution conditions.

For some CDXs high enantioselectivity and resolution were obtained.

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PC-50

Caracterização do perfil carbonílico em cafés por GDME-HPLC-DAD-MS/MS para correlação com diferentes parâmetros de qualidade

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Estima-se que aproximadamente 15% da fração volátil do café (verde e torrado) é constituída por compostos carbonílicos [1], pelo que, a caracterização desta fração e correlação com a qualidade do café é de todo o interesse para a indústria deste sector. Neste trabalho, é apresentada uma metodologia analítica para a determinação de compostos carbonílicos voláteis em café verde e torrado. A metodologia baseia-se na técnica de GDME (microextração por difusão gasosa) para efeitos de pré-concentração da fração volátil que, posteriormente, é analisada por HPLC-DAD-MS/MS.

A metodologia desenvolvida permitiu identificar 27 compostos carbonílicos. Alguns desses compostos têm sido correlacionados com importantes aspetos de qualidade do café, dos quais se destacam o impacto organolético, a autenticidade quanto à proveniência, o tempo de armazenamento pós-colheita, o tipo de torra, o tratamento pós-colheita e a presença de grãos defeituosos.

Esta metodologia contrasta com os métodos descritos para a análise de voláteis, habitualmente baseados na técnica de SPME, quanto à simplicidade de execução e custo por análise, constituindo deste modo uma ferramenta de elevada utilidade para avaliação simultânea de diversas características do café.

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PC-51

Establishment and differentiation of the volatomic composition of juice and peel from Tahiti lime (*Citrus × latifolia*) based on HS-SPME/GC-qMS analysis

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The Tahiti lime (*Citrus* × *latifolia*), the most widely cultivated lime species for commercial use, is a hybrid citrus fruit, derived from Key lime and lemon, present an intense citric aroma less acidic and more sweet than Key lime and lemon. Constitute an important source of secondary metabolites for nutrition, health, and industrial applications, being widely used in the beverage industry, but also in culinary (namely the peel). Lime extracts and lime essential oils are frequently used in cosmetics, cleaning products and aromatherapy.

In this study headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-quadrupole mass spectrometry (GC–qMS) was used as a powerful strategy to establish the volatomic composition of juice and peel of Tahiti lime. Multivariate statistical analysis (MVA) was performed to select the most powerful volatile metabolites able to differentiate among Tahiti lime juice and peel. Up to 150 volatile organic metabolites (VOMs) belonging to different chemical groups, namely monoterpenes (responsible for more than 70% of the total peak area), sesquiterpenes, terpenoids, alcohols and carbonyl compounds, were identified. The major identified volatile in both, juice and peel of lime, were limonene, γ-terpinene, α-bergamotene, β-bisabolene, sabinene and β-pinene. In addition, although juice has a higher number of volatile metabolites identified than peel (120 vs. 86), the concentration of the volatile components identified in peel is about 20 times higher than that determined in juice. The data matrix was submitted to principal component analysis (PCA) and the most associated VOMs to both lime components investigated were selected.

The results reveal the great potential of chromatography on food analysis providing useful results toward food typicity and authenticity.

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PC-52

Coupling HPLC and GC-FID for the monitorization of oxidized intermediates from wet peroxide biphasic oxidation

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A GC-FID method capable to detect 2-nitrophenol (2-NP) and the resulting intermediates from its oxidation with H_2O_2 was developed and validated under the typical criteria for in-house pre-validation [1]. Linearity was demonstrated by F-test; accuracy and precision were assessed in three concentration levels. The oxidation process under study consists on the peroxidation of 2-NP, used as target lipophilic pollutant, in a biphasic mixture of cyclohexane(cC_6)/water to simulate contaminated oily streams. The oxidized intermediates produced by the peroxidation of 2-NP (P_{OW} = 51.3) in aqueous phase are phenol (Ph, P_{OW} = 30.2), hydroquinone (HQ, P_{OW} = 4.4), p-benzoquinone (BQ, P_{OW} = 1.9), catechol (CTL, P_{OW} = 7.6) and carboxylic acids [1]. Those compounds can be quantified in the aqueous phase by previous developed HPLC methods [2]. In the biphasic medium of reaction, the oxidation of cC_6 can also take place to produce cyclohexanone (cC_6O, P_{OW} = 5.8) and cyclohexanol (cC_6OH, P_{OW} = 21.9). The cC_6-water partition coefficient of 2-NP and the oxidized cyclic intermediates, *a priori* all lipophilic compounds, were assessed by analysis of the aqueous phase before and after addition of different volumes of cC_6. HQ and CTL keep their concentration in the aqueous phase after cC_6 addition and only the analysis of the other analytes (dissolved in cC_6) were done by GC-FID (Scion 436-GC, Bruker), without derivatization and adding a little quantity of Na_2SO_4 to remove the moisture. The injector and detector temperatures were set at 260 ºC and 270ºC, respectively. Separation was performed on a 50 m x 0.25 mm CP-Sil 88 column using the following temperature program: a first isotherm step at 160 ºC, followed by a heating ramp at 5 ºC/min, then at 10 ºC/min and a final isotherm step at 220 ºC during 5, 2, 5 and 5 min, respectively. At these conditions, the cC_6, cC_6O, cC_6OH, BQ, 2-NP and Ph compounds show well resolved peaks, and the method is capable to detect the compounds with maximum concentrations of 4.7, 2.3, 1.3, 1.0 and 7.1 g/L (in the same order) without overlap. The developed method, coupled with the HPLC methods, allows to follow the evolution of all compounds in the biphasic oxidation system.

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PC-53

Fingerprint targeted compounds for use in authenticity of sugarcane honey – an approach based on chromatographic and statistical data

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Sugarcane honey (SCH) is a black syrup recognized by its excellent quality, being produced in Madeira Island from regional sugarcane cultivars through a traditional and peculiar manufacturing and storage processes. However, some low-quality commercial products have been labeled as SCH but do not respect its criteria, revealing the need of development of powerful strategies in order to detect and prevent adulterations. The knowledge of furanic derivatives (FDs) pattern, produced during browning reactions that occurs during food processing and storage, emerged as a promising strategy in food quality and fraud prevention. Therefore, the aim of this study was to establish the FDs pattern of typical SCH produced by certified and non-certified producers, and in different geographic regions (Madeira and Brazil), based on microextraction by packed sorbent (MEPS) combined with UHPLC as a useful tool to define its typicality and traceability. These parameters are defined through the differentiation and discrimination of FDs profiles among other sugarcane-derived products using multivariate statistical analysis (ANOVA with post-hoc Tukey, principal components analysis, partial least square, linear discriminant analysis and hierarchical clustering). The results demonstrated that SCH samples from noncertified producers present the highest levels of FDs whereas SCH samples from Brazil present higher levels of FDs than samples from Madeira region. The proposed approach revealed a valuable strategy to establish the typicality of SCH, ensuring its quality, authenticity, safety control and to support the application of Madeira region SCH to EU certification.

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PC-54

Caracterização química de uma coleção de germoplasma de variedades tradicionais de tomate com recurso a diferentes técnicas cromatográficas

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O tomate (*Solanum lycopersicum* L.) é uma importante fonte de nutrição para a população mundial [1]. Ao longo do seu processo evolutivo e de domesticação, esta espécie sofreu alterações genéticas e fenómenos de endogamia severa que levaram ao surgimento de variedades com diferentes atributos morfológicos e sensoriais [2]. Estas variedades têm sido cultivadas localmente e representam um reservatório de diversidade genética com enorme potencial. No entanto, uma vez que a informação sobre a variação química entre populações de tomateiro é limitada, este estudo teve como objetivo caracterizar quimicamente diferentes variedades portuguesas de tomate. Para isso, as sementes de cinco acessos de tomate da região de Santarém (conhecidos por "coração-de-boi", "maçã", "tomate", "redondo" e "vermelho"), que se encontravam conservada *ex situ* no Banco Português de Germoplasma Vegetal, em Braga, foram regeneradas para obter material vegetal para análise. Os frutos maduros foram colhidos à mão e analisados quanto aos teores de açúcares (por HPLC-RI), ácidos orgânicos (por HPLC-PDA), tocoferóis (por HPLC-fluorescência), ácidos gordos (por GC-FID) e compostos fenólicos (por HPLC-DAD-ESI/MS) [3]. As variedades analisadas apresentaram diferenças nos teores de compostos sem o efeito de variação das condições edafoclimáticas. Os teores mais elevados de ácido ascórbico e β-tocoferol e os mais baixos de ácidos gordos polinsaturados (PUFA) foram detetados na variedade "maçã". A variedade "tomate" apresentou os teores mais altos de frutose, glucose e δ-tocoferol e os mais baixos de α-tocoferol, tocoferóis totais e ácido málico. Em contrapartida, os teores mais baixos de açúcares, ácido ascórbico e ácido cítrico e as concentrações mais elevadas de ácido málico, α- e γ-tocoferóis, PUFA e ácidos fenólicos foram quantificados na variedade "vermelho". Os compostos fenólicos foram particularmente abundantes na variedade "redondo". Os resultados deste estudo serão úteis para estabelecer critérios para uma seleção racional das variedades de tomate mais promissoras do ponto de vista químico.

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PC-55

Assessment of biogenic amines profile in biological samples from Holstein dry-cows

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Biogenic amines result from the decarboxylation of amino acids and by amination or transamination of aldehydes and ketones [1]. They are converted in the digestive tract of ruminants by microbial decomposition of dietary proteins and amino acids. For that reason, biogenic amines presence and content can be used as an indicator of the protein degradation of feed in ruminant animals. In addition, the influence of biogenic amines on the health of ruminants has been motivating their study [2].

The presented work aimed to characterize the content of biogenic amines in biological samples (urine, feces and ruminal content) collected from fistulated Holstein dry-cows fed with different diets (hay-silage (5,9% PB e 63,4% NDF) and straw-based (4% PB e 83,4% NDF) diets). The biogenic amines were extracted from the samples, derivatized and the analysis was performed by high performance liquid chromatography with fluorimetric detection (HPLC-FLD).The identity of unknown chromatographic peaks was confirmed using mass spectrometry analysis. In the studied samples several amines were detected and identified, mainly methylamine, 2-phenylethylamine, putrescine, spermidine and iso-pentylamine.

Figure 1. *Fistulated Holstein dry-cows*

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PC-56

The effects of starter culture on the biogenic amine accumulation in traditional Portuguese dry-sausages

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Linguiça is a highly popular and appreciated traditional Portuguese dry fermented sausage. Its production involves a ripening step, which provides favorable conditions for biogenic amines formation due to microbial growth, acidification and proteolysis. The levels of biogenic amines in dry-fermented sausages are highly dependent on the type of product, producer and could even vary from batch to batch. The microbiological quality of raw materials, technological process and growth/type of microbial flora are some factors that may explain this variability. To the authors' best knowledge, only few studies focused on the quantification of biogenic amines in Portuguese traditional sausages, reporting variable levels of accumulation, being the tyramine the most abundant followed by putrescine and cadaverine. Starter cultures have been used aiming to prevent or reduce the formation of biogenic amines during the manufacture of dry-fermented sausages. Based on the results reported in the literature, the use of starter cultures may reduce or not the biogenic amines accumulation during the fermentation of sausages.
In this work, it was evaluated the influence of one commercial starter culture (Texel®ELCE Br, Danisco) on biogenic amine accumulation during manufacture process and storage. Parameters such as pH value, water activity and microbial counts were also assessed. In general the results pointed out that the starter culture inhibited the accumulation of biogenic amines (putrescine, cadaverine and tyramine) as well as the growth of *S. aureus* and Enterobacterias. This inhibitory effect was clear during ripening and storage periods. On the other hand the starter culture did not have a significant effect on spermidine and spermine concentrations.

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PC-57

High-throughput method for the analysis of sterols in food samples by gas chromatography without previous fractionation steps

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Sterols are characterized by the presence of four rings and an alcohol group. Cholesterol (CHO) is the most common found sterol in animal sources while -sitosterol, campesterol, and estigmasterol are in plants and ergosterol in fungi [1]. They have attracted much attention in the last years, since CHO has been associated to cardiovascular diseases, while phytoesterols have shown anti-inflammatory properties [2]. The analysis of these compounds is quite complicated as they have low volatility and solid phase extraction purification steps are required. This research works aims to develop an analysis method by GC avoiding fractionation steps.

Cooked tuna (CT) and commercial canned tuna (NT), fish (FO), soya (SY) and krill (KR) oils were assayed as follows: lipids were isolated according to Matyash *et al.* [3]; afterwards 5β-cholestan-3α-ol was added to samples (internal standard) and all fatty acids (free and/or esterified) were converted into fatty acyl methyl esters (FAME) [4]. Finally, the sterol fraction was derivatized into trimethylsilyl derivatives (TMS) using bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) [5]. Identification was carried out by injection of pure sterol standards.

Preliminary trials showed presence of interference peaks not corresponding to sterol compounds. However, when derivatization was carried out using only glassware such peaks did not appear in the chromatogram. Determination of linearity, recovery and precision showed satisfactory values according to previously recommended acceptance criteria [6]. FAME derivatization allowed to eliminate any interference for lipids eluting in the sterol region. The obtained data showed that sterol fraction from CT and FO, was only composed of CHO while SY and NT had also campesterol, stigmasterol and -sitosterol. In KR samples, besides CHO, desmosterol was detected.

The proposed method allowed reliable analysis of sterols by GC as TMS in food samples bypassing the need of fractionation steps by using FAME derivatization.

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PC-58

Free fatty acids profiling in olive oil and olives from the Trás-os-Montes Portuguese region

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Olive oil and olives are food products highly appreciated by consumers for both organoleptic (e.g. taste/flavour) and technological properties (i.e. cooking). Moreover, during the last years several research works have also reported presence of phenols, tocopherols, squalene, sterols and fatty acids (e.g. oleic acid) with important positive health effects (e.g. anti-inflammatory, antiarrhythmic and vasodilatory) [1]. Although free fatty acids (FFA) in olive oil are an important quality parameter, this analysis is highly challenging as it involves isolation, fractionation and derivatization steps. Interestingly, some studies suggest that free polyunsaturated FA can be recognized by GPR120 receptors triggering anti-inflammatory processes [2]. Thus, a single-step method for the FFA analysis in both biological and foodstuffs was recently developed by authors of this current work [3].

The Portuguese region of Trás-os-Montes is an important producer of high quality olive oil and olives but to date, its FFA composition has been poorly studied. The detailed composition of such nutritional parameter would help increase the value of these products to promote the region and the producers. Therefore, different monovarietal olive oils of Cobrançosa (n=2), Madural (n=2), Verdeal (n=5) and Santulhana (n=6) were collected, in duplicate, directly from various local olive mills. Corresponding olive samples were also obtained. The procedure described by Matyash *et al.* [4] was used for lipid isolation. FFA in all samples were analyzed by GC-FID as fatty acid methyl esters (FAME) according to the above commented method of Pimentel *et al.* [3].

Olive oil and olives had the same qualitative composition and oleic acid was the main FFA as expected. Results showed intra-varietal differences that may be associated to location. When compared with olives, oil elaboration decreased the concentration of all FFA as expected, but homogenized the composition of the assayed oils.

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PC-59

Application of an HPLC method for the quality control of vitamin C content in foods for infants

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Vitamin C is an important water-soluble vitamin that humans are not able to synthesize, so it needs to be provided by the diet. Babies less than 1 year usually do not eat foods naturally rich in vitamin C, so the main sources are breast milk and/or infant formulas. Regulation (EU) No. 609/2013, Commission Delegated Regulation (EU) No 2016/127 and Ministério da Agricultura, do Desenvolvimento Rural e das Pescas (2008) establishes compositional and information requirements for food intended for infants and young children [1-3]. Adequate intake of vitamin C is very important to assure children's good health and development, so it is crucial to evaluate vitamin C content in this type of foods.

The aim of this study was to validate a high-performance liquid chromatography (HPLC) method for the quality control of vitamin C content in infant foods (2 infant formulae (IF), 2 follow-on formulae (FF), 2 processed cereal-based foods (PCF) and 2 baby foods (BF)).

In 2016, the infant foods were collected from major supermarket chains and parapharmacies in the region of Lisbon (Portugal). Two of the acquired samples were already ready-to-use, while the remaining samples were prepared according to manufacturer's instructions. Separation and quantification of vitamin C was carried out on an Alliance 2695 HPLC system, with diode array detection (DAD), using a Synergi™ Hydro-RP analytical column (150 x 4.6 mm I.D., 4.0 µm particle size). Samples were monitored at 245 nm.

The HPLC-DAD analytical method was validated for selectivity, linearity, LOD, LOQ, precision and accuracy, using an infant formula. Calibration curves were linear over the range 1-100 µg/mL. The achieved LOD and LOQ were 0.026 and 0.086 µg/mL, respectively. Vitamin C content in the analysed samples ranged from 1.5 ± 0.01 to 178 ± 1.01 mg/100 g for BF1 and PCF2, respectively.

The developed method is rapid, specific, precise and accurate, for the quantification of vitamin C in different categories of foods for infants and young children, showing satisfactory data for all the tested parameters. Since this type of food products are very important for such young and vulnerable consumers, regulation and assurance of an adequate intake of essential nutrients, like vitamin C, is extremely important for a healthy development.

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PC-60

Valorization of apple wood wastes from traditional and exotic Portuguese varieties: phenolic profile and antioxidant activity

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Recently, the extraction of phenolic compounds from apple wood wastes is gaining considerable attention [1]. These compounds are known to have many health-promoting activities, especially anticancer, antiradical and antioxidant effects. Although, the referred benefits can vary greatly between different apple varieties [2]. In this work, two traditional Portuguese apple wood cultivars (Porta-da-Loja and Pipo-de-Basto) and four commercial varieties (Golden, Jonagold, Fuji and Gala) were investigated in terms of their phenolic composition and antioxidant activity. For that, two extraction techniques, namely conventional extraction (CE) and microwave-assisted extraction (MAE), were employed for the recovery of phenolic compounds. Further, different spectrophotometric assays, namely total phenolic (TPC) and total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-RSA) and ferric reducing antioxidant power (FRAP) assays, were used to characterize the apple wood wastes. The highest TPC and TFC were reported for Fuji extract obtained by MAE (52.9±3.4 mg GAE/g DW and 17.2±1.4 mg EE/g DW for TPC and TFC, respectively). Concerning the antioxidant activity, the apple wood variety presenting the highest values was Gala also extracted by MAE (58.2±6.3 mg TE/g DW and 66.7±3.9 mg AAE/g DW for DPPH-RSA and FRAP assays, respectively). Regarding the traditional Portuguese apple wood varieties studied, the highest phenolic and flavonoid content, as well as antioxidant activity was obtained for Porta da Loja variety (TPC: 45.9±2.0 mg GAE/g DW; TFC: 15.8±1.1 mg EE/g DW; DPPH-RSA: 42.6±2.8 mg TE/g DW and FRAP: 52.2±4.7 mg AAE/g DW). Work is in progress to identify by HPLC which compounds are the main responsible for the antioxidant activity of the analyzed apple wood wastes. However, the previous results demonstrate the potential of Portuguese apple wood wastes from different varieties to be used as a promising source of phenolic compounds with antioxidant activity, for further application in cosmetic and/or pharmaceutical industries.

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PC-61

Comparison of different extraction solvents for characterization of phenolic compounds *Geranium robertianum* L. extracts

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It is well known that the extraction procedures used for the isolation of phenolic compounds from plant materials will dictate the nature and quantity of the phenolic compounds obtained in the extracts, which in turn will have a significant impact on the outcome of any investigation aimed at evaluating the profile and properties of the plant phenolic compounds [1]. In this context, the aim of this study was to compare the phenolic profile of two extracts from *Geranium robertianum* L., obtained with two different solvents.

Extract 1 was prepared by decoction of *G. robertianum* dried powder while the extract 2 was prepared with ethanol in a soxhlet extractor. Afterwards, both extracts were analyzed through high performance liquid chromatography with diode array detector coupled to an electrospray ionization mass spectrometer operating in negative mode.

Overall, the phenolic profile of the two extracts were notably different (Figure 1), although corilagin (peak 2, λ_{max} at 269 nm, $[M–H]^-$ at *m/z* 633301, 463, 275) and ellagic (peak 4, λ_{max} at 253 nm, $[M–H]^-$ at *m/z* 301229) were the two most intense peaks identified in both extracts. Brevifolin carboxylic acid (peak 1, λ_{max} at 276, 351 nm, $[M–H]^-$ at *m/z* 291247) was exclusively identified in extract 1, while brevifolin (peak 3, λ_{max} at 276, 351 nm, $[M–H]^-$ at *m/z* 247219) and an unidentified compound at *m/z* 319 (peak 5, λ_{max} at 276, 353 nm, $[M–H]^-$ at *m/z* 319275245) were found only in extract 2.

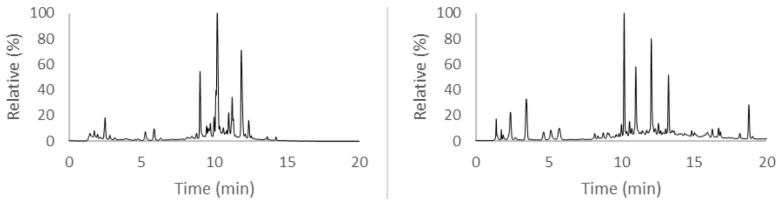

Figure 1. *Chromatographic profile at 280 nm of the extract 1 (A) and extract 2 (B)*

Presence of flavonoids was only detectable in extract 2, in which luteolin (peak 6, λ_{max} at 265, 367 nm, $[M–H]^-$ at *m/z* 285151) was found to be the most intense signal. With this work, it was possible to conclude that different extraction procedures may generate extracts with different phenolic compounds. Further studies are being carried out in order to understand the impact that this might have in the extracts bioactivities.

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PC-62

Validação do método de aflatoxinas por cromatografia - HPLC

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A avaliação dos parâmetros de validação seguiu o preconizado pela Resolução da ANVISA nº 899, de 29 de maio de 2003 [1]. A linearidade do método das curvas de calibração foi comprovada pelos coeficientes de determinação (R^2), obtidos por meio da análise de regressão linear das curvas de calibração. Os coeficientes obtidos para as aflatoxinas B1, B2,G1 e G2 foram 0,9951, 0,9986, 0,9941 e 0,9975, respectivamente. Os resultados foram considerados satisfatórios, estando de acordo com o critério mínimo aceitável do coeficiente de determinação (0,99). Foi realizada a seletividade do método comparando-se uma matriz em branco com e sem a adição dos analitos em estudo conforme a metodologia determinada. O resultado destas medições foi avaliado considerando-se os tempos característicos de retenção dos picos. Conforme figura 1, os tempos de retenção foram para G2: 6,874; G1: 7,813; B2: 10,664 e B1: 12,071 minutos, e quando avaliando a matriz em branco sem adição do analito não foi evidenciado a formação de picos no tempo citado (figura 2). Para o estabelecimento dos limites de detecção e quantificação levou-se em consideração o menor sinal detectável nas condições do método, sendo estabelecido os valores de B1= 0,5; B2= 0,05; G1= 0,5 e G2= 0,5 $\mu g.kg^{-1}$. A recuperação foi avaliada comparando-se os resultados analíticos de amostras extraídas na concentração 5 $mg.L^{-1}$, com os resultados obtidos de soluções padrão não extraídas, que representam 100% de recuperação. Para as aflatoxinas B1,B2,G1 e G2, encontrou-se recuperação média de 68, 93, 71 e 68%, respectivamente.

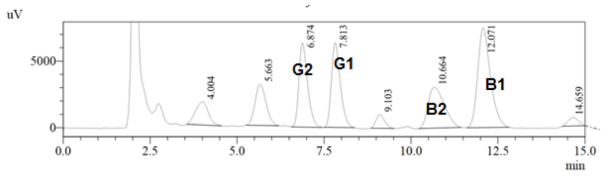

Figure 1. *Estudo de seletividade analisando matriz com adição do analito*

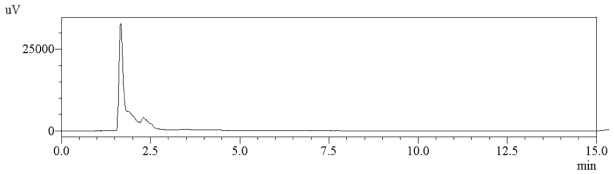

Figure 2. Estudo de seletividade analisando matriz sem adição do analito

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PC-63

Monovarietal olive pomaces: stability prediction based on fatty acid profile and oleic/linoleic ratio

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Olive pomace (OP) is the main by-product of the olive oil chain production. OP composition can influence external and intrinsic oxidative factors related to their degradation, influencing, for instance, the fatty acids profile of the remaining oil. Despite OP phytotoxicity, the recovering of OP bioactive components for innovative applications in food and cosmetic products has increasing in the last few years [1].

Taking into accounts that some olive mills are producing monovarietal olive oils, two questions emerged: are there significant differences in the fatty acid profiles of OP derived from different olive varieties? Will it be reasonable to select OP from specific olive varieties accordingly to its putative stability?

This work presents the fatty acid profiles of OP obtained from different olive varieties from Portugal (Arbosana, Arbequina, Oliana and Koroneiki), as well as the respective C18:1/C18:2 (oleic/linoleic) ratios as indicative marker of oil stability [2].

OP oil was obtained by Soxhlet extraction [3]. The fatty acids were converted to their methyl esters (FAMEs) [4] and analyzed by Gas Chromatography with Flame Ionization Detection. FAMEs were identified by comparison with commercial standards and expressed in relative percentage.

In all samples, the major fatty acid was C18:1. Koroneiki pomace oil was the richest in C18:1, presenting the lowest levels of C18:2, showing the highest C18:1/C18:2 ratio (9.25%). In turn, Arbequina pomace oil presented the lowest percentage of C18:1 and higher amount of C18:2. A Principal Component Analysis allowed to group Arbosana and Arbequina samples, while Oliana and Koroneiki formed other separated groups.

In sum, the results show significant differences between the fatty acid profiles of OP from different varieties. Furthermore, it was possible to select Koroneiki pomace oil as the sample with more stability characteristics based on the C18:1/C18:2 ratio.

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PC-64

Influence*of Bactrocera oleae* infestation on the fatty acids profile of two Algerian olive cultivars: *Limli* and *Rougette de Metidja*

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The sensory quality and nutritional/health properties of virgin olive oil (VOO) are related to the presence of different bioactive compounds, such as certain fatty acids.
VOO properties can be compromised by the olive fruit fly (*Bactrocera oleae*), the most serious insect pest of the cultivated olive fruits worldwide, and the degree of drupe attack is strongly correlated to the VOO quality [1].
This work aimed to study the influence of the *Bactrocera oleae* attack on the fatty acid composition of two Algerian olive cultivars - *Limli* and *Rougette de Metidja*. Olives were divided into three different groups according to the infestation: i) non- attacked olives; ii) non-selected olives, which reflects the real attack rate on the fruits; iii) attacked olives. The oil was extracted using a laboratory mill. The fatty acid methyl esters (FAMEs) were determined accordingly to the EU Regulation 796/2002 [2]. FAMEs were analyzed in a Shimadzu GC-2010 Plus Gas Chromatograph equipped with an auto-injector with a split/splitless injector and a flame ionization detector. Helium was the carrier gas at an internal pressure of 120 kPa and a flow rate of 40 mL/min. A CP-Sil 88 silica capillary column for FAME analysis (50 m length x 0.25 mm i.d, 0.20 µm film thickness) was used. The temperature program was as follows: 120°C, for 5 min, programmed to increase to 220°C at 3°C per min, and a constant temperature of 220°C during 10 min; injector and detector temperatures were 250 and 270°C, respectively; run time, 48.33 min. The split ratio was 1:25 and the injected volume was 1.0 µL. FAMEs were identified by comparison with standard mixtures (FAME 37, Supelco) and analyzed using the Shimadzu software GC Solution. The results were expressed in relative percentage of each fatty acid.
The oil yield varied between attacked and non-attacked olives. The oil loss in *Rougette de Metidja* was higher than in *Limli* (respectively, 39 and 27%). Concerning fatty acids composition, *Rougette de Metidja* presented high levels of C18:1(≈80%), the major monounsaturated fatty acid, whereas *Limli* presents ≈67%. Regarding C18:2, relatively low percentages were observed in the analyzed samples with a highest value observed in *Limli* (≈13%).
Overall, the infestation did not cause significant variations in the fatty acid composition of the studied olive cultivars. Nevertheless, olive oil varieties showed differences in their fatty acid profiles due to the cultivar.

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PC-65

Contribution of a liquid chromatographic method to evaluate if Portuguese vegetables are a good source of vitamin C?

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Vitamin C is a water-soluble vitamin which cannot be synthesized by the human body [1]. It is a highly thermolabile vitamin and it has two biologically active forms, L-ascorbic acid and its oxidation product, dehydroascorbic acid [1, 2]. This vitamin is naturally present in various vegetables and fruits, and is an excellent antioxidant to scavenge free radicals and to prevent oxidative stress. The daily reference intake established for adults in Regulation (EU) No 1169/2016 for this vitamin is 80 mg/day [3]. The aim of this study was to determine the vitamin C content in different vegetables varieties and to evaluate their contribution to the daily reference intake of vitamin C for adults.

In 2016, 11 types of raw vegetables (beetroot, broccoli, carrot, courgette, cucumber, ginger, iceberg lettuce, pumpkin, purple cabbage, spinach and tomato) were acquired in commercial areas in Lisbon. Samples were manually separated between edible portion and non-edible portion, and their vitamin C content (L-ascorbic acid and dehydroascorbic acid) was determined by a previously validated HPLC method. The nutritional contribution was calculated using the daily reference intake defined in Regulation (EU) No 1169/2011 [3].

The vitamin C content in the analysed samples ranged from 0.259 ± 0.04 to 64.5 ± 1.5 mg/100 g of edible portion for beetroot and broccoli, respectively. According to the results obtained, 100 g of raw broccoli contributes approximately with 81% of the daily intake for vitamin C, whereas the same amount of beetroot only contributes with approximately 0.3%. Considering the vitamin C content per 1 serving portion of vegetables (180 g of raw vegetables), it was found that broccoli was the only sample analysed that could exceed the daily reference intake (80 mg/day) [4].

Comparing the analytical content of vitamin C in the analysed vegetables with the daily reference intake established for this vitamin, we can conclude that for the majority of the vegetables it is necessary to consume more than one portion to reach the recommended value. However, it is necessary take into account that the vitamin C of these foods can be affected by many factors, such as climate, soil, and harvest, storage and processing methods [5].

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PC-66

Influência da temperatura de secagem nos compostos fenólicos e nas propriedades bioativas de folhas, caules e casca de *Croton urucurana* Baill

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A secagem de plantas medicinais (tecnologia física de processamento térmico) é um processo crucial que visa a manutenção da qualidade pós-colheita, bem como a conservação de compostos bioativos com propriedades fitoterapêuticas [1,2]. *Croton urucurana* Baill, conhecida popularmente no Brasil como sangra d'água, é principalmente utilizada pelas suas propriedades anti-hemorrágicas, anti-inflamatórias, anti-sépticas, cicatrizantes e pela sua ação antifúngica e entomológica [3,4]. O presente trabalho teve como objectivo verificar a influência da temperatura de secagem (40, 50, 60 e 70 °C) no perfil fenólico (obtido por HPLC-DAD-ESI/MS) de folhas, caules e casca da espécie anteriormente referida; bem como nas propriedades antioxidantes e citotóxicas dos seus extratos hidroetanólicos e aquosos (obtidos por decocção). Relativamente aos compostos fenólicos, o perfil foi muito semelhante em todas as amostras, havendo somente diferenças na quantidade de cada um dos compostos; as folhas secas a 40°C revelaram a maior concentração de compostos fenólicos. Foram identificados flavan-3-óis, flavonas, flavonóis e ácidos fenólicos (o ácido gálico foi somente detetado nos caules). A maior atividade antioxidante foi observada nos extratos hidroetanólicos de folhas e caules secos a 50 °C e de casca seca a 40 °C. Todas as amostras revelaram atividade citotóxica, sendo que os GI_{50} mais baixos foram também obtidos nos extratos hidroetanólicas e a temperaturas de secagem mais baixas. Com estes resultados podemos concluir que as altas temperaturas de secagem influenciam o conteúdo de compostos fenólicos e as propriedades bioativas de Sangra d'água. A adequabilidade das temperaturas de secagem para o processamento de plantas medicinais é de extrema importância para a indústria de forma a preservar as suas propriedades bioativas.

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PC-67

Volatile profile of different monovarietal olive oils by HS-SPME-GC/MS

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Olive oil is the most consumed vegetable oil in the Mediterranean basin, being considered extremely important economically. In recent years, its consumption and production has spread to other countries outside Mediterranean Basin, due to its health benefits and nutritional properties. The differentiation between olive oil categories depends on its sensorial analysis, and this is related to olive oil aroma. The composition of volatile compounds in olive oil depends fundamentally on the cultivar, the ripening degree of the fruits and processing conditions [1], among other factors. So, the present study aims to determine the volatile composition of monovarietal olive oils produced with different varieties of olives (Blanqueta, Verdeal Alentejana, Madural, Picual, Arbequina, Cordovil de Serpa, Cobrançosa, Carrasquenha, and Galega Vulgar). Volatile profile of 22 monovarietal olive oils was performed by head-space solid phase microextraction hyphenated with gas chromatography/ mass spectrometry (HS-SPME-GC/MS). A total of 112 volatile compounds belonging mainly to the family of the aldehydes, hydrocarbons, alcohols, terpenoids, ketones, sulphurous compounds, acids and esters have been identified and semi-quantified. 21 compounds were detected belonging to the aldehydes family, the most abundant one, accounting for 42% of the total of volatile compounds. Hydrocarbons accounted for 23% of the total and 22 compounds were identified belonging to this family. Regarding alcohols, 24 volatile compounds were found, representing approximately 9% of the total volatile compounds. The remaining classes accounted for only 26% of the total of volatile compounds present in the olive oils studied. Some differences were found among varieties. Galega Vulgar presented the highest percentage of volatile compounds, followed by Madural, Verdeal Alentejana and Blanqueta, while Cordovil de Serpa had the lowest percentage of volatile compounds. However, Cordovil de Serpa had a greater diversity of volatile compounds (99), while Galega Vulgar and Madural show the lowest number of volatile compounds (85) among the analyzed olive oil varieties.

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PC-68

Assessment of volatile composition in amphora wines by HS-SPME-GC/MS

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Amphora wines are known in Portugal as “Vinhos de Talha” since in this particular technology alcoholic fermentation takes place in clay vessels in the shape of amphoras. In the south of Portugal, in Alentejo region, always existed a strong tradition of making wine in the ancient way using clay amphoras, like the Greeks and the Romans did in the past. Nowadays, we are witnessing a renaissance of amphora wines. These wines are matchless, overflowing with all the character and identity of the Alentejo, since the clay allows for some oxygen transfer, the extended skin contact following the alcoholic fermentation will add complexity and body to the wine and the minimalist intervention with low or none sulphur dioxide will contribute to character and authenticity of the final product. So, the present research aimed to evaluate the volatile composition of amphora wines. To achieve this goal, the volatile fraction of the different types of amphora wines (red, white and palhete wines) was done by head-space solid phase microextraction hyphenated with gas chromatography/ mass spectrometry (HS-SPME-GC/MS). A total of 134 volatile compounds, belonging to the family of the alcohols, esters, terpenoids, benzenoids, norisoprenoids, carbonyl and sulphurous compounds, sesquiterpenes, lactones, carboxylic acids and other acids, were identified. The volatile compounds belonging to the family of higher alcohols and esters represent approximately 80% of the total concentration of all wines analyzed, of which nearly 55% are referent to the higher alcohols. Additionally, a principal component analysis (PCA) was conducted in order to evaluate if the volatile profile of the wines were different regarding the type of the wine. The PCA results showed that although only 31 % of the variance existing among these samples were explain by the 2 principal components, we could observe a clear separation of the wines.

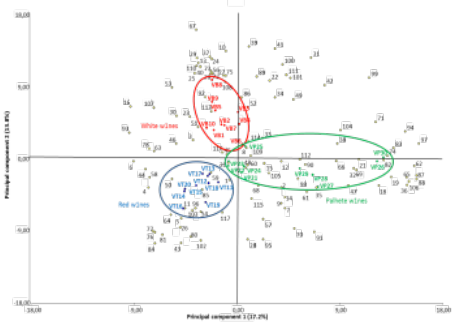

Figure 1. *Principal component bi-plot analysis (PCA) illustrating the simultaneous projection of the wines and volatile compounds*

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PC-69

Optimization of the extraction of phenolic compounds from walnut leaves using DES

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Deep eutectic solvents (DES) are a new generation of alternative solvents resulting from the mixture of two (or more) starting materials where the eutectic temperature of the mixture is considerably lower than the melting point of the individual components [1]. DES can be considered "designer solvents" due to the possibility of combining different hydrogen bond acceptors and donors, to obtain solvents with specific affinity to the target molecules. Among their many applications is the potential use of DES as both solvents and formulation media of extracts rich in phenolic compounds [2].

In this work, the heat assisted extraction of phenolic compounds from walnut leaves was optimized, using DES based on choline chloride (CC) and carboxylic acids. To evaluate the response, the main phenolic compounds present in the extract (acid 3-*O*-caffeyloquinic acid, quercetin 3-*O*-glucoside and quercetin *O*-pentoside) were determined by HPLC-DAD.

From a preliminary solvent screening, butyric acid (BA) and phenylpropionic acid (PPA) were selected as hydrogen bond donors. The extraction conditions (time, temperature and water content) were then optimized by an experimental design, assisted by response surface methodology. Water content was the most relevant extraction variable, followed by temperature and, lastly, extraction time.

Under the optimized conditions, it was possible to obtain a response of 37.9±4.0 mg/g dw for CC:BA and 31.7±4.2 mg/g dw, for CC:PPA. Compared to the traditional water + ethanol reference solvent, similar or higher extraction yields were obtained using the selected DES.

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PC-70

Óleo essencial de *Chenopodium ambrosioides*: perfil químico em CG/EM e influência na resposta imune em ratos infectados com *Trypanosoma cruzi*

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O mastruz (*Chenopodium ambrosioides* - Amanranthaceace) é uma planta medicinal amplamente distribuída no planeta. Seu óleo essencial é rico em ascaridol e já foram reportadas diversas atividades biológicas vinculadas a esta substância. O objetivo deste trabalho foi estudar o perfil químico do óleo de mastruz, por meio de cromatografia gasosa acoplada a espectro de massas (CG/EM), bem como avaliar alguns parâmetros imunológicos relacionados à população de células NK e NKT de ratos infectados com *Trypanosoma cruzi* nas fases aguda e crônica.

A extração do óleo foi realizada por meio da técnica de hidrodestilação, do tipo Clevenger, partindo-se de 40g de folhas de mastruz. A análise química do óleo foi feita por meio de cromatografia gasosa acoplado a espectro de massas (CG/EM), equipamento Shimadzu CG-17A, com detector seletivo de massa QP 2010, coluna EN5MS e fluxo de 1,5 mL/min. O parâmetro imunológico selecionado foi a análise da população de células NK e NKT (*Natural Killer*) de ratos infectados com *T. cruzi,* nas fases aguda e crônica, quantificadas por citometria de fluxo.

A análise do cromatograma permitiu a identificação de 9 picos, com diferentes tempos de retenção. Para cada um destes foi gerado um espectro de massas, cuja análise foi baseada na comparação com espectros padrões das bases de dados WILEY7, FFNSC1.3 e NIST08, além do cálculo do índice de retenção para cada substância [1]. Desta forma, foi possível a identificação de 7 componentes químicos presentes na amostra: α-terpineno, *p*-cimeno, limoneno, γ-terpineno, ascaridol, piperitona e isoascaridol.

Quanto ao parâmetro imunológico analisado, verificou-se que o tratamento do óleo essencial na fase aguda não proporcionou um aumento significativo das células NK. No entanto, na fase crônica, verificou-se diminuição da porcentagem destas células quando comparados aos animais sem infecção, sendo considerado um efeito positivo na resposta imune nesta fase da doença. As células NK integram a população de linfócitos grandes granulares e contribuem efetivamente na eliminação de agentes infecciosos. Quanto ao papel das células NKT durante a fase aguda da infecção, o tratamento com o óleo essencial levou a um aumento significativo dessas células, contribuindo para a contenção da replicação parasitária.

Acknowledgements:
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PC-71

Influence of storage conditions on polyphenolic, terpenoids and sensory profile from *Cymbopogon citratus* infusions

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Cymbopogon citratus, commonly known as lemongrass, is a tropical plant from Asia. The dried leaves can be brewed into an infusion and benefits, like cytoprotective, antioxidant and anti-inflammatory properties have been described.

The present work aims to evaluate the characteristics of the lots of aromatic plants throughout the storage using mainly chromatographic and sensory analysis in order to define a period of consumption preference. The plants were packed and stored in a can under forced conditions for 30 days. To evaluate the individual polyphenols and terpenoids compounds a HPLC-DAD and GC-MS analysis were performed, respectively. Additionally, antioxidant capacity, total phenolic compounds and sensory analysis were performed. For the sensory analysis, a panel of 60 consumers evaluated the samples using a 9-point hedonic scale. A trained panel, with 10 judges, evaluated the infusions sensory profile, through a QDA method.

From the results it was possible to verify that only the polyphenol profile presented significant changes. The main individual polyphenolic compounds identified were the chlorogenic, caffeic and rosmarinic acids that decreased over time together with antioxidant capacity results. Several aromatic compounds were found, such as citral, 6-methyl-6heptone-2-one, linalool, nerol, geraniol, eucalyptol, but no significant changes were observed throughout storage time.

Based on the overall liking, no significant differences were found over time. Also, in the QDA analysis no significant differences were found between samples for the attributes evaluated, confirming the correlation with the stable aromatic compounds.

In conclusion, despite the decrease in the phenolic profile the maintenance of antioxidant activity is justified by the stability of the aromatic profile, which also has an antioxidant potential, during storage time. Furthermore, the storage time didn't have a perceptible effect on the infusions sensory profile and consumers liking.

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PC-72

Preparation, purification and chromatographic fraction of hydrophobins from biomass of fungus *Aspergillus niger*

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Hydrophobins are a large class of low-molecular structural fungal proteins, which have extremely high surface activity. Hydrophobins can form amphipathic membranes at the interface, change the nature of the surface from hydrophobic to hydrophilic (and vice versa), and also organize strong structures on the surface [1].

This protein can form highly stable foams and emulsions even at low concentrations in the solution. Hydrophobins surfaces coatings can prevent adhesion and prevent the formation of biofilms, on the surface caused by bacteria. Also hydrophobins can be used to immobilize various substances, such as enzymes, antibodies, peptides, etc. on surfaces.

As a source of hydrophobins, we used mycelium of Aspergillus niger. which is a waste product of citric acid production. For the extraction of hydrophobins 1% sodium dodecyl sulphate (SDS) in 100mM Tris-HCl buffer (pH 9) was used. SDS was removed from the extract with a 2M KCl. After that, the extract was foamed using an aerator and the foam was treated with 60% ethanol solution. The residue was separated from the solution with centrifugation. Ethanol was removed by evaporation and hydrophobine soulution was purified from low-molecular impurities by exclusion chromatography, using Sephadex-G25.

The determination of the molecular weight and fractionation of the obtained hydrophobin extract was carried out using the method of exclusion chromatography.

Exclusive chromatography was performed on Sephadex G-75. A 1 ml sample was applied to the colum (Concentration of protein = 0.2 mg / ml). Elution was performed with sodium phosphate buffer (pH 7.4). The resulting fractions forming 1 and 2 peak were dried. Their surface activity was measured. The proteins forming fraction 2 of the peak showed greater surface activity(Figure 1). The mass of proteins forming fraction 2 of the peak was 9.2 ± 3 kDa, which corresponded to the mass of hydrophobins.

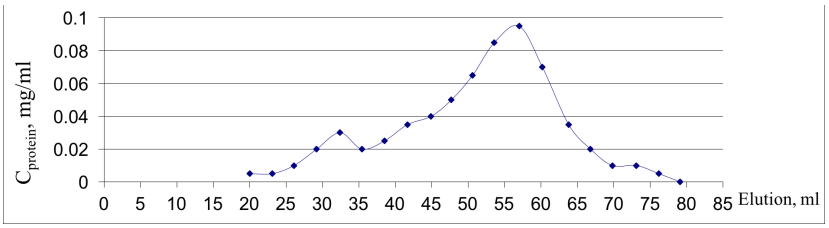

Figure 1. *Chromatogram of the obtained extract hydrophobins*

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PC-73

Análise cromatográfica de iogurte funcionalizado com extrato etanólico de *Agaricus bisporus*

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Agaricus bisporus (Lange) Imbach é um cogumelo rico em compostos bioativos e os seus extratos podem ser utilizados na funcionalização de alimentos. A caracterização dos extratos e dos alimentos funcionalizados é de extrema importância, principalmente em relação ao teor em compostos bioativos (e.g., ergosterol) e à composição nutricional, respetivamente. Neste trabalho, foi obtido um extrato etanólico de *A. bisporus* utilizando a técnica de ultrassons para funcionalização de iogurte. O teor em ergosterol foi determinado por cromatografia líquida de alta eficiência (HPLC-UV, Knauer Smartiline 1000) em coluna de fase reversa (Inertsil 100A ODS-3) e acetonitrilo/metanol (70:30, *v/v*) como fase móvel. O extrato apresentou uma concentração de 19,4 mg $_{ergosterol}$/g $_{extrato}$, tendo sido as bandas de absorção características identificadas por FTIR (caracterização estrutural). Posteriormente, o iogurte funcionalizado foi caracterizado em relação à sua composição nutricional e perfis cromatográficos de ácidos gordos e açúcares. O perfil de ácidos gordos foi determinado por cromatografia gasosa (equipamento DANI 1000, Contone) equipado com um detetor de ionização em chama (GC-FID) a 260°C, enquanto que os açúcares foram analisados por HPLC acoplado a um detetor de índice de refração, utilizando como acetonitrilo/água (70:30, *v/v*) como fase móvel [2]. Não foram observadas alterações significativas em relação ao valor nutricional dos iogurtes funcionalizados em comparação com o controlo (iogurte sem adição do extrato); apresentaram ácido mirístico (C14:0, 11.1±0.4%), ácido palmítico (C16:0, 31±1%), ácido esteárico (C18:0, 10.0±0.5 %) e ácido oleico (C18:1n9, 23±1 %) como ácidos gordos maioritários, e galactose (0.82±0.05 g/100 g) e lactose (4.6±0.2 g/100 g) como açúcares predominantes. No entanto, o extrato conferiu propriedades antioxidantes ao iogurte, demonstrando que a estratégia adotada é promissora para a obtenção de alimentos funcionais.

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PC-74

Cromatografia em Camada Fina e Cromatografia em Coluna utilizadas na síntese química de derivados do ergosterol

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O ergosterol é o esteroide mais abundante nos fungos e apresenta diversas propriedades bioativas, nomeadamente atividade hipocolesterolémica, que o tornam um agente funcionalizante interessante para a área alimentar. Contudo, este é insolúvel em água e apresenta solubilidade limitada em meios apolares, sendo a via da modificação química utilizada frequentemente para ultrapassar este problema. A cromatografia revela-se essencial no acompanhamento das reações de modificação química e na purificação dos produtos obtidos. Este trabalho teve como objetivo proceder a reações de acetilação [1], esterificação [2] e metilação [3] da molécula de ergosterol. A conversão do ergosterol no decorrer das reações foi avaliada por Cromatografia em Camada Fina (TLC, placas DC-Fertigfolien Alugram® Xtra SIL G/UV254; éter de petróleo/acetato de etilo (9:1, *v/v*) como eluente). A mistura obtida no final de cada reação foi diluída em éter de petróleo/acetato de etilo e aplicada numa coluna cromatográfica contendo sílica em gel (14x230 mm, Geduran® Si 60). O eluente foi recolhido e a pureza do produto monitorizada por TLC por comparação com os fatores de retenção dos reagentes iniciais. As frações contendo os produtos de interesse foram separadas e avaliadas em relação às propriedades citotóxicas em linhas tumorais (MCF-7, NCI-H460, HeLa, HepG2) e não-tumorais (PLP2). Após isolamento, os compostos foram caracterizados por ressonância magnética nuclear de protão (^{1}H RMN, $CDCl_3$). Estes apresentaram menor atividade citotóxica do que a molécula de ergosterol parental, em todas as linhas celulares tumorais. No entanto, ao contrário desta que apresentou toxicidade em células não-tumorais (GI_{50} = 89 μg/mL), nenhum dos compostos sintetizados apresentou toxicidade para células normais à concentração máxima testada (400 μg/mL). Assim, as moléculas obtidas por modificação química poderão ser utilizadas em matrizes alimentares lipofílicas.

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PC-75

Cosmeceutical properties of phenolic acids and use of microencapsulation to ensure controlled release

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Skin care formulations are designed to exert multifunctional benefits to the skin, promoting the interest on natural bioactive compounds as cosmeceutical ingredients. However, the utilization of such natural ingredients can present constraints related to their stability (e.g. against pH and temperature) being microencapsulation useful to overlap some of these limitations [1].

The present work describes the anti-inflammatory, anti-tyrosinase and antimicrobial activities of *p*-hydroxybenzoic, *p*-coumaric, protocatechuic and cinnamic acids. These compounds were microencapsulated using the atomization/coagulation method with sodium alginate coagulated with calcium chloride. The obtained microspheres were characterized in terms of morphology, particle size distribution, FTIR and encapsulation efficiency. Free and microencapsulated individual compounds were then incorporated into a semi-solid cosmetic base formulation. HPLC-DAD was used to check the presence of the compounds in the formulations.

Considering anti-inflammatory activity, *p*-Coumaric acid presented the lowest EC_{50} value (152 ± 6 µg/mL) (NO production inhibition), followed by cinnamic acid (180 ± 14 µg/mL), which was also the most active in the anti-tyrosinase assay (EC_{50} = 310 ± 50 µg/mL). All tested compounds displayed antimicrobial activity against Gram positive and Gram negative bacteria. The microparticles showed spherical morphology, various sizes (20-260 µm) with little agglomeration and a unimodal and bimodal particle size distribution (number and volume, respectively). The encapsulation efficiency was above 50 % in all cases. After incorporation, free compounds still maintained some of its bioactive properties, while the encapsulated forms preserved the bioactivity showing a slow release profile of the compounds. Concluding, this encapsulation procedure provides a suitable alternative to prolong retention of bioactive compounds for subsequent release (sustained release).

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PC-76

A QuEChERS method followed by liquid chromatography for the quantification of three organic contaminants in soil samples

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Soil fertility is a condition regarded by the European Environment Agency as a natural capital. Sewage sludge is the residue originated from the wastewater treatment. The EU encourages treated sewage sludge (biosolids) use in agriculture (Directive86/278/EEC) as they are rich in organic matter also containing essential elements (e.g. nutrients). Although biosolids can improve soil fertility, there is an environmental and human risk due to the presence of organic contaminants (OCs), namely pharmaceutical and personal care products (PPCPs).

PPCPs are emerging OCs being frequently detected in biosolids worldwide. Once in the amended soils, PPCPs can be mobilized contaminating groundwater or be accumulated by living organisms, representing both an environmental and a health risk.

In this work, an analytical methodology is presented enabling the determination of three PPCPs in soil samples. The target analytes are a nonsteroidal anti-inflammatory, an antibiotic and an antibacterial and antifungal agent: Ibuprofen, sulfamethoxazole and triclosan.

The sample preparation procedure is based on the quick, easy, cheap, effective, rugged and safe (QuEChERS) principle based on a salting-out extraction with a solvent (acetonitrile) followed by a dispersive solid phase extraction (d-SPE). Analysis is performed by high-performance liquid chromatography (HPLC) with diode array (DAD) and fluorescence (FLD) detectors.

The method for the extraction, separation, detection and identification of these organic contaminants in the soil will provide a powerful tool to trace them in the environment and monitor the development of remediation technologies.

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PC-77

Estudo e identificação de compostos bioativos na casca de pinheiro (*Pinus pinaster* Aiton subsp. *Atlantica*)

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A casca de pinheiro é um resíduo agroindustrial proveniente da indústria madeireira e que representa uma fonte de compostos fenólicos. Estes compostos têm várias propriedades benéficas sendo elas antioxidantes, antimicrobianas, anti-inflamatórias, cardiovasculares, entre outras.
O objetivo deste trabalho é extrair compostos fenólicos com propriedades antioxidantes da casca de pinheiro bravo e o seu estudo por HPLC.
Os compostos fenólicos foram extraídos por *Soxhlet* aplicando 5% (*m/v*) de material sólido com diâmetro da partícula 0.25-0.80 µm. Os solventes utilizados foram 100% água, 100% etanol e 50% etanol aquoso, durante 4 horas em refluxo. O rendimento da extração e o estudo da composição dos extratos foram avaliados por HPLC. O rendimento da extração com o solvente 100% água é mais baixo do que a extração com os restantes solventes, não havendo diferenças significativas entre eles. Identificaram-se a catequina e os ácidos ferrúlico, gálico e siríngico.

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PC-78

Optimization of key parameters influencing the chromatographic analysis of phenolic compounds in beverages after isolation by μ-SPEed

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Phenolic compounds are secondary metabolites produced by plants. They are constituents of several food matrices, including juices and beverages such as coffee and wine. Despite their wide distribution, the health effects of dietary phenolic compounds have come to the attention of nutritionists particularly in recent years. This interest was driven by epidemiologic data as well as in vitro and in vivo studies, suggesting that compounds have a preventive effect in cancer and cardiovascular diseases. The selection of the proper analytical strategy for phenolic characterization of food matrices depends on the purpose of the study as well the nature of the sample and the target analyte. In this study the potential of ultrahigh performance liquid chromatography (UPLC) combined with an innovative and promising microextraction technique, µ-SPEed, for the isolation and quantification of phenolic compounds in beverages, namely teas, was investigated. Some key parameters influencing the chromatographic analysis namely the type of column (BEH, HSST3 and Cortecs), elution system and elution mode, were tested and optimized, in order to maximize the chromatographic resolution. Regarding the extraction technique, some parameters were also assayed and optimized, including the nature of sorbent, elution solvent, the volume of loading sample and number of extraction cycles.
The performance of the analytical approach was assessed in terms of limits of detection (LOD) and quantification (LOQ), linear dynamic range (LDR), precision and matrix effect. The results obtained are very interesting and the newly proposed strategy, µ-SPEed/UHPLC-PDA -FLR, presented good analytical performance with very satisfactory results for recovery (> 96%), precision (% RSD < 7%), linearity (r^2 > 0.99) and LODs and LOQs. Moreover, the developed methodology is ultrafast, semi-automatic, involving minimal sample pre-treatment and solvent usage, in comparison with conventional techniques, allowing a rapid, simultaneous and highly sensitive quantification of different phenolic compounds in food matrices.

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PC-79

Fingerprint of phenolic compounds in *Osyris quadripartite* Salzm. ex Decne. from Algeria

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Medicinal plants are known as being a rich source of bioactive components, which can be correlated with their biological effects. The present study focused on the valorization of the leaves of *Osyris quadripartite* Salzm. ex Decne. with recognized folk medicinal uses in some Africa countries, namely Algeria [1, 2]. Therefore, the aim was to perform a phytochemical characterization, regarding phenolic composition, of *O. quadripartita* aqueous extracts and two different organic fractions (n-butanol and ethyl acetate), by using high-performance liquid chromatography coupled to a diode array detection and electrospray ionisation mass spectrometry (HPLC-DAD–ESI/MS). The separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (4.6 mm × 150 mm, 3 µm), using a gradient elution, with 0.1% formic acid in water and acetonitrile. Twenty-eight individual phenolic compounds were identified: fifteen flavan-3-ols, six flavones, four flavonols, two phenolic acids and one flavanone derivative. The ethyl acetate fraction presented the highest concentration in phenolic compounds, being (+)-catechin and procyanidin dimer B1 (EC-4,8-C) the most abundant compounds (110.5 ± 0.3 and 100.5 ± 0.3 mg/g extract, respectively). The aqueous extract and the n-butanol fraction presented similar contents of phenolic compounds, being quercetin-3-*O*-rutinoside the main molecule present (33.8 ± 0.1 and 17.70 ± 0.02 mg/g extract, respectively). Moreover, the phenolic profiles of all fractions and extracts presented similarities in their qualitative composition, although some differences were observed, especially in the ethyl acetate fraction, where no flavone derivatives were present. This study highlights the potential of *O. quadripartita* fractions, rich in phenolic compounds, to be used in food, pharmaceutical and cosmetic fields.

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PC-80

Determination of residual amounts of acetamiprid in crops by high-performance liquid chromatography

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In the present work, a technique has been developed to determine the residual amounts of acetamiprid in pea, cabbage, carrot and onion grains using high-performance liquid chromatography. Acetamiprid is an insecticide of contact, intestinal and systemic action. Identification of acetamiprid is carried out by retention time, quantitative determination by absolute calibration method. Selectivity of the method is provided by a combination of conditions for sample preparation and chromatography [1].

For the analysis, a high-perfomance liquid chromatograph "Alliance" (Waters) was used with a UV detector, a degasser, an automatic sampler and a column thermostat. Chromatographic column ACQUITY UPLC BEH C18 (100x2.1) mm, 1.7 µm (Waters).

Optimal conditions for sample preparation and chromatography were selected. The percentage yield of acetamiprid, in compliance with all regulated conditions of the analysis in exact accordance with the developed methodology, exceeded 80%. The results obtained are presented in table 1.

Table 1. *Recovery, standard deviation, confidence interval for n=20, P=0.95*

Analyzed object	Range of determined concentrations, mg / kg	Recovery, %	Standart deviation, S	Confidence interval of the average result, ± %
Pea	0,025 – 0,25	85,2	7,70	3,37
Cabbage	0,025 – 0,25	87,0	5,85	2,56
Carrot	0,025 – 0,25	81,5	3,04	1,33
Onion	0,025 – 0,25	82,6	4,78	2,09

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PC-81

Miniaturized Techniques for the determination of Antidepressants in plasma

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Antidepressants (ATD) are a group of drugs used for the treatment of depressive disorders. Based on the data provided by OCDE, the consumption of antidepressants has increased in the last 15 years, especially in Spain according to the data of Ministry of Health, Social Services and Equality [1,2].
The liquid chromatography is the technique more used for the separation of different compounds, due to it is not necessary a derivatization step required in gas chromatography. The miniaturization of analytical instrumentation is considered in this study, including the sample preparation and the column particle size and its dimensions. Dispersive Liquid Liquid Microextraction (DLLME) developed by Rezaee [3] has been successfully applied in the analysis of benzodiazepines in plasma [4].
A simple Ultrasounds Assisted (UA)-DLLME method is developed for the simultaneous determination of six second-generation ATD in plasma by Ultra Performance Liquid Chromatography with Photodiode Array Detector (UPLC-PDA) [5]. UPLC–MS/MS operating in positive electrospray ionization (ESI) mode was used as a confirmatory technique.
Analysis was performed with Acquity UPLC®BEH Shield RP18 (100 mm × 2.1 mm ID, 1.7 µm particle size). The methods were validated for linearity, limits of detection and quantification, precision and accuracy. The limits of detection were in the range 4–5 ng mL^{-1} for UPLC-PDA and 0.01-0.1 ng mL^{-1} for UPLC-MS/MS. The method precision and accuracy were made by spiking human plasma with ATDs at three concentration levels. Intra-day and inter-day precision (n = 5) was evaluated showing relative standard deviations (RSD) lower than 8.1% for UPLC-PDA and 14.8% for UPLC-MS/MS. The average recoveries ranged from 92.5% for fluoxetine to 110% for mirtazapine, using UPLC-PDA and 85.9% for escitalopram to 104.5% for mirtazapine, using UPLC-MS/MS.

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PC-82

An Improvement of Lab Efficiency in Liquid Chromatography

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The advances in technology and the consequent increase in costs associated with Good Manufacturing Practices (GMP) have been growing over the years in the Pharmaceutical Industry. In this highly competitive sector, it is vital to ensure the highest quality of the manufactured products and to minimize the costs associated with production and quality control analysis. On the other hand, the Pharmaceutical Industry has been concerned about the environmental impact of its activity. Consequently, looking for alternative methods that allow for greater productivity, efficiency, lower associated costs and an environment-friendly approach has been a priority.

Liquid chromatography is one of the most used techniques in quality control. Due to the reasons pointed out above, and despite being a challenge in some cases, the transfer of the conventional chromatographic methods (high performance liquid chromatography - HPLC) to ultra-fast liquid chromatographic methods (Ultra Performance Liquid Chromatography - UPLC) became very tempting. UPLC is a technique that allows the decrease of time and solvent consumption [1], at the same time offering greater chromatographic resolution and higher sensitivity (as it was designed to work with system back-pressures).

The aim of this work was the transfer of a method from the HPLC to the UPLC, by applying a software that helps on the adaption of the best chromatographic conditions in terms of chromatographic column, run time, flow and injection volume, while maintaining the temperature profile.

In the table below, it is possible to check the changed parameters (run time and flow), comparatively to the initial conditions on HPLC method, presenting an improvement per injection in terms of time and solvent reduction. Additionally, using a column with a lower pore size, it was possible too the improvement of the efficiency and resolution. The transfer was successful by achieving the complete resolution of the Active Pharmaceutical Ingredient (API) and the related substances presented on the formulation. It demonstrates the importance of a continuous improvement and innovation, so that in the future this technology will be increasingly used.

Table 1. *Improvements done during an HPLC Method transfer to UPLC Method, in terms of time and solvent reduction.*

Changed Parameters	HPLC Method	UPLC Method	% improvement /injection
Run time	50 min	22 min	44%
Flow	1 mL/min	0.3 mL/min	70%

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PC-83

Aplicação da metodologia SALLE para a determinação de aminas biogénicas em produtos alimentares de origem animal

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A extração líquido-líquido assistida por *salting-out* (SALLE) [1,2] refere-se a uma extração envolvendo água e solventes orgânicos miscíveis com água, em que a separação das fases é induzida pelo efeito de *salting-out*. O processo de *salting-out* consiste na adição de um eletrólito a uma solução aquosa de forma a modificar o coeficiente de distribuição de um dado soluto entre duas fases podendo, de uma forma genérica, alterar as forças de solvatação entre os solutos e a água. Esta metodologia apresenta um conjunto de vantagens importantes, entre as quais se destacam a simplicidade, custos reduzidos, baixa utilização de solventes e a possibilidade de ser aplicada a outros analitos de diferentes polaridades, relativamente às aplicações possíveis através da extração líquido-líquido clássica.
As aminas biogénicas (AB) podem ser encontradas em vários produtos alimentares de origem animal, tais como queijos, carnes ou peixes, sendo formadas através da descarboxilação enzimática de aminoácidos em alimentos que contêm proteínas ou aminoácidos livres. A sua presença em produtos alimentares é considerada indesejável, pois em concentrações elevadas podem induzir diversos problemas de saúde e alterar as caraterísticas organoléticas dos alimentos [3].
Neste trabalho, desenvolveu-se uma metodologia baseada no processo de SALLE para a extração de AB em produtos alimentares de origem animal. Simultaneamente com o processo de SALLE é efetuada uma derivatização das AB com o cloreto de dansilo, seguido da determinação dos derivados por cromatografia líquida de alta eficiência de fase reversa. Os principais parâmetros experimentais com influência no processo de extração foram otimizados (pH, tempo de derivatização, concentração do reagente derivatizante, sal utilizado para o *salting-out*); simultaneamente, estudou-se a influência das condições de preparação e armazenamento do produto no conteúdo de AB do alimento.

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PC-84

High-performance liquid chromatography in routine environmental analysis: in-house validation of analytical methods

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High-performance liquid chromatography (HPLC) is an important tool for the development of environmental technologies, since it allows the identification and quantification of specific organic pollutants. However, HPLC analytical procedures must be perceived as a chain of error-prone steps; there might be errors due to method conception, calibration and/or misuse [1]. Therefore, HPLC methods for routine analysis should be previously validated against test protocols with systematic experiments designed to challenge the methods in all the aspects that are subject to error [1]. An acceptance criteria should be previously established for each experiment, so that the results can indicate if the method works accordingly to the expectations, i.e., if the method does not originates faulty or misleading results [1].
Bearing this in mind, a reversed-phase single-component HPLC analytical method suitable for the determination of 4-nitrophenol (4-NP) – a compound used as model pollutant for the evaluation of water treatment technologies, was developed and validated in this work, under the typical criteria for in-house pre-validations. Likewise, two multi-component HPLC analytical methods devoted to the determination of aromatic and non-aromatic by-products of 4-NP degradation were also developed and validated. Figure 1 describes the main tasks involved in this work.

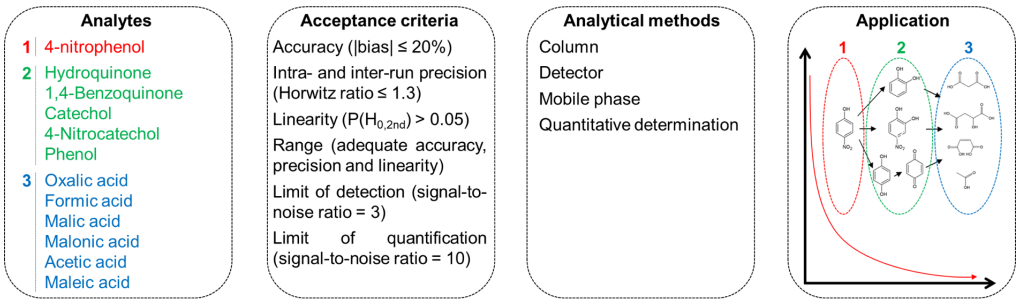

Figure 1. *Steps involved in the development and in-house validation of 3 analytical HPLC methods.*

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PC-85

Identification and quantification of phenolic compounds present in three different cultivars from *Sambucus nigra* L.

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European elderberry (*Sambucus nigra* L.) is a deciduous shrub, widespread throughout the temperate and subtropical regions, being present in several European countries, also in Asia, North Africa and North America [1]. Extracts of elderberries are a rich source of polyphenols presenting antioxidant and anti-inflammatory activities, as well as atheroprotective and chemopreventive potential [1,2].
The objective of this work was to identify and quantify the phenolic profile of mature fruit obtained from three different cultivars ('Sabugueiro', 'Sabugueira' and 'Bastardeira') of *Sambucus nigra* L., using HPLC-DAD.
The results indicated that chlorogenic acid and quercetin-3-rutinoside were the major cinammic acid and flavonol (respectively), present in the extracts of elderberries (Table1). Regarding the anthocyanins content, cyanidin-3 glucoside and cyanidin-3-sambubioside were the major cyanidins present in the extracts of elderberries (Table 2). This trend is in accordance with previous reports [1,2,3], being elderberries a good source of polyphenols, mainly anthocyanins.

Table 1. *Concentration (g/100g DW) of cinammic acids, cryptochlorogenic acid (CCA) and chlorogenic acid (CA) and flavonols, quercetin-3-glucoside (Q-3-G), quercetin-3-rutinoside (Q-3-R) and isorhramnetin-3-glucoside (I-3-G), present in elderberries extracts from S. nigra cultivars.*

Sample	CCA	CA	Q-3-G	Q-3-R	I-3-G
Sabugueiro'	0.009±0.003	0.098±0.075	0.195±0.092	3.395±0.319	0.425±0.064
Sabugueira'	0.010±0.010	0.111±0.034	0.422±0.253	3.330±1.201	0.309±0.150
Bastardeira'	0.025±0.008	0.109±0.028	0.354±0.113	3.022±1.129	0.331±0.131

Table 2. *Concentration (g/100g DW) of anthocyanins, cyanidin-3,5-diglucoside (C-3,5-dG), cyanidin-3-sambubioside-5-glucoside (C-3-S-5-G), cyanidin-3-glucoside (C-3-G) and cyanidin-3-sambubioside (C-3-S) present in elderberries extracts from S. nigra cultivars.*

Sample	C-3,5-dG	C-3-S-5-G	C-3-G	C-3-S
Sabugueiro'	0.217±0.031	1.520±0.253	6.524±3.690	9.981±3.272
Sabugueira'	0.385±0.235	2.048±1.212	6.295±1.785	9.757±1.493
Bastardeira'	0.361±0.169	2.795±0.801	3.663±0.971	7.916±1.603

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PC-86

Anthocyanins profile of *Sambucus nigra* L. harvested in three different years

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European elderberry (*Sambucus nigra* L.) is a deciduous shrub, widespread throughout the temperate and subtropical regions, being present in several European countries, also in Asia, North Africa and North America [1]. Extracts of elderberries are a rich source of polyphenols, mainly anthocyanins, presenting several bioactivities [1,2].

The objective of this work was to identify and quantify the amount of anthocyanins in mature fruit of elderberries harvested in three different years, using HPLC-DAD.

As expected, the results indicated that the amount of anthocyanins changed with the different years studied. However, the profile in anthocyanins were similar during the three years studied, being cyanidin-3-sambubioside and cyanidin-3-glucoside the major anthocyanins, followed by cyanidin-3-sambubioside-5-glucoside and cyanidin-3,5-diglucoside (Figure 1). This trend is in accordance with previous reports [1,2,3]. Thus, extracts of elderberries are a good source of anthocyanins.

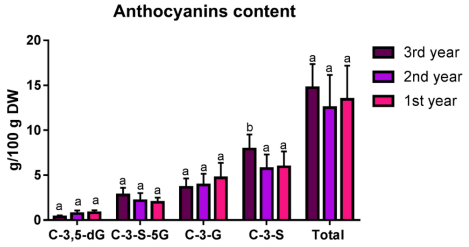

Figure 1. *Amount of anthocyanins (g/100d DW) present in elderberries extracts from S. nigra harvested in three different years. C-3-S: cyanidin-3-sambubioside; C-3-G: cyanidin-3-glucoside; C-3-S-5-G: cyanidin-3-sambubioside-5-glucoside and C-3,5-dG: cyanidin-3,5-diglucoside.*

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PC-87

Asthma urinary metabotyping: strategies for data normalization

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Asthma is an inflammatory, chronic and heterogeneous respiratory disorder, with high socioeconomic impacts, and growing incidence and prevalence. Several challenges related with disease management have been reported, namely the implementation of methodologies that allow the understanding of the related metabolic pathway disorders, using non-invasive sampling body fluids. On this context, urine seems to be a suitable fluid; however, its volume can vary widely based upon water consumption and other physiological factors. As a result, the concentrations of its endogenous metabolites could vary and normalization strategies may be taken into consideration. The main objective of this work was the implementation of a methodology for the simultaneous determination of a set of metabolites with potential relevance in the understanding of asthma, such as amino acids, organic acid, fatty acids and sugars. Also, normalization approaches that utilized targeted metabolites content common to all samples (expressed as equivalent of internal standard), urinary conductivity and creatinine concentration were compared in order to determine which strategies could be successfully used to differentiate between patients and control groups. Firstly, different silylation conditions were tested, prior the GC-qMS analysis (gas chromatography - mass spectrometry with quadrupole), based on previous study [1]. After that, a set of urine specimens, collected in hospital context, which included a group of asthmatics, including subjects with various allergies and healthy subjects, used as control, were analysed. Urinary conductivity was also determined. Variability observed in GC-MS results obtained from targeted metabolomic analyses was highly dependent on the strategy used for normalization, and urinary conductivity and creatinine concentration are significantly different between the groups under study, which may be attributable to renal disorders that have been reported for asthma [2]. Thus, these parameters may be themselves used as differentiating factors. The concentration of the targeted metabolites expressed as equivalents of internal standard was recommend in order to enhance the identification of statistically significant changes in the endogenous metabolite profile between asthmatic patients and controls. Despite the variability on targeted metabolomic profiles within asthma group, which may be explained by the several asthma phenotypes and endotypes, significant differences were identified in relation to control group, namely on a set of metabolites associated to the lipids, amino acids and carbohydrates metabolism, as well as energetic metabolism and oxidative stress.

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PC-88

Combined application of two-dimensional gas chromatography and headspace solid phase microextraction unravels changes in the volatiles of *Rhizobium* exposed to cadmium

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Rhizobacteria live in close association with plant roots and some of them, such as *Rhizobium* sp. strain E20-8, are able to establish a well-known endosymbiotic relationship with plants, with beneficial environmental effects. The use of fertilizers, such as phosphates, is a major input of Cd into agricultural soils, as well as the industrial wastes. These Cd sources contribute to high Cd concentrations in soils throughout Europe with the exception of Scandinavia, Scotland and west Iberian Peninsula [1].
As, no information is available on bacterial VOCs related to environmental stresses such as metals exposure, the volatile metabolome of *Rhizobium* sp. strain E20-8 exposed to three concentrations of cadmium (2.5, 5.0 and 7.5 μM) was screened using comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry (GC×GC–ToFMS), combined with headspace solid phase microextraction (HS-SPME). Cd exposure induced a global increase in the concentration of volatile organic compounds (VOCs) both intra and extracellularly. Peak areas of several linear alkanes, ketones, aldehydes, alcohols, terpenic and volatile sulfur compounds, and one ester (ethyl acetate), were especially increased when compared with the control condition (no Cd). These compounds might originate from the metabolization of toxic membrane peroxidation products, the proteolysis of oxidized proteins or the alteration of metabolic pathways, resulting from the oxidative stress imposed by Cd. Several VOCs are related to oxidative damage, but the production of VOCs involved in antioxidant response (menthol, α-pinene, dimethyl sulfide, disulfide and trisulfide, 1-butanol and 2-butanone) and in cell aggregation (2,3-butanedione, 3-methyl-1-butanol and 2-butanone) is also observed. These results bring new information that highlights the role of VOCs on bacteria response to Cd stress, identify a novel set of biomarkers related with metal stress and provide information to be applied in biotechnological and remediation contexts.

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PC-89

Fatty acids as potential chemical marker to discriminate robusta coffee silverskin from different geographical origins

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Silverskin, a coffee roasted by-product, is a potential ingredient for food and cosmetic purposes. Attending lipid fraction, coffee silverskin has a fat content around 2.42 g/100g [1]. Fatty acid profile has been described as a chemical parameter that can be used to assess the authenticity of Arabica and Robusta coffee beans [2]. The aim of this work was to evaluate the possibility of fatty acids profile to discriminate different geographical origins of Robusta coffee silverskin (*C. canephora*). For that, silverskin samples from five different origins (Brazil, Vietnam, Cameroon, Indonesia, and India) were acquired in a national coffee roaster industry (BICAFÉ) and analysed. Coffee beans from which silverskin samples derived were roasted by the same procedure, in order to avoid variations due to the roast degree.

Lipids were extracted and fatty acid methyl esters (FAME) prepared as described to Costa *et al.* [1]. FAME were analyzed in a Shimadzu GC-2010 Plus gas chromatograph equipped with a Shimadzu AOC-20i auto-injector and a flame ionization detector (Shimadzu, Japan). A CP-Sil 88 silica capillary column (50 x 0.25 mm i.d, 0.20 µm) from Varian (Middelburg, Netherlands) was used for FAME separation. The temperature program was as follows: 120°C (5 min), up to 220 °C (3°C/min), and constant temperature (220°C) for 15 min. Injector and detector temperatures were 250 and 270 °C, respectively. The compounds were identified by comparison with standards (FAME 37, Supelco, Bellefonte, PA, USA) and data analysed using the Shimadzu software GC Solution (v. 2.30, Shimadzu, Japan). The results were expressed in relative percentage of each fatty acid.

The results show significant differences between the fatty acid profile of the samples. Palmitic acid (C16:0) was the major fatty acid (21.7-27.6%) in four of the samples (Brazil, Vietnam, Cameroon, Indonesia), followed by linoleic acid (C18:*2n6*, 17.5-26.0%). In the case of Indian silverskin, C20:0 (22.3%) and C16:0 (21.4%) were the major compounds, followed by C22:0 (20.5%) and C18:*2n6* (8.7%). These differences allowed the discrimination of samples using a Principal Component Analysis, in which the Indian silverskin was separately grouped from the other samples.

Based on these results, the fatty acid profile of coffee silverskin can be pointed out as a potential chemical marker to discriminate different geographical origins.

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PC-90

Vitamin E profile of melon seed oils

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Melon (*Cucumis melo* L.) contains a large amount of seeds considered as a waste. These seeds are rich in oil (30%) [1] with a characteristic fatty acid profile (high content in linoleic acid, followed by oleic acid) [2].

In order to evaluate the gastronomic potential of the melon seed oils, the vitamin E profile of the oil extracted from seeds of 7 different melon varieties (*Amarillo Canario*, *Arizo*, *Blanco de Ribatejo*, *Charentais*, *Piel de Sapo*, *Piñonet* and *Tendral*) has been analyzed. The oil was extracted only by mechanical processes, using a screw press, to obtain virgin oils ready for consumption.

Vitamin E was determined by normal-phase HPLC, using a diode-array detector (to obtain UV-Vis spectra and confirm the compounds identity) connected in series with a fluorescence detector, programmed for excitation at 290 nm and emission at 330 nm (for compounds quantification). Tocol was used as internal standard.

Total vitamin E varied from 220 to 530 mg/kg oil. Different vitamer profiles were obtained for different varieties, however the predominant form was always γ-tocopherol (100-460 mg/kg), followed by α-tocopherol (37-74 mg/kg). All the samples contained minor amounts of β-tocotrienol and δ-tocopherol.

Compared to other vegetable oils with similar fatty acid profiles (such as corn, sunflower or soybean oils) [3], the majority of the samples contained lower vitamin E contents, which can suggest, for instance, lower stability to heat. Nevertheless, based on their fatty acid composition, they can be considered an interesting option to be included in a healthy diet, when consumed without refining (i.e. to season salads).

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PC-91

Comparison of *Ulva rigida* fatty acid profile in summer and winter seasons

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Although seaweeds have low fat content, they can be a source of essential fatty acids, turning them into a suitable and nutritional, health and readily available supplement to convention food. As for other nutrients, the fatty acid composition of macroalgae can however vary with several factors, including seasonality. The present work highlights the fatty acid (FA) profile variations of *Ulva rigida* grown under Integrated Multi-Trophic Aquaculture conditions, established at ALGAplus, Lda. (Aveiro, Portugal), in summer and winter seasons.

Ulva rigida (10 g) was extracted by petroleum ether on a soxhlet system, and the fatty acids composition was analyzed by gas-chromatography mass spectrometry, after derivatization by silylation reaction.

The fat content of *Ulva rigida* represented 0.32% and 1.21%, in summer and winter samples. In general, the fatty acid profile of the macroalgae was similar for the two seasons, with saturated fatty acids representing about 62- 64 % of total FA. Despite this, both saturated and insaturated fatty acids were increased by about 2.6 times in winter, with respect to the summer. In particular, the levels of the main fatty acids in this macroalgae ie palmitic acid, oleic acid and α-linolenic amounted for 458, 149 and 81 mg/kg dry base, respectively, in summer, while their levels in the winter were 2-3 times higher (1099, 326 and 171 mg/kg dry base, respectively).

Although unsaturated fat has accounted for less than 20% of the total FA content in the samples, the ratio Ω6/Ω3 reflected higher levels of omega 3 FA in relation to omega 6 FA, with the equal values of 0.2 regardless the seasons. In conclusion, seasonal variations were detected in terms of total fat content, without any significant differences in lipid compounds proportions of seaweed profile.

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PC-92

Lipophilic profile of four relevant European macroalgae species

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Macroalgae are a rich source of distinct bioactive components, including phenolics, terpenes and polyunsaturated fatty acids, although their composition is greatly variable between macroalge species. The present work is focused on the comparison of the lipophilic profile of four relevant European species, namely, *Saccharina latissima* and *Fucus vesiculosus* (brown algaes), *Ulva rigida* (green algae) and *Gracilaria* sp. (red algae).

In all macroalgae lipid fraction was extracted with petroleum ether on soxhlet system, and the fatty acids composition was analyzed through derivatization by silylation reaction in gas-chromatography mass spectrometry.

The lipidic fraction of the four macroalgae represented was since 0,9 in *U. rigida*, 0,7 in *Gracilaria* sp, and 0.5 g/100g dw in S*. latissima* to 3.0 g/100 dw in *F. vesiculosus*. In general, each phylum of macroalgae usually presented a characteristic profile of fatty acids. The present study shown that, among the saturated fatty acids, palmitic acid was the most abundant one, regardless of the sample, representing roughly half of the total quantified saturated fatty acids. In fact, palmitic acid was mainly in *U. rigida* (78.4%) and *Gracilaira* sp (69,7%), while the miristic acid was abundant in brown macroalgae *F. vesiculosus* and *S. latissima* samples, with 927and 185,2 mg/Kg, respectively.

The unsaturated fatty acids (UFA) fraction in the four algae was mainly comprised by oleic, linolenic, and linoleic acid. Oleic acid was the most abundant fatty acid in *U. rigida* and *Gracilaria* sp achieved main content in *U. rigida* (47.9 % of UFA) while linolenic acid was predominant among UFA in brown macroalgae (1760 mg/Kg in *F. vesiculosus* and 385,0 mg/Kg in *S. latissim*, followed by linoleic acid achieved 46,8% and 41,3%, respectively, in the same macroalgae. Additionally, omega 6/omega 3 ratio was below 0.5, since 0.36 of *U. rigida* to 0.40 and 0.48 *F. vesiculosus* and *S. latissimi*, respectively, and particularly low in *Gracilaria* (0.22).

In addition to fatty acids, the lipid fraction of the four macroalgae also contained considerable amounts of other compounds. Interestingly, *U. rigida* samples have almost the same content of butyl 6,9,12,15-octadecatetraenoate, as *F. vesiculosys* samples, with 160.8 and 182.4 mg/kg seaweed dw. Despite the total lipid content of *F. vesiculosus* samples being 3-times larger, the same is verified for neophytadiene (172.3 and 182.5 mg/kg seaweed dw, respectively). Another interesting observation is the high phytol content verified in *Gracilaria* samples at 279.7 mg/kg seaweed dw, even though its lipid content is only 0.7 g/100g seaweed dw.

In conclusion, the quantities of overall compounds were concordat with que total fat content, with exception of linoleic and linoleic, stearic and myristic acids present mainly in brown seaweeds.

PC-93

Caracterização de compostos antociânicos em flores comestíveis

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As antocianinas são pigmentos de origem vegetal, solúveis em água e responsáveis por uma gama de cores que vai desde a coloração azul, roxa até à vermelha. Este grupo de compostos é amplamente encontrado em frutos, bagas, flores e folhas, estando descrito que o seu consumo apresenta vários efeitos benéficos para a saúde [1]. A preocupação crescente dos consumidores relativamente à utilização de corantes artificiais na indústria alimentar, incentivou a procura de alternativas naturais [2]. Neste sentido, o objetivo do presente estudo foi a caracterização antociânica de pétalas de flores comestíveis de *Dalia mignon*, *Rosa Damascena* 'Alexandria' e *R. Gallica* 'Francesa' enxertada em *R. canina* e *Centaurea cyanus* L., de forma a avaliar a possibilidade da sua utilização como fonte de corantes naturais.

Os extratos obtidos por maceração metanol/água acidificada (80:20, *v/v*, com 0.01% de ácido cítrico) foram analisados quanto à sua composição em compostos antociânicos por cromatografia líquida de alta eficiência acoplada a um detetor de díodos e a um espectrómetro de massa - HPLC-DAD-ESI/MSn. O gradiente aplicado utilizou os seguintes solventes: (A) 0,1% TFA em água e (B) 100% acetonitrilo. A deteção dos compostos fenólicos foi realizada através de um detetor de díodos a 520 nm e de um espectrómetro de massa operando em modo de ionização positivo. As antocianinas presentes nas amostras foram caracterizadas de acordo com os seus espetros de massa e de UV-Vis, e o seu tempo de retenção, por comparação com padrões, quando disponíveis. A análise quantitativa foi realizada de acordo com as curvas de calibração das soluções-padrão de antocianinas.

Foram identificados nove compostos antociânicos nas pétalas de dália sendo a cianidina-acetil-hexóxido o composto maioritário; nas pétalas de rosa foram identificados 2 compostos, sendo a cianidina-di-hexósido o maioritário, e nas pétalas de centáurea 8 compostos com a cianidina-malonilglucorónido-hexósido a destacar-se. Foi esta última amostra aquela que revelou maior concentração de compostos antociânicos (26 ± 1 µg/g de extrato).

Deste modo, as flores comestíveis podem ser consideradas uma fonte de pigmentos naturais com potencial de aplicação na indústria alimentar e farmacêutica.

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PC-94

Gas chromatography: a useful tool for bakery products differentiation

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Bakery products (cookies, biscuits, cakes, crackers, bread and savoury snacks) are highly appreciated and frequently consumed by all age consumers, usually as part of breakfast, mid-morning and afternoon snacks. The main ingredients of bakery products are: wheat flour, sugar, fat, water and salt. With respect to fat, besides the total amount, it is very important to evaluate its quality, namely the fatty acid profile. One of the most suitable techniques to evaluate the fatty acids composition of a food is gas chromatography coupled to flame ionization detection [1]. The aim of this study was to evaluate if gas chromatography is a useful tool for bakery products differentiation, with respect to their fatty acids profile and impact on human nutrition. Therefore, 30 bakery products from six categories ("Maria" cookies, plain salty cookies, coated chocolate cookies, brioche filled with chocolate, brioche without filling and with chocolate chips, and French croissants) and from different brands, were acquired in supermarkets from Lisbon region. Total fat content was determined by acid hydrolysis and Soxhlet extraction with petroleum ether [2]. Fatty acids methyl esters were obtained by cold transesterification and analysed by gas chromatography with flame ionization detection [3]. Most of the analysed foods (83%) have a higher content of saturated than monounsaturated and polyunsaturated fatty acids. For all the studied categories considerable differences were observed in the fatty acid profile, except for brioche with chocolate chips. In "Maria" cookies, plain salty cookies and coated chocolate cookies, at least one sample had as major fatty acids the monounsaturated instead of saturated. In the brioche filled with chocolate category, for two of the analysed samples the major fatty acids were saturated, while for the other sample predominated the polyunsaturated fatty acids. In conclusion, gas chromatography is a useful technique to differentiate the nutritional quality of bakery products, as well as to estimate the potential impact of these foods on public health. Furthermore, it was possible to conclude that food industry should address new strategies to reformulate their products. According to the obtained results, it is possible to produce similar foods with healthier nutritional features.

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PC-95

Profile of Bound Phenolic Compounds from Olive Pomace

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The olive oil production is characterized by significant amounts of residues and their management is an economic and environmental challenge issue for the olive mills. Nowadays, the more relevant waste of olive oil extraction is Olive Pomace (OP), due to the large implementation of two-phase system [1]. OP contains large amount of water (50-70%), fibre [2] and carbohydrates [3], but also phenolic compounds (~98% of olive's phenolic compounds remains in OP) [4]. Several studies have been performed with OP as a source of polyphenol compounds. However, an appreciable amount of polyphenols, called "Non-Extractable Polyphenols" or "Bound Polyphenols" (BP) can remain on the extraction residue [5]. The BP are associated with fibre and/or proteins and its extraction can't be performed using the common aqueous-organic solvents. In rich fibre plant foods like olives, BP and fibre are intimately associated [6], therefore the polyphenol content of OP could be underestimated. In several studies have been demonstrated that BP are representative group of total polyphenols. Besides that BP could be bioaccessible and bioavailable in the human gut and they may have an important role in gastrointestinal health and contribute to the systemic effects associated with dietary antioxidants [5].

The overall objective of present study was to study the relevance of Bound Phenolics (BP) of OP. Therefore, the present study aimed to determine the content of phenolics (BP and FP) using chromatographic method (HPLC and LC/MS) to identify the major classes of phenolic compounds present in BP and FP. The antioxidant activity of FP and BP was also studied.

The OP samples were supplied from olive mills (2-phase system) from Coimbra district and was stored in in a freezer at -80 ºC. Extraction procedures of polyphenols (FP and BP) followed the method described by Xie *et al.* (2015) with some modifications [3]. Total Phenolic Compounds (TPC) and Antioxidant Activity (AOX) of FP and BP were analysed using the methods Folin-Ciocalteau [mg gallic acid equivalents (GAE)/ g DW] and ABTS [mg Trolox equivalents/g DW], respectively. FPC and BPC analyses were performed by HPLC followed the method described by Oliveira *et al.* [8].

The TPC and AOX of FP were greater (at least 6 times) than BP. However, the amount of BP was significant (3,62 ± 0,38 and 3,20 ± 0,12 mg GAE/g DW) as source of phenolic compounds. The principal FP quantified in OP samples was 3-hydroxytyrosol (FP: 2.07 ± 0.14 mg/g DW; BP: 0.19 ± 0.05) and the principal BP was caffeic acid (greater in BP – 0.25 ± 0.02 mg/g DW - than in FP – 0,22 ± 0.02 mg/g DW). The protocatechuic acid only was found in BP in the range of 0.11 ± 0.01 to 0.08 ± 0.01 mg/g DW. Derivatives of hydroxybenzoic acid, such as the protocatechuic acid, can be found as part of complex structures such as lignin, while the derivatives of hydroxycinnamic acid such as caffeic acid, are mainly linked to structural components of cell wall (cellulose, lignin and protein) through ester linkages [9].

Although, the OP's amount of BP was lower than FP, they could significantly contribute to polyphenol intake from OP and provide a significant biological activity associated with gastrointestinal health and potential systemic effects in large intestine. Based on previous studies BP reach the colon, where they are released and metabolized in different bioavailable metabolites by the action of bacterial microbiota [5].

Further studies to determine contribution to the health-related properties of BP from OP are needed, namely simulation of gastrointestinal tract (GIT).

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PC-96

Application of GC-MS to characterize the volatile composition of fruit distillates made with honey

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Distilled spirits could be made from several fermented sugar-based materials. Many of the spirits produced with fruits have the disadvantage of a higher methanol content [1] as result of enzymatic degradation of the pectins [2], which are present in high amounts on the seeds and skins of the fruits [3]. By the contrary the honey spirit, with an alcoholic strength ranging between 37.4% and 53.0%, exhibited a methanol content quite null which is an advantage for these beverages, due to well know and studied neurotoxicity of this compound [4]. Additionally this beverage has a very interest sensory attributes, namely: fruity, floral, sweet, vegetative/herbaceous, smoky, sweet and bitter [5].
In this context, the aim of this study was to evaluate the volatile composition of some spirits combining fruit and honey.
It was quantified the methanol, acetaldehyde, ethyl acetate and higher alcohols content of four spirits produced: with cherry; with cherry and honey; with madrono; madrono and honey.
The methanol, acetaldehyde, ethyl acetate and higher alcohols were quantified by GC-MS equipped with a fused silica capillary column of polyethylene glycol. Compound concentrations were determined by direct injection of the distillate and compared the peak area of each sample with the calibration curve obtained with the standards. The carrier gas used was helium.
The results showed a decrease of the total fusel alcohols for the spirits with honey adition in comparison to the corresponding fruit spirits without honey. The concentration of volatile compounds variation depend of the fruit used in the spirit prodution. However, the 1-Propanol content decrease in the fruit spirit produced with honey adition. The best advantage for these new beverages is the decrease of the methanol content as well a small decrease of the acetaldehyde content.

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PC-97

HPLC/DAD fingerprint of standardize extracts from *Ligustrum lucidum* Aiton berries, for bioactive activity screening

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Ligustrum lucidum Aiton is a China-native tree, whose fruits provide a reddish black dye that, according to some authors, was used in the past to intensify wine's color. These fruits were also applied in traditional Chinese medicine with the aim of curing fatigue, hemoptysis and dropsy [1]. Studies developed on the last two decades provided scientific evidence regarding its biological activities [2,3], as well as, the presence of several biologically active compounds among which dammarane triterpenes and the flavonoids quercetine, apigenin and luteoline glycosides [1].

This work aims to provide the fingerprint of selected extracts by HPLC-DAD of Portuguese *L. lucidum* berries from two regions and to characterize its bioactive potential.

Profiles were established with three different extraction methods: 1- boiled water; 2- ethanol (100%); 3- ethanol/water (1:1) (*v/v*) 15 hours of stirring at room temperature at 120 rpm [3]. Phenolic acids and flavone/flavonols structures were determined by UV absorption spectroscopy according to Campos and Markham [4].

Results revealed that fingerprint of the extracts were very similar, however, both regions showed some differences on the concentration of the compounds. Boiling water extract was the more concentrated. Even so, the flavonoids found in all extracts as presented the same structure and the derivatives of caffeic acid, apigenine and luteoline were confirmed as minor constituents. Further studies may be carried out in order to explore the bioactive potential of *L. lucidum* berries.

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PC-98

Similarity analysis between four Portuguese propolis samples using UHPLC-DAD-ESI-MS^n chromatographic profiles of phenolic compounds

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Propolis is a complex resinous substance produced by bees (*Apis mellifera* L.) and used in the hive to seal openings and cracks, protecting it from fungi and bacteria, embalm dead insects that cannot be removed from the hive, thus preventing its putrefaction and spread of disease within the hive [1,2]. It is considered a "natural antibiotic" since its compounds are collected from floral/ leaf buds and are mixed with pollen, wax and enzymes of bees' saliva [3,4].The major components are phenolic acids (hydroxybenzoic and hydroxycinnamic acid) and flavonoids that are closely associated to propolis biological properties such as, antioxidant, antimicrobial and anti-inflammatory activities [4,5].Since it is a resinous substance, extraction of the phenolic compounds and their identification is a necessary step for considering this product as a food additive and protective cover to improve the stability and shelf-life of food products [6].

In this work, four Portuguese propolis samples were collected in different regions: Braga, Lousã, Macedo de Cavaleiros e Montesinho. Samples were cleaned, extracted and purified using *n*-hexane and ethanol/ water (4:1) solvents. The phenolic compounds extracts were analysed by UHPLC-DAD-ESI-MS^n and the overall results showed a chromatographic profile containing 55 phenolic compounds, from which, 44 were identified. Usually, visual evaluation of the chromatographic profile allows their discrimination when differences are large and the number of samples is limited. However, even if the number of samples is limited but differences are subtle, mathematical approaches are necessary. Considering this, cluster analysis (similarity study) was applied to the four propolis UHPLC-DAD chromatograms using the areas of each peak as data matrix. Moreover, since some peaks were not in all propolis chromatograms, a Yeo-Johnson transformation was used in order to accommodate predictors with zero values. Finally, results showed that Lousã propolis was the sample with higher differences in the chromatographic profile, followed by the Braga sample. Macedo de Cavaleiros and Montesinho samples were the most similar with regard to the phenolic profile.

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PC-99

Determination of organophosphorus pesticides in strawberries using modified QuEChERS method with magnetic nanoparticles and GC–FPD

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In recent years, there has been growing environmental concern, especially regarding the use of toxic substances. Hence, there is a growing need to monitor organic micropollutants in environmental studies and to control the quality of food. Fresh vegetables, fruits and pulses are the important part of a healthy diet because of the presence of significant amount of nutrients and minerals in them. However, at the same time, they can also turn out to be source of toxic substances such as pesticides. Among various pesticides, organophosphorus pesticides (OPPs), derivative of phosphoric acid, are the most extensively used insecticides or acaricides in many crops. Due to low persistency and high killing efficiency of OPPs, many farmers regularly use this group of pesticides for various vegetables and fruits crops. The continuous use of pesticides has caused the deleterious effects to ecosystem. In response to this, different methods have been developed and are applied routinely for the quantification and monitoring of multi pesticide residues in fruits and crops. The analysis of OPPs is highly challenging due to the tendency of pesticides losses during the several steps of the methodology: sample preparation, clean up, storage of sample extracts and standard solutions as well as during GC analysis. Seeing the current need to address the awareness for long term moderate exposure of pesticides and high analytical requirements, the following study on evaluation of the different OPPs in strawberries will be performed using new strategies in analytical procedures. Based on a modified quick, easy, effective, rugged and safe (QuEChERS) sample preparation method with magnetic nanoparticles (MNPs) as the adsorbing material and gas chromatography with a Flame Photometric Detector (GC-FPD), we stablished a new method for OPPs determination in strawberry samples. The extraction conditions were studied using different QuEChERS and novel cleanups compositions and different extraction times were evaluated.

The method proved to be simple and gave quantitative results for the assayed analytes, providing good validation parameters, such as linearity, precision, limits of detection and quantification. The MNPs used for removing impurities improved the speed of sample pre-treatment and exhibited an enhanced performance and purifying effect. The preliminary results reinforce the relevance of this study for food chemistry namely food safety analysis.

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PC-100

Occurrence of Organophosphorus pesticide in sediments from Portuguese rivers

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Pesticides include a large group of organic compounds belonging to different chemical families, which play an important role in increasing agricultural productivity. Among various pesticides, organophosphorus pesticides (OPPs), derivative of phosphoric acid, are one of the most extensively used insecticides or acaricides in many crops. However, they are environmental hazards due to their stability, possible persistence and toxicity and they pose a tremendous danger to wildlife. Some of these pesticides are bioaccumulative and due to their vertebrate and non-vertebrate toxicity can affect non-target organisms. Water is one of the primary pathway of pesticides dissemination from their application areas to other parcels of the environment. As a result, the presence of pesticides in environmental samples, namely sediments, typically in the lower ng L^{-1} concentration ranges, have been reported. In Europe, there are few studies that determine the occurrence of currently used pesticides in water and related environmental samples. The study cases are two Northern Portuguese rivers (Âncora and Ferreira River) where physico-chemical water parameters and biological indexes based on the benthic macroinvertebrate community were already determined. The analysis of pesticides in sediment is also important to obtain a more complete spectrum of information of the ecological status of the studied rivers located in areas with high biodiversity value (Natura 2000 Network, PTCON0039 and PTCON0024). In response to this, different methods have been developed and are applied routinely for the quantification and monitoring of multi pesticide residues in environmental samples. Although QuEChERS method (Quick, Easy, Cheap Effective Rugged and Safe) is a reference for foodstuff, it has been used for the extraction of pesticides from sediment, soil and sludges. The major advantages of QuEChERS are low solvent consumption (low costs), speed, high sample throughput and possibility to obtain high recoveries for a wide spectrum of compounds. After selective sample extraction (QuEChERS), seven OPPs were quantified by gas chromatography with a Flame Photometric Detector (GC-FPD). The method proved to be simple and gave quantitative results for the assayed analytes, providing good validation parameters, such as linearity, precision, limits of detection and quantification. The contamination profile in sediments is marked by the presence of an OPP (chloropyriphos). The mean concentrations ranged from 16 – 24 ng/g d.w. in sediment. The spatial distribution of the pesticide was consistent with the agricultural activities of the studied area (e.g., corn crop).

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PC-101

Total fat content and fatty acid profile of pseudocereals

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Amaranth, quinoa, and buckwheat are called "pseudocereals" since they produce starch-rich seeds like cereals but they are dicotyledonous plants (and not monocotyledonous as cereals). According to some phylogenetic classifications, amaranth (*Amaranthus*) and quinoa (*Chenopodium*) genera belong to the order *Caryophyllales*, whereas buckwheat (*Fagopyrum*) belongs to *Polygonales* [1]. Pseudocereals have recently gained more popularity as a part of human diet thanks to their chemical composition, particularly as a source of protein, vitamins of B group, minerals and also for their gluten-free flour [2]. Some pseudocereals can even present a fat content three times higher than cereals, with a fatty acid profile dominated by unsaturated fatty acids [3-5].

The total fat content was determined using Soxhlet method, and the fatty acid profile was subsequently determined using gas chromatography with flame ionization detection (GC-FID). The results for total fat content ranged from 3.1% to 8.0%. and evidenced a significant predominance of unsaturated fatty acids. For amaranth and buckwheat unsaturated fatty acids composed up to 81% of the total chromatographic area while for quinoa this value ranged from 87.6% to 90.4%, depending on variety. Polyunsaturated fatty acids formed 48.8% in amaranth, 61.1-64.9% in quinoa and 40.9% while monounsaturated fatty acids compose 32.2% in amaranth, 26.7-29.2% in quinoa and 40.2% in buckwheat. Main unsaturated fatty acids consisted of linoleic acid followed by oleic, linolenic and nervonic acids. Main dominance for saturated fatty acids was for palmitic acid. Buckwheat shown significant differences in fatty acid composition having much higher amount of oleic acid but lower amount of linoleic acid and no signs of nervonic acid. This distinct fatty acid profile may be partially explained by the different botanical origin of buckwheat, which belongs to the *Polygonales* order, while amaranth and quinoa are classified into the *Caryophyllales* order.

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PC-102

Enantiomeric separation and chiral recognition mechanisms of different macrocyclic glycopeptide-based chiral stationary phases

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Macrocyclic glycopeptide-based chiral stationary phases (CSPs), developed by Armstrong *et al.* in 1994 [1], are considered one of the most versatile and efficient for enantiomeric separation. Moreover, their applicability can be increased considering the complementary profile in enantioselectivity of the different macrocyclic glycopeptide selectors [2].

In this work, a systematic study of enantioresolution of a library of enantiomeric mixtures of oxygen heterocyclic compounds prepared "in-house" was successfully carried out using four commercially available macrocyclic glycopeptide-based CSPs, namely Chirobiotic T™, Chirobiotic R™, Chirobiotic V™ and Chirobiotic TAG™. Multimodal elution conditions (normal-phase, polar organic, polar ionic and reversed-phase) were evaluated.

The effects of the mobile phase composition, the percentage of organic modifier, the nature and concentration of different mobile phase additives on the chromatographic parameters are discussed. The elution order, was evaluated in all cases. Chirobiotic T™, under normal-phase and reversed-phase modes, demonstrated to be the most effective for the tested enantiomeric mixtures.

Considering the importance of understanding the chiral recognition mechanisms associated with the chromatographic enantioresolution, and the scarce computational studies available on macrocyclic glycopeptide-based CSPs, computational studies by molecular docking were also carried out.

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PC-103

Pyrolytic markers of effect of agricultural practices in the chemical composition of soil organic matter

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Chemical characterization of soil organic matter (SOM) by analytical pyrolysis has been extensively used to evaluate the effect of land-use changes and vegetation on the soil C balance, specifically for assessing the degradation of plant biomacromolecules or the formation of new organic constituents [1]. In this study, the chemical composition of SOM in agricultural soils was characterized by analytical pyrolysis. Analytical pyrolysis was carried out at 500 ºC in the soil humic acids (soil-HA, the alkali-soluble, acid-insoluble SOM fraction). Up to 130 pyrolytic products were identified and grouped into five classes reflecting the different origin of SOM. Then, the pyrolytic profiles were compared by an upgraded graphical-statistical method (Van Krevelen plot) representing the molecular composition of the SOM accumulated in the different soils [2] (Fig. 1). The results of multivariate data treatments suggested different pyrolytic markers of agricultural practices, viz: i) abundance of methoxyphenols indicating the frequent periodical incorporation of organic amendments, ii) practical lack of lignin-derived compounds and the increase of non-methoxylated aromatic compounds as consequence of inputs of charred materials, iii) amount of polysaccharides and protein derived compounds in soils with amorphous minerals highlighted the importance of water retained in soil microporous iv) and finally, a substantial yield of allylic compounds (alkanes, alkenes and fatty acids) showing a high microbial activity in disturbed soils. As a whole, the intensity of agricultural practices could be grouped into biogeochemical processes [3], domained either diagenesis or pyrogenesis of soil C.

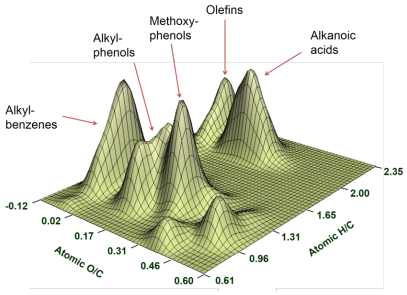

Figure 1. *Surface density map displaying cumulative abundances of soil-HA pyrolysis compounds represented in the space defined by their H/C and O/C atomic ratios in a Van Krevelen diagram.*

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PC-104

Gas chromatographic signature of soil lipids associated to land-use changes

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The molecular characterization of the biomarker assemblages in soil lipid extracts provides environmental and biogeochemical information about the structure and dynamics of the soil trophic system. In this research field, compounds such as triterpenes from soil lipid extracts have been found highly responsive to land use and agricultural practices [1]. In order to monitor changes in soil functioning resulting from transformation of secondary forests into cultivated soils, the lipids from soil samples collected in Tenerife Island (Spain) were analyzed by gas chromatography–mass spectrometry (GC/MS).

The lipids were extracted at room temperature using dichloromethane-methanol 3:1 by vol. under ultrasonic shaking, methylated with trimethylsilyldiazomethane and the compounds were separated in an HP 5890 chromatograph connected to an HP 5971 mass detector (EI, 70 eV) with a 25-m, 0.22 mm i.d., cross-linked OV-1 column. Helium flow was adjusted to 1 cm^3 min^{-1}; the oven temperature was fitted from 70º to 220 ºC at 4 ºC min^{-1} during the chromatographic run. Lipid compounds were identified from both their retention time and mass spectra stored in spectral libraries.

The main lipid compounds identified consisted of *n*-alkanes and *n*-fatty acids, but important amounts of cyclic compounds, mainly triterpenes (lanostenol > ursenone > taraxerol > friedelanone > amyrin > lupanone) were characteristically found in seminatural forest soils (Laurel forest). In addition, other diagnostic compounds (e.g., diterpenes and a variety of steroids: stigmasterol, ergosterol, cholesterol) were found in variable amounts, depending on vegetation type. Multivariate statistical data analyses showed how the chromatographic signature of lipid compounds quantitatively reflected the impact of agricultural practices. After clearing the Laurel forest, an increased carbon preference index (even-to-odd C-number preference, CPI) of alkanes was observed. As regards the relative chain length (RCL), both in the case of alkanes and fatty acids, a dominance of short-chain length compounds was observed in cultivated soils. Finally, the most conspicuous quantitative indicator of the changes in land-use was the significant decrease in the total amount of cyclic constituents, mainly triterpenes, which can be interpreted as the result of a simplification in the soil trophic system.

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PC-105

Influência do método de secagem no perfil fenólico e propriedades bioativas de *Galium aparine* L.

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Galium aparine L. é uma planta conhecida como amor-de-hortelão pertencente à família das Rubiaceae. É uma erva indesejada em diversas plantações, conhecida por provocar um impacto prejudicial em campos de cereais, colza e beterraba [1]. No entanto, possui reconhecidas aplicações medicinais, sendo utilizada em casos de febre, infeções do trato urinário, doenças da pele, purificação do sangue e aumento do fluxo linfático, entre outras [2]. Neste trabalho, foram estudados diferentes extratos (infusões e extratos hidrometanólicos) de amostras de *G. aparine* processadas de duas formas: secas à sombra e liofilizadas. O perfil fenólico dos diversos extratos foi analisado por cromatografia líquida de alta eficiência acoplada a detetor de díodos e de espetrometria de massa (HPLC-DAD-ESI/MS); as propriedades antioxidantes foram avaliadas pela atividade captadora de radicais livres, pelo poder redutor e pela capacidade de inibição da peroxidação lipídica em homogeneizados cerebrais.

Os diferentes extratos de *G. aparine* revelaram perfis fenólicos muito semelhantes, apresentando apenas variações na concentração dos compostos detetados. Os extratos da amostra liofilizada revelaram teores totais de compostos fenólicos ligeiramente superiores aos obtidos a partir da amostra seca à sombra. Para ambos os métodos de secagem, os extratos hidrometanólicos revelaram concentrações totais de compostos fenólicos superiores (191 e 181 mg/g de extrato, na amostra liofilizada e seca à sombra, respetivamente) aos observados nas infusões (183 e 164 mg/g) da mesma amostra. O ácido 5-*O*-cafeoilquínico foi o composto maioritário em todos os extratos estudados (163 e 158, 157 e 145 mg/g de extrato hidrometanólico e infusão de *G. aparine* liofilizada e seca à sombra, respetivamente). Relativamente à atividade antioxidante, o extrato hidrometanólico da planta liofilizada revelou melhores resultados nos ensaios de poder redutor (EC_{50}=175 µg/mL), inibição da descoloração do β-caroteno (EC_{50}=83 µg/mL) e inibição da peroxidação lipídica (EC_{50}=14 µg/mL). No entanto, a infusão de planta seca à sombra revelou melhores resultados de atividade captadora de radicais 2,2-difenil-1-picril-hidrazilo (EC_{50}=467 µg/mL).

Os resultados obtidos neste trabalho contribuem para um conhecimento mais aprofundado da composição fenólica e das propriedades bioativas de *G. aparine*.

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PC-106

A novel natural colouring strategy for ice cream: effects on the profiles of individual sugars

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The production of ice cream consists of two essential steps: firstly, the mixture is prepared and aerated, and afterwards it is frozen. This complex frozen dessert consists of several components, such as air bubbles (to give volume), water (in the form of ice crystals to confer weight), a partially frozen serum phase, and small amounts of emulsifiers, stabilizers and colourants [1]. Probably, ice cream is not the best product to answer to the growing health concerns, mainly due to the increased rate of obesity, metabolic syndrome and diabetes. This has been bothering consumers, leading them to seek healthier foods [2]. Nevertheless, ice cream is a very appreciated desert all over the world, representing a suitable food matrix to be submitted to any improvement process, such as the addition of natural additives with acknowledged bioactive properties. Currently, there is a trend to replace the artificial colourants used in ice cream preparation. The flowers of *Gomphrena globosa* L., contain high levels of betacyanins, which have a strong colouring capacity, in addition to their high antioxidant activity and chemopreventive effects [3]. Accordingly, aqueous extracts from *G. globosa* flowers were incorporated in ice cream, benefiting from their dual colouring/functionalizing effects. To validate their suitability, the prepared formulations were compared to other ice cream (i) free of any colourant, (ii) added with commercial betalain, or (iii) added with B*eta vulgaris* extract (E-162). Among other parameters, the individual sugar profiles were evaluated by high-performance liquid chromatography (HPLC) coupled to a refractive index detector (RI) throughout storage time (up to 60 days). Overall, the tested formulations did not induce significant changes in individual sugars profile, except for the lower sucrose contents detected in ice creams prepared with *B. vulgaris* extract. Therefore, the extracts of *G. globosa* might be considered as an effective colouring solution, without compromising individual sugars profile, one of the most important parameters to assess ice cream quality.

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PC-107

Development of a MHS-SPME-GC/MS method for analysis of volatile composition of Tawny Port wine

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Port wine is a fortified wine produced in the Douro Demarcated Region (DDR) of Portugal, widely known throughout the world. This type of wine is produced from authorized grape varieties grown in the DDR and by a specific winemaking practice. Particularly, Tawny Port wines are stored in small wood barrels during several years and bottled at commercialization time [1]. The extent of this aging process is essential to the quality of the final product. In this oxidative aging process, Tawny Port wine undergoes through many changes in colour and aroma, with the levels of some compounds decreasing over time while other increase or appear [2]. Some of the compounds formed or accumulated over the aging time can be considered age markers being the wine aging process a complex system [3], so it is important to know more about the evolution of the aroma composition of Tawny Port wines. In line with that, it is necessary the optimization of a method to identify the compounds present in Tawny Port wines aroma. Some parameters like sample dilution, extraction temperature and time and ionic strength effect from adding different amounts of NaCl were optimized using a four three-level box factorial with three blocks design to determine the optimal experimental conditions for analysing the volatile compounds in Tawny Port wine aroma by HS-SPME-GC/MS (headspace - solid phase microextraction – gas chromatography mass spectrometry) using a DVB/CAR/PDMS fibre. The optimal conditions were as follows: sample dilution of 5 mL of wine to 5 mL of water with 3.5 g of NaCl and the extraction during 90 min at 30 °C. In order to eliminate possible matrix interferences, MHS-SPME (multiple headspace - solid phase microextraction) was performed to analyse Tawny Port wine samples.

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PC-108

Translocation study of pesticides applied by endotherapy in coconut palm (*Cocos nucifera* Linn.) and determination of residues by UHPLC-MS/MS

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The coconut palm (*Cocos nucifera* Linn.) is the most important palm in tropical and subtropical ecosystems because different parts of it can be useful, due to the exploitation of its fruit in the form of coconut water, oil and solid albumen. The application of pesticides is still one of the practices used to control pests and diseases. Moreover, conventional treatments using the spraying and/or application of pesticides in the soil have not controlled or eliminated some persistent pests and diseases in the coconut palms. The main objective of this work was to analyze the aspects of movement/translocation of pesticides in coconut palm, after the application of pesticides by alternative techniques as endotherapy, and subsequent evaluation of the contamination of the coconut water and pulp.

The pesticides selected were: carbosulfan, cyproconazole, imidacloprid, difenoconazole, thiabendazole, thiamethoxam and spirodiclofen. The mix was prepared with all pesticides with the *Break-thru®*, which was the adjuvant chosen. Through a randomized design, the mix was applied in 30 plants with the commercial equipment *Bite Infusion®* version *Di Palma*, using a volume of 10mL of mix. The stem samples were collected 50 and 100 cm above the point of application with intervals from 2 to 45 days, while the fruit had intervals of 45 to 120 days after the applications. All methods for the determination of pesticides in coconut stem, coconut water and pulp used the QuEChERS (Quick Easy, Cheap, Effective, Rugged and Safe) modified acetate extraction technique and ultrahigh efficiency liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) as the analysis technique. [1,2]

In most cases, the samples stem 50cm above the point of application showed higher concentrations than those at 100cm (near cup coconut). Based on these results, the hypotheses were that the communication between the vascular bundles may have been a distribution/dilution of pesticides and the pH difference found in the stem extension may have some influence on the translocation of it. Thus, except for spirodiclofen (non-systemic pesticide) and 3-OH-carbofuran, carbosulfan metabolite, all pesticides were quantified/detected in the samples analyzed 45 days after the applications. Pesticides were not detected in the fruit, indicating that the endotherapic treatment can be effective for the control of diseases and pests without the risk of contaminating the fruit.

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PC-109

The impact of extrusion on the organics acids composition of gluten-free snacks based on rice, bean and carob flour blends.

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Due to their nutritional value and chemical profile, legumes, such as beans and carob, have a great potential to be explored leading to the development of novel foods for being included in healthy diets. Organics acids are biomolecules, indispensable for the human body, since they are essential intermediates in cell metabolism. Some of these molecules exhibit antioxidant potential, since they are capable of chelating metals or delocalizing electronic charge from free radicals. Thus, they can be applied in a wide range of industries including food, pharmaceuticals, cosmetics, detergents, polymers and textiles [1]. The aim of this study was to evaluate the changes induced by extrusion processing on organics acids in novel formulations containing different ratios of rice (50-80%), bean (20-40%), and carob (5-10%). The methodology based on Barros *et al.* [2] for organics acids' extraction was applied and the analysis was performed by using ultra-fast-liquid-chromatography coupled to a photodiode array detector (UFLC–PAD). Generally, seven organic acids were identified, namely oxalic, quinic, malic, shikimic, citric, succinic and fumaric acids. However, the composition of these molecules in the studied samples was heterogeneous, being citric acid the major organic acid found in all samples, with the exception of rice and carob where succinic and quinic acids prevailed, respectively. Bean sample was the raw material with the highest organics acids content (3.46 ± 0.01 g/100 g dry weight). On the other hand, commercial extruded rice was the sample that showed the lowest content of total organics acids, presenting trace amounts of all the identified molecules. In general, the higher amount of legume in the non-extruded and extruded samples, the higher concentration of organics acids content detected. In general, the total content of organics acids was not significantly affected by food processing, which is in accordance with other reported works [3]. With this study, it was possible to conclude that the incorporation of legumes may improve the nutritional value of the studied snacks, increasing the levels of organic acids, in comparison with those only made with rice.

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PC-110

Tocopherols content in gluten-free extruded composite flours of rice and different legumes

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Extrusion cooking is a high temperature short time process, which modifies flour properties through starch gelatinization, protein denaturation, complex formation between amylose and lipids, degradation of pigments and improvement of sensory characteristics [1]. Vegetables contain numerous phytochemicals, such as tocopherols, useful for their nutritional and nutraceutical properties. Tocopherols (constituents of vitamin E) appear in several active forms, presenting α-tocopherol the highest biological activity, and being γ-tocopherol the most abundant in vegetable foods, such as sesame seed, soybean, black bean and peanut. Due to its action as a free radical scavenger, vitamin E also plays a role on body protecting against degenerative abnormalities, mainly cancer and cardiovascular diseases [2]. The aim of this study was to evaluate the changes induced by extrusion-cooking on tocopherols content in functional novel formulations of flours containing different proportions of rice (50-80%), bean (20-40%), and carob (5-10%) using the raw materials as control. Tocopherols were determined in the different flours mixtures of rice-legumes by high performance liquid chromatography coupled to a fluorescence detector (HPLC-FL) programmed for excitation at 290 nm and emission at 330 nm, following a procedure previously described by Barros *et al.* [3]. In general, the samples showed low levels of tocopherols and, in some cases, namely in extrusion samples, the total absence of this vitamin was verified. α-, γ- and δ-Tocopherols were the vitamers detected in several flours, highlighting bean with the highest concentration of total tocopherols (180 ± 1 μg/100 g). In the samples where tocopherols were detected, the raw materials and in all the evaluated mixtures, γ-tocopherol was the predominant vitamer, being present in greater concentration in bean with values of 172 ± 1 μg/100 g. In this study it was also observed that, after extrusion, a significant reduction occurred in the total tocopherols content, being verified the absence of these molecules in different flour mixtures.
In addition, the sensitivity of vitamin E to extrusion cooking depends on the extrusion processing variables and conditions used, particularly extrusion temperatures (that promotes the decrease in α-tocopherol) and moisture during extrusion (decreasing γ-tocopherol content).

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PC-111

Phytochemical characterization of *Opuntia macrorhiza* (Engelm.) and *Opuntia microdasys* (Lehm.) cladodes

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The genus *Opuntia* belongs to the Cactaceae family. The cladodes, also known as "nopalitos", have been widely used, in many countries, for their beneficial effects and phytochemical composition. It is used as a food, as a source of nutrients, and also in folk medicine [1]. In the present work, the cladodes of *Opuntia macrorhiza* (Engelm.) and *Opuntia microdasys* (Lehm.) were characterized in terms of free sugars, organic acids, fatty acids, tocopherols and phenolic compounds. The individual sugars' profile was determined by HPLC-RI, organic acids by HPLC-DAD, fatty acids by GC-FID, and tocopherols by HPLC-fluorescence. The phenolic compounds profile was determined by HPLC-DAD-MS/ESI. Despite the phylogenetic proximity and similar geographical origin the samples of *O. microdasys* and *O. macrorhiza* showed significant differences in the profiles of sugars, organic acids, fatty acids, tocopherols and phenolic compounds. While, *O. microdasys* presented higher sugars and tocopherols content, *O. macrorhiza* revealed higher concentration in organic acids, poly and monounsaturated fatty acids. The cladodes of *O. microdasys* (10 compounds: 6 phenolic acid derivatives and 4 favonols) revealed a higher amount of phenolic compounds in comparison with *O. macrorhiza* (7 compounds: 6 phenolic acid derivatives and 1 favonol); only four of the identified compounds were detected simultaneously in both species. Piscidic acid (3400±236 μg/g of extract) and eucomic acid (1688±26 μg/g of extract) were the main phenolic compounds in *O. macrorhiza*, while isorhamnetin-*O*-(rhamnosyl)-rutinoside was the most abundant in *O. microdasys* (2507±73 μg/g of extract). Hence, the elucidation of the most abundant compounds might constitute useful information to select the best species regarding specific applications of these natural extracts.

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PC-112

Chemical characterization of *Opuntia* sp. by-products

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The species from the Cactaceae family are important invaders in the Mediterranean. The seeds are usually discarded and proper utilisation of these waste products could lead to an important source of bioactive compounds. The seeds have been described to be rich in health-promoting polyunsaturated fatty acids and may potentially be included in animal and human diets [1]. Moreover, seeds could also be recovered for their high nutritional value, as sources of sugars, tocopherols, dietary fiber and polyphenols instead of being discarded, as it currently occurs.

In this study, the composition in soluble sugars, fatty acids, organic acids, tocopherols and phenolic composition were determined in seeds of *Opuntia macrorhiza* (Engelm.) and *Opuntia microdasys* (Lehm.). The individual sugars profile was determined by HPLC-RI, fatty acids by GC-FID, organic acids by HPLC-DAD, tocopherols by HPLC-fluorescence and phenolic compounds by HPLC-DAD-ESI/MS.

In both cases, the overall concentrations of soluble sugars in seeds were quite lower than those detected in other *Opuntia* sp. parts. Quinic (0.30 g/100 g dw) and oxalic (0.32 g/100 g dw) acids were the major organic acids detected in *O. microdasys* and *O. macrorhiza*, respectively. The fatty acid profiles detected in the seeds of both species were similar, with linoleic acid (C18:2n6) as the major fatty acid (71% in O. microdasys; 74% in *O. macrorhiza*). γ-Tocopherol was the most abundant tocopherol vitamer in both species (7.4 mg/100 g dw in *O. microdasys*; 5.8 mg/100 g dw in *O. macrorhiza*). Regarding the phenolic profile, eight compounds were identified: seven phenolic acid derivatives and one flavonoid, and *O. macrorhiza* seeds (1016 µg/g extract) revealed a higher concentration than *O. microdasys* (450 µg/g extract). Feruloyl di-hexoside was the most abundant phenolic acid in both species (225 µg/g extract in *O. microdasys* and 718 µg/g extract in *O. macrorhiza*).

The seeds of *Opuntia* sp. presented several bioactive phytochemicals, which may allow considering this botanical part as highly interesting by-products.

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PC-113

Extractability of rosmarinic acid by using three different aqueous based extraction procedures

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Rosmarinic acid is the main constituent of the rosemary extract (E392), which is the only natural extract allowed in the European Union to be used as food preservative. Furthermore, rosmarinic acid has many bioactivities, namely astringent, antioxidative, anti-inflammatory, antimutagenic, antibacterial and antiviral. It is also used against dyspepsia, dysmenorrhea, rheumatic diseases and Herpes simples, among others [1]. Various techniques can be employed to extract compounds from different plant parts, including traditional extraction protocols through maceration, as also extractions assisted by microwaves and supercritical fluids, among others. In this study, four different plant species were submitted to aqueous based extractions in order to determine the best conditions to obtain the highest quantity of rosmarinic acid. Rosemary (*Rosmarinus officinalis* L.) was chosen due to its permission to be used in the food industry and also for its high content of rosmarinic acid. Basil (*Ocimum basilicum* L.), Oregano (*Origanum vulgare* L.), and Sage (*Salvia officinalis* L.) were chosen for being putative sources of natural food preservatives in the near future, having also interesting bioactivities, no toxicity and a fair amount of rosmarinic acid in relation to rosemary [2]. The three chosen aqueous extraction systems were infusion, decoction and hydroethanol extraction (80:20, v/v). All the extracts were analysed using a High-Performance Liquid Chromatography coupled to a Diode Array Detector and an Electrospray Ionization Mass Spectrometer (HPLC-DAD-ESI/MS). Table 1 shows the amount of rosmarinic acid in each extraction conditions (mg/g).

Table 1. *Quantities of rosmarinic acid in different plants and extraction procedures.*

	Rosemary	Basil	Oregano	Sage
Infusion	43±1^{c}	22.23±0.01^{b}	20.5±0.7^{c}	36±2^{b}
Decoction	22.1±0.3^{a}	10.64±0.05^{a}	15±2^{b}	25±1^{a}
Hydroethanolic	29.3±0.3^{b}	35±1^{c}	1.11±0.02^{a}	42±1^{c}

It is clear that infusion and hydroethanol extractions are best suited to obtain rosmarinic acid. Infusions proved to be more suitable in the cases of rosemary and oregano, while the hydroethanol mixture extracted higher contents in basil and sage. This study highlights infusion as the most adequate extraction procedure to obtain rosmarinic acid, a compound with great interest in the food industry.

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PC-114

Rosmarinic acid contents in putative natural food preservatives

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Rosmarinic acid is one of the main constituents of the only allowed food preservative extract within the European Union, Rosemary Extract (E392) [1, 2]. This additive is extracted from rosemary (*Rosmarinus officinalis* L.) leaves and stems. Thousands of plants have been screened for suitability to be used as preservatives in the food industry, and although many are hypothetically suitable, some authors refer sage and oregano, as potential future approved extracts, given their similarity with rosemary, in terms of chemical composition [3]. As previously mentioned, rosmarinic acid is the most abundant phenolic compound in rosemary extract, along with carnosol and carnosic acid, which are diterpenes [1]. Thus, most of its antioxidant and preserving capacity are related to this molecule [4].

In this study, the authors screened rosemary aqueous extract obtained by infusions along with other plant species that are candidates to be approved for use in the near future, namely oregano (*Origanum vulgare* L.), sage (*Salvia officinalis* L.) and basil (*Ocimum basilicum* L.) in order to verify the quantities of rosmarinic acid. Rosmarinic acid content was determined by using High Performance Liquid Chromatography coupled to a Diode Array Detector and an Electrospray Ionization Mass Spectrometer (HPLC-DAD-ESI/MS).

Overall, the highest amount of rosmarinic acid was detected in the rosemary extract (43±1 mg/g of lyophilized infusion), followed by sage (36±2 mg/g). Basil and oregano showed a lower content, 22.23±0.01 and 20.5±0.7 mg/g, respectively.

The benefits and bioactivities of these three aromatic plants are quite vast and have been described in recent scientific literature, and they do not pose any type of toxicity, given their use in culinary preparations all over the world for many centuries [5, 6]. This work proves that there are other natural extracts that can be used as food additives similarly to rosemary extract, and further diversify the offer of natural preservative extracts in the European market.

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PC-115

Medicinal properties of biologically active substances derived from basidiomycetes

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Bazidiomycetes are widely used now not only for food, but also as a source of biologically active substances released from the fruit body, submerged culture and native solution. Of the major modern fields of application of basidiomycetes, researchers primarily mention dietary food, mushroom pharmaceuticals, plant protection products against pathogens and cosmeceutical preparations. Among the biologically active substances released from fungi, special attention is given to polysaccharides and polysaccharide-protein complexes [1]. According to approximate estimates, medicinal mushrooms have about 130 therapeutic functions, including antitumor, immunomodulating, antihypercholesterolemic, antibacterial, antiparasitic, antifungal, detoxicating, hepatoprotective, antidiabetic and many others functions [2].

It is believed that among all fungal polysaccharides for the implementation of a number of activities, such as immunomodulating, primarly β-glucans are respond. Depending on the type of basidiomycetes, the structure of β-glucans is differently, what also leads to a change in its biological activity. Nowaday, it has been determined that only β-glucans of basidiomycetes have the following activities: Immunological, antitumour, antimicrobial, antiallergic, anti-inflammatory, anti- atherogenic, antiobesity, hypoglycemic, hepatoprotective activities, cardiovascular, antihypercholesterolemia and radioprotective effects [3].

However, there is a lack of research of β-glucans and fungal polysaccharides in general, especially in the treatment of metabolic syndrome and a decrease of cholesterol level [2, 3]. Some researchers argue that the studies of pharmaceutical activities of new species should be a priority in science [2].

Thus, biologically active substances obtained from basidiomycetes remain an interesting and relevant object for research, as they can be associated with some healthful benefits for different systems of human body. Therefore, more studies are needed regarding the isolation and identification of new compounds by means of different chromatographic techniques since these substances can be prospective basis for developing new treatments.

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PC-116

Holistic strategy using HPLC-QqQ-MS and GC-qMS towards the screening of bioactive compounds from *Salicornia ramosissima*

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In order to bioprospect the potential biological active compounds from *S. ramosissima*, a holistic strategy based on GC-qMS and HPLC-QqQ-MS was established. Beyond chromatographic data, in vitro biological assays were performed contributing to explain *S. ramosissima* health benefits, including antioxidant (FRAP, ABTS, DPPH, NO and lipid peroxidation) and anti-inflammatory (inhibition of thromboxane A2 production) assays. Glasswort (*Salicornia* spp.) has been used in folk medicine and human diet, being reported, among others, anti-inflammatory, antihyperglycemic and antioxidant activities [1]. These biological effects may be attributed to a wide range of biomolecules including phenolic compounds [1-2], and other phytochemicals as sterols and unsaturated fatty acids [1-2]. These molecules present different physico-chemical properties, including the carbon skeleton (from C_7 to C_{30}), polarity, volatility and thermal stability, implying the establishment of chromatographic solutions that can perform an integrated profiling. Therefore, a strategy was designed to screen the components from a polar fraction (methanol and petroleum ether/water fractionation), i.e., phenolics (non-volatile and thermally labile compounds) using HPLC-QqQ-MS; and from a lipophilic fraction (dichloromethane extraction and derivatization, silylation), the sterols, fatty acids and alcohols (less polar and thermally stable compounds) using GC-qMS. Wild samples inherent variability implied three harvesting locals at Ria de Aveiro, Portugal. From the 16 fatty acids, 12 alcohols, 5 sterols and 31 phenolic identified compounds, 22 were reported for the first time in this matrix. Lipophilic content ranged from 0.54 to 5.4 mg/g dry plant, being the linoleic and linolenic acids and the stigmasterol and β-sitosterol the main components. Phenolics ranged from 13.9 and 33.9 mg/g dry plant, with the flavonols isorhamnetin and quercetin 3-*O*-glucoside as the main components. The established strategy permitted to identify a panoply of bioactive compounds belonging to different chemical families, which is supported by *in vitro* biological data, contributing to understand *S. ramosissima* potential health benefits.

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PC-117

Análise cromatográfica de compostos hidrofílicos em acessos de tomate (*Solanum lycopersicum* L.) conservados *ex-situ*

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O Banco Português de Germoplasma Vegetal (BPGV), situado em Braga, acolhe e conserva coleções representativas de germoplasma dos mais importantes recursos agrícolas de Portugal Continental e Ilhas. A manutenção destas coleções exige que o material vegetal seja regenerado e caracterizado periodicamente. Neste sentido, este trabalho teve como objetivo fazer uma caracterização química dos acessos das variedades tradicionais de tomate (*Solanum lycopersicum* L.) apresentados na Tabela 1.

Tabela 1. *Acessos de tomate regenerados e caracterizados.*

Código do acesso	Nome da variedade	Origem
12260	Tomate coração de boi	Bragança, Portugal
12437	Tomate amarelo	Bragança, Portugal
12906	Tomate	Aveiro, Portugal
13034	Tomate coração de boi	Guarda, Portugal

As sementes conservadas *ex-situ* foram regeneradas e multiplicadas nos campos experimentais do BPGV e os frutos maduros foram analisados quanto à sua composição em açúcares livres e ácidos orgânicos. Estes compostos hidrofílicos foram determinados por cromatografia líquida de alta eficiência (HPLC) acoplada a um detetor de índice de refração ou a um detetor de fotodíodos, respetivamente [1]. Com base nesta análise foi possível verificar que o acesso 12260 tinha os maiores teores de frutose, glucose e açúcares totais, enquanto que a sacarose foi particularmente abundante no acesso 13034. Foi possível identificar os ácidos oxálico, málico, ascórbico e cítrico (o mais abundante) em todas as amostras. O acesso 13034 apresentou as maiores concentrações de ácido oxálico, ácido cítrico e ácidos orgânicos totais. O ácido málico e o ácido ascórbico foram particularmente abundantes nos acessos 12260 e 12437, respetivamente. Este trabalho destacou os acessos de tomate coração-de-boi (originários de Bragança e Guarda) como sendo ricos em açúcares e ácidos orgânicos, compostos relevantes do ponto de vista organolético e nutricional.

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PC-118

Caracterização do perfil em tocoferóis e ácidos gordos de uma coleção de germoplasma de tomate (*Solanum lycopersicum* L.)

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O tomateiro (*Solanum lycopersicum* L.) é uma espécie domesticada muito presente no nosso cotidiano. O fruto é consumido fresco e processado, sendo um elemento chave da Dieta Mediterrânica, que tem sido associada a várias propriedades benéficas para a saúde [1]. Com o objetivo de comprovar a presença de compostos bioativos em variedades tradicionais de tomate, os perfis em tocoferóis e ácidos gordos foram determinados em quatro acessos [12260 - coração de boi; 12437 - amarelo; 12906 - tomate; e 13034 - coração de boi] cujas sementes se encontram conservadas *ex-situ* no Banco Português de Germoplasma Vegetal (BPGV), em Braga. A partir de um ensaio de regeneração e multiplicação de germoplasma, realizado nos campos experimentais do BPGV selecionaram-se os frutos maduros para análise. Os perfis em tocoferóis e ácidos gordos foram determinados por cromatográfica líquida de alta eficiência acoplada a um detetor de fluorescência (HPLC-FL) e cromatografia gasosa acoplada a um detetor de ionização por chama (GC-FID) depois um processo de derivatização, respetivamente [2]. Após tratamento estatístico dos resultados, o acesso 12906 destacou-se com os teores mais elevados de γ- e δ- tocoferol e de tocoferóis totais. Já os vitâmeros α- e β-tocoferol foram detetados em maior quantidade nos frutos do acesso 12260. Relativamente aos ácidos gordos, os mais abundantes foram os ácidos linoleico (C18:2n6), palmítico (C16:0), α-linolénico (C18:3n3) e oleico (C18:1n9). Os acessos 12260, 12906 e 13034 apresentaram teores elevados de C18:2n6, enquanto que o ácido gordo saturado C16:0 abundou nos frutos do acesso 12437. Os resultados deste estudo contribuem para completar a informação relativa aos acessos de germoplasma de tomateiro conservado no BPGV, facilitando o estabelecimento de critérios para uma seleção racional de germoplasma com interesse tanto para reintrodução em cultivo como para programas de melhoramento vegetal.

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PC-119

Epicarpo de *Diospyros kaki* L. como uma fonte de vitaminas: análise cromatográfica de ácido ascórbico e de tocoferóis

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Diospyros kaki L. é a espécie mais cultivada para a produção de frutos do género Diospyros e pertence à família *Ebenaceae*. Apesar de ser nativa do continente Asiático, esta espécie tem-se disseminado por todo o mundo [1]. O fruto, conhecido como dióspiro, apresenta um grande valor económico, associado às suas propriedades bioativas, baixo valor energético e teor muito reduzido de gordura. Neste contexto, foram encontrados estudos que evidenciam a sua elevada atividade antioxidante, citotóxica e antidiabética, bem como um efeito benéfico em doenças coronárias [2]. Ao consumir o fruto, o epicarpo é retirado e descartado, contudo, poderá apresentar um elevado teor de compostos bioativos. Assim, o objetivo deste trabalho foi analisar e caracterizar a presença de tocoferóis (vitamina E) e ácido ascórbico (vitamina C) nos bioresíduos do diospiro. Os frutos foram adquiridos comercialmente em Bragança, em outubro de 2017. Retirou-se o epicarpo manualmente, que apresentava uma espessura média de 2 mm, tendo sido posteriormente congelado e liofilizado. Os tocoferóis foram determinados por cromatografia líquida de alta eficiência acoplada a um detetor de fluorescência (HPLC-FL) e o ácido ascórbico foi determinado por cromatografia líquida ultra rápida acoplada a um detetor de díodos (UPLC-DAD).
Foram quantificadas os quatro vitâmeros de tocoferóis (α-, β-, γ- e δ-tocoferóis), tendo sido o α-tocoferol encontrado em concentrações mais elevadas (9,4±0,1 mg/100 g matéria seca). O teor em ácido ascórbico foi de 125,6±0,2 mg/100 g matéria seca.
Este estudo destaca a importância da recuperação e valorização de bioresíduos do dióspiro como uma fonte de vitaminas, podendo ser interessante para o desenvolvimento de alimentos funcionais.

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PC-120

Propriedades nutricionais de croissants aditivados com sumo de sabugueiro

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Apesar da grande apetência dos consumidores por croissants, este produto não tipifica, definitivamente, aquilo que pode considerar-se como um alimento promotor da saúde (1). A associação destes dois fatores atribui um especial interesse ao estudo da incorporação de componentes bioativos, como os que estão presentes no sumo de baga de sabugueiro, pela sua dupla função de prevenir oxidações e atuar como corante (2).

Neste sentido, prepararam-se diferentes formulações de croissants com concentrações de sumo de sabugueiro distintas e foram também preparadas formulações de croissant tradicional, bem como outras incorporando corante comercial de cenoura preta, já utilizado noutras aplicações alimentares, para ter uma ideia mais exata do verdadeiro potencial do ingrediente selecionado. Além de outros parâmetros, os ácidos gordos foram determinados por cromatografia gasosa com detetor de ionização em chama (FID), e a composição em açúcares foi determinada por cromatografia líquida de alta eficiência com um detetor de índice de refração (HPLC-RI). Do ponto de vista nutricional, os croissants não apresentaram uma variação muito relevante entre as diferentes formulações. Foram detetados 4 açúcares livres nos croissants: frutose, glucose, maltose e trealose, sempre com as mesmas proporções independentemente da formulação (como se pode observar na tabela 1). Entre os ácidos gordos detetados, o ácido oleico (30%), o ácido linoleico (entre 20 e 30%) e o ácido palmítico (entre 17 e 24%) foram os mais abundantes, não se tendo verificado diferenças significativas entre formulações.

Tabela 1. *Perfil dos ácidos gordos maioritários e de açúcares livres nas diferentes formulações de croissants (CB: Croissants base; CSS4: Croissants com 4% de sumo de sabugueiro; CCC: Croissants com 4% de corante comercial de cenoura preta)*

	C16:0	C18:1n9c+t	C18:2	Frutose	Glucose	Maltose	Trealose
CB	21±1	28±1	28±1	4,1±0,1	2,4±0,1	3,9±0,2	1,8±0,1
CSS4	19±1	30±1	22±1	5,6±0,5	3,4±0,5	3,6±0,1	1,4±0,1
CCC	21±1	29±1	22±2	4,2±0,2	1,6±0,1	3,6±0,1	1,2±0,2

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PC-121

Utilização de subprodutos de bagas de sabugueiro como fonte de corantes naturais

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Indo de encontro a um público cada vez mais disposto a consumir alimentos isentos de produtos artificiais, dando preferência ao natural e saudável, surge a necessidade de encontrar aditivos naturais em substituição dos artificiais.

As antocianinas revelam ser um importante substituinte dos corantes artificiais, sendo estudadas em todo o mundo como agentes de coloração natural em alimentos, em particular na gama do vermelho e a azul em muitos alimentos [1].

O processamento de frutas e legumes gera normalmente uma quantidade apreciável de subprodutos, tais como cascas e sementes que poderão conter importantes quantidades de compostos bioativos e com enorme capacidade corante [2]. Assim, no presente trabalho o "bagaço" remanescente da extração do sumo de bagas de *Sambucus nigra* L. (sabugueiro) foi estudado como fonte de corantes naturais, em particular como fonte de antocianinas, para aplicação num produto alimentar de pastelaria, o profiterole.

A caracterização das antocianinas foi efetuada por cromatografia líquida de alta eficiência acoplada a um detetor de díodos e a um espetrómetro de massa (LC-DAD-ESI/MS).

Foram detetados 3 compostos distintos, todos derivados da cianidina: cianidina-3-*O*-sambubiósido-5-*O*-glucósido, cianidina-3-*O*-sambubiósido e cianidina-3-*O*-glucósido. A cianidina-3-*O*-glucósido foi identificada por comparação com o padrão comercial, enquanto os outros dois compostos foram confirmados por comparação das características cromatográficas e espetrais (UV e massa) com dados da nossa base de dados de compostos e com a literatura disponível.

Após a sua incorporação no alimento foi possível verificar que aproximadamente 60% da concentração em antocianinas incorporada foi mantida nas formulações de profiteroles. Tal como era objetivo, a adição do corante do extrato aquoso de "bagaço" de sabugueiro provocou uma alteração de cor significativa provando ter um valioso poder corante.

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PC-122

Perfil cromatográfico de ácidos orgânicos e tocoferóis de *Umbilicus rupestris* (Salisb.) *e Raphanus raphanistrum* L.

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A utilização de plantas silvestres na dieta alimentar esteve em declínio, no entanto, a globalização estimulou a recuperação do uso de espécies tradicionalmente consumidas [1]. Por outro lado, o interesse dos consumidores por uma alimentação cada vez mais saudável, leva ao estudo de plantas silvestres variadas onde se incluem as espécies *Umbilicus rupestris* (Salisb.) Dandy e *Raphanus raphanistrum* L., preferencialmente consumidas em saladas. Assim, no presente trabalho fez-se a caracterização do seu perfil cromatográfico em termos de tocoferóis e ácidos orgânicos. As amostras de *U. rupestris* e *R. raphanistrum* foram colhidas em 2016 e 2017, respetivamente, na região de Trás-os-Montes. A determinação dos tocoferóis foi realizada por cromatografia líquida de alta eficiência acoplada a um detetor de fluorescência (HPLC-FL) e dos ácidos orgânicos por cromatografia líquida ultra rápida acoplada a um detetor de díodos (UPLC-DAD). Em ambas as amostras foram identificadas os quatro vitâmeros do tocoferol (α-, β-, γ- e δ-tocoferóis), sendo o γ-tocoferol o mais abundante na amostra de *U. rupestris* (13,24±0,09 mg/100 g matéria seca), e o α-tocoferol o maioritário na amostra de *R. raphanistrum* (8,8±0,1 mg/100 g). Foram identificados cinco ácidos orgânicos na amostra de *U. rupestris* e sete na amostra de *R. raphanistrum*, entre os quais os ácidos oxálico, quinico, málico, ascórbico e cítrico; na amostra de *R. raphanistrum* foram identificados também os ácidos sucínico e fumárico. O ácido cítrico (7,258±0,004 g/100 g matéria seca) e o ácido quínico (7,1±0,2 g/100 g) foram os compostos maioritários na amostra de *U. rupestris*, enquanto que a amostra de *R. raphanistrum* apresentou os ácidos oxálico (7,01±0,01 g/100 g), quínico (6,1±0,2 g/100 g) e cítrico (6,09±0,05 g/100 g) como compostos maioritários.

Este trabalho permitiu verificar que as espécies *U. rupestris* e *R. raphanistrum* são uma fonte de ácidos orgânicos e de tocoferóis com importante função antioxidante, podendo ser incorporadas na dieta contemporânea.

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PC-123

Perfil cromatográfico em tocoferóis e ácidos orgânicos da microalga *Spirulina platensis*

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A microalga *Spirulina platensis* é uma cianobactéria filamentosa que apresenta coloração azul esverdeada e elevada concentração de macro e micronutrientes, tais como proteínas, aminoácidos, ácidos gordos insaturados, vitaminas, minerais e pigmentos [1]. O interesse pela *Spirulina* deve-se essencialmente ao facto de esta apresentar cerca de 60 a 70% de proteínas e ser uma fonte importante de vitaminas. Em particular, apresenta um elevado teor de vitamina E, comparável ao trigo germinado, que é considerado uma fonte natural desta vitamina [2, 3]. Também por apresentarem diversos compostos bioativos, as microalgas são consideradas ingredientes promissores para aditivação/ enriquecimento de alimentos [4].

Assim, no presente trabalho estudou-se o perfil cromatográfico em tocoferóis e ácidos orgânicos da microalga *S. platensis*. A amostra desidratada e pulverizada foi adquirida numa Ervanária de Bragança (Portugal), em outubro de 2017. Os tocoferóis foram determinados por cromatografia líquida de alta eficiência acoplada a um detetor de fluorescência (HPLC-FL) e os ácidos orgânicos foram analisados por cromatografia líquida ultra rápida acoplada a um detetor de díodos (UFLC-DAD). Foram identificados três vitâmeros do tocoferol (α-, β- e δ-tocoferóis), sendo o α-tocoferol (388±8 µg/100g), seguido do δ-tocoferol (139±5 µg/100g) os maioritários. Relativamente, aos ácidos orgânicos, foram identificados e quantificados dois compostos, o ácido quínico (792±19 mg/100g) e o ácido oxálico (apenas em quantidades vestigiais).

Em conclusão, a microalga *S. platensis* apresenta elevados teores de α-tocoferol e de ácido quínico, podendo ser um ingrediente promissor na funcionalização de matrizes alimentares.

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PC-124

Lipophilic and phenolic compounds from *Eucalyptus grandis* wood cultivated in Portugal, Brazil and South Africa

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Eucalyptus species are the most extensively cultivated hardwood trees for pulp and paper production due to their adaptability to different edaphoclimatic conditions, fast growing and excellent wood properties, and have therefore an important economic impact worldwide. In this work, a detailed study of the dissimilarities in the lipophilic and phenolic fractions of *E. grandis* wood extractives from three different geographic origins, namely Portugal, Brazil and South Africa, using gas chromatography-mass spectrometry (GC–MS) and ultra-high-performance liquid chromatography-mass spectrometry (UHPLC–MS) is reported. The lipophilic fraction of the studied *E. grandis* wood is mainly composed of sterols, fatty acids and phenolic compounds. Three triterpenic acids were detected for the first time in the wood extracts from Brazil. *E. grandis* wood from Portugal presents the highest lipophilic content (1.67 g kg^{-1} of dry wood), followed by South Africa (1.56 g kg^{-1} of dry wood) and Brazil (1.05 g kg^{-1} of dry wood). 51 Phenolic compounds were identified in *E. grandis* wood MeOH:H2O extracts, from which 11 are reported for the first time as *E. grandis* constituents and 4 are firstly reported as *Eucalyptus* genus components. *E. grandis* wood from Brazil shows the highest phenolic content (~2.36 g kg^{-1} of dry wood), followed by South Africa (~1.90 g kg^{-1} of dry wood) and Portugal (~1.30 g kg^{-1} of dry wood), which shows the influence of the geographic origin over *E. grandis* wood extractives composition and abundance, as well as on the antioxidant activities of the phenolic fractions. The detailed knowledge of these extracts can contribute on the one hand to prevent their impact in the bleaching process, and, on the other demonstrates the potential of *E. grandis* species as a source of bioactive phytochemicals for nutraceutical applications [1].

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PC-125

Detailed composition and biological properties of lipophilic fraction of *Bifurcaria bifurcata* macroalga

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In recent years, marine resources, and macroalgae in particular, have become alternative sources of several value-added compounds. In Portugal, the exploitation of these resources is also one of the critical sectors for the economic development, given the extent and richness of its Exclusive Economic Zone (EEZ). In this study, the lipophilic fraction of short-term (three weeks) cultivated *Bifurcaria bifurcata* was characterized in detail by gas chromatography–mass spectrometry (GC-MS). *B. bifurcata* dichloromethane extract was composed mainly by diterpenes (1892.78 ± 133.97 mg kg^{-1} dry weight (DW)), followed by fatty acids, both saturated (550.35 ± 15.67 mg kg^{-1} DW) and unsaturated (397.06 ± 18.44 mg kg^{-1} DW). Considerable amounts of sterols, namely fucosterol (317.68 ± 26.11 mg kg^{-1} DW) were also found. In vitro tests demonstrated that the *B. bifurcata* lipophilic extract have antioxidant, anti-inflammatory and antibacterial activities (against both Gram-positive and Gram-negative bacteria), using low extract concentrations (in the order of µg mL^{-1}). Additionally, the use of *B. bifurcata* extract enhanced the antibiotic activity of drug families of major clinical importance. This enhancement depends on the microbial strain and on the antibiotic. This work represents the first detailed phytochemical study of the lipophilic extract of *B. bifurcata* and is, therefore, an important contribution for the valorization of this macroalga, with promising applications in functional foods, nutraceutical, cosmetic and biomedical fields [1].

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PC-126

Tocopherols content of different wheat varieties: differences between refined and whole-wheat flour

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Wheat is an important cereal worldwide that plays an outstanding role in human nutrition [1]. Whole grain consumption has been associated with reduced risk of developing chronic diseases and this health benefit may be due to different phytochemical compounds, including tocopherols [2]. The aim of this study was to identify and quantify the tocopherols presentin four different varieties of wheat flours (Cajeme and Marius as soft wheat and Endural and Aldura as durum wheat),comparing whole grain flour and refined flour. Tocopherols were determined by using HPLC coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA). The quantification was based on the fluorescence signal response of each standard, using the internal standard (tocol) and by using calibration curves obtained from commercial standards of each compound [3]. In the present study α–, β– and γ–tocopherols were identified and quantified, while γ–tocopherol was not found in the analysed samples. The refined Cajeme flour was the only exception in which none of the vitamers were detected. In all samples, α–tocopherol was the major compound (0,53 mg/100g and 0,17 mg/100g, in Marius whole grain flour and Aldura refined flour, respectively).Total tocopherols content ranged from 0.81 mg/100g to 0.23 mg/100g in Marius whole grain flour and Aldura refined flour, respectively. Tocopherols content was significantly higher ($p < 0.05$) in all whole-wheat flour in comparison with refined wheat flours. Comparing durum wheat flour with soft wheat flour, the present study showed that the content of α–tocopherol is higher in soft wheat varieties. The Relative vitamin E activity (REA) of the analyzed samples ranged from 0,2 mg to 0,7 mg (Aldura refined flour and Marius whole grain flour, respectively), covering up to 5,5% of Nutrient Reference Value (NRV) according to the EU Regulation1169/2011 [4]

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PC-127

Analysis of tocopherols and phenolic compounds in extruded lentil flour formulations for development of snack-type functional foods

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Pulses are currently considered as functional gluten-free foods, which could be included in extrusion formulations in order to develop functional products with high nutritional value [1]. In the present study individual phenolic compounds and tocopherols were evaluated in different lentil flours (raw and extruded at 140 and 160ºC) formulated with nutritional yeasts.Phenolic compounds were analysed by using a HPLC equipped with a diode-array detector and coupled to a mass detector [2].For the analysis of tocopherols, it was used HPLC coupled to a fluorescence detector [1].

Extrusion cooking may affect bioavailability of phenolic compounds due to high temperature that causes decomposition of heat-labile phenolic compounds and may also lead to polymerization of some phenolic compounds under high pressure [3]. Catechin hexoside was the most abundant phenolic compound, with values around 30.7 to 66.1 mg/100g dw in raw samples. After the extrusion process, it was observed the highest decrease in the lentil flours formulated with the highest amount of nutritional yeast (16%). The other phenolic compounds, also experiment a decrease but in a less extend, probably due to partial hydrolysis of conjugated phenolics[4].Particularly in the case of kaempferol-*O*-desoxyhexide-*O*-hexoside-*O*-rutinoside, quercetin-3-*O*-glucoside, quercetin-*O*-hexoside and quercetin-*O*-pentoside.

Regarding tocopherols content, there was a significant decrease in all tocopherol vitamer safter the extrusion process.In the studied raw samples, total tocopherols content ranged from 2.05 to 3.02 mg/100g (dw). The results showed a reduction of 81.5-92% in total tocopherols, which is in accordance with other authors [3].

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Design of an one-step platform purification of STEAP1 using octyl-sepharose

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Prostate cancer (PCa) is one of the most lethal and prevalent carcinoma among elder men worldwide. Currently, PCa diagnosis based on prostate-specific antigen (PSA) levels is unspecific and not completely efficient, mainly in advanced stages of cancer. Thus, there is a need to identify and characterize specific and reliable protein biomarkers for PCa [1]. Six transmembrane epithelial antigen of the prostate 1 (STEAP1) is a transmembrane protein whose high expression levels were correlated with PCa. STEAP1 may take part in intracellular and intercellular communication in cancer cells by modulating cell proliferation and tumor invasiveness through its potential activity as ion channel or transporter [2]. So, the characterization of STEAP1 structure might allow the design of specific inhibitors that decrease and modulate its oncogenic function. The structural and functional studies require high purified amounts of protein, which can be obtained through a recombinant production of human STEAP1 protein combined with a sustainable chromatographic strategy. In this work, the performance of octyl sepharose was evaluated according to binding and elution conditions required for STEAP1 isolation from cell lysates obtained in mini-bioreactor *Pichia pastoris* X33 methanol-induced cultures. The concentration of sodium phosphate in the equilibration buffer was optimized in order to promote a complete STEAP1 adsorption on the hydrophobic support. By the analysis of SDS-PAGE gel and western blot, a higher retention of STEAP1 was observed with concentrations above 500 mM of sodium phosphate buffer, pH 8.0. If the adsorption is achieved at high concentrations of sodium phosphate buffer, the elution must be performed with increasing concentrations of Triton X-100 in 50 mM phosphate buffer. These results indicate that the exposition of membrane binding domains of STEAP1 to octyl sepharose requires high salt concentrations due the strong interactions established between them. However, after its complete adsorption, STEAP1 elution requires strong agents such as detergents. Although successful applications of HIC in the purification of integral membrane proteins are uncommon, our results indicate that traditional hydrophobic matrices can open a promising alternative for the isolation of STEAP1.

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PC-129

Valorising leaves of *Garciniabrasiliensi* Mart as sources of bioactive compounds

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Discovery of new drugs from natural products is becoming widespread and an example of a promising plant is the *bacupari-anão* or *bacupari mirim* that belongs to the species: *Garcinia brasiliensi* Mart, family *Myrtaceae*. This tree is native to the forests of the Amazonian and also to the Atlantic Forest. Since ancient time, the leaves of this tree have been reported to have numerous functionalities, correlated with the presence of bioactive compounds [1]. Thus, this study aimed to evaluate the bioactive properties of *G. brasiliensi* leaves regarding to its possible antioxidant activity and cytotoxic properties in human tumor cells. Three different extracts of the leaves were tested: hexane, dichloromethane and ethyl acetate. Furthermore, the most active extract was characterized in terms of its phenolic compounds content.Chemical profile of the extracts was obtained using an HPLC system coupled to a diode array detector (DAD) and mass spectrometry (MS) with an electrospray ionization interface (ESI). The antioxidant activity was evaluated by four in vitro assays:DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power, inhibition of β-carotene bleaching and inhibition of lipid peroxidation by the thiobarbituric acid reactive substances (TBARS) assay.The cytotoxicity was tested in MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung carcinoma), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma) cell lines and in a non-tumor primary culture (porcine liver primary culture PLP2). Among all the tested samples, ethyl acetate extract presented the highest DPPH scavenging activity (EC_{50} value = 31,2 ± 0,2μg/mL), reducing power (68,8± 0,2 μg/mL), β-carotene bleaching inhibition capacity (15,9 ± 0,3μg/mL) and TBARS (4,6 ± 0,2 μg/mL). It was also able to inhibit all the tested human tumor cells and none of the samples revealed toxicity for the non-tumor cell line PLP2 (GI_{50}<400 μg/mL). The ethyl acetate extract was the most active extract and analysis of HPLC-DAD-MS data revealed a total of twelve phenolic compounds, comprising five bioflavonoids, four flavones, two flavonols and a flavan-3-ol. The most abundant phenolic compound was the bioflavonoid morelloflavone-7-*O*-glucoside. This study highlights the importance of the recovery and valorisation of *G. brasiliensi* leaves, in order to obtain valuable products, which can be explored in the development of functional foods.

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Evaluation of fatty acids of salmon from different origins: comparison of extraction and derivatization methodologies

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Global demand for fish and fish products has increased significantly over the last decades, which led to a simultaneous increase of aquaculture production around the world, currently corresponding to almost 50% of the global fish market [1]. Among different concerns regarding the fish that consumers are eating, nowadays, there is a demand for correct information about the species, production method (farmed vs. wild) and the catch origin/provenience of fish. Salmon, one of the most popular fish in Europe, can have different geographical origins and generally command higher prices when caught in the wild. Moreover, the commercially important species of salmon belong to different genus, namely *Salmo* and *Oncorhynchus*. Therefore, this work intended to compare the fatty acid composition of salmon from diverse origins, testing different extraction and derivatization methodologies.

Farmed salmon specimens were obtaining from Chile, Canada and Norway. Two lipid extraction methods, namely conventional Soxhlet extraction using *n*-hexane added with butylated hydroxytoluene (BHT) and an adaptation of the Bligh and Dyer extraction using ultra-turrax homogenisation with 1% NaCl, followed by extraction with chloroform and methanol, were tested. Additionally, fatty acid methyl esters (FAME) were prepared by two methodologies, namely by alkaline transmethylation using KOH and by acid-catalysed transmethylation using boron trifluoride-methanol solution. FAME were analysed in a Shimadzu GC-2010 Plus gas chromatograph equipped with a Shimadzu AOC-20i auto-injector and a flame ionisation detector (Shimadzu, Japan). A CP-Sil 88 silica capillary column (50 x 0.25 mm i.d, 0.20 µm) from Varian (Middelburg, Netherlands) was used for FAME separation. Injector and detector temperatures were 250 and 270 °C, respectively. The compounds were identified by comparison with standards (FAME 37, Supelco, Bellefonte, PA, USA).

Based on the obtained results, the ultra-turrax method was chosen for lipid extraction since it allowed obtaining higher amounts of long chain unsaturated fatty acids, particularly of docosahexaenoic acid (DHA). Similar results were obtained for both tested derivatization methodologies. Nonadecanoic acid (C19:0) was submitted to BF_3/MeOH derivatization resulting in a high transmethylation yield (90.3%). In general, salmon samples showed high contents of polyunsaturated fatty acids, including ω-3 fatty acids, which supports its consumption as part of a healthy diet.

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PC-131

A new multiple reaction monitoring method for the assessment of catechol-*O*-methyltransferase Val/ Met108

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Catechol-*O*-methyltransferase (COMT, EC 2.1.1.6) is an S-adenosyl-L-methionine-dependent methyltransferase enzyme expressed in two isoforms in human tissues, a soluble (SCOMT) and a membrane-bound form (MBCOMT). Due to its physiological role in the methylation, and subsequent elimination of biologically active or toxic catechols, it has been implicated in several human disorders, including Parkinson's disease. COMT inhibitors has been effectively used as adjuvants in treatment for Parkinson's disease but its cytotoxicity may be influenced by polymorphic and less active variants, including Met108/158 variant [1]. Therefore, adequate levels of purified Met108/158 variant are required for study the response of this polymorphic form to the different inhibitors commercially available. So, this work aims to produce, purify and assess the recombinant COMT108Met. The valine were replaced by a methionine using the DNA mutagenesis kit and a biologically active hexa-histidine-tagged COMT108Met, with specific activity of 16.28 nmol $h^{-1}mg^{-1}$, was expressed in methanol-induced Pichia pastoris cultures for 72h, based on a previous strategy described by our research group [2]. Immobilized metal affinity chromatography was used to purify the recombinant target enzyme, which is typically recovered at 300 mmol L^{-1} of imidazole. Finally, a methodology based on multiple reaction monitoring (MRM) mass spectrometry were developed for detection and assessment of COMT isoforms. The MRM method were in conformity with the criteria accepted in bioanalytical method validation, with a linearity between 20 and 200 µg mL^{-1} and with LOD and LOQ of, respectively, 3.3 and 10.0 µg mL^{-1}. The obtained concentration for SCOMT Val108 in Pichia pastoris lysatesis 62.35 µg mL^{-1}. However more work must be done in the adjustment of the methodology to the polymorphic variant Met108.

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