



CHALLENGES AND OPPORTUNITIES FOR THE INNOVATION WITH PHENOLIC COMPOUNDS

IBERPHENOL. IBERIAN CONGRESS ON PHENOLIC COMPOUNDS

BOOK OF ABSTRACTS

Ourense, 2nd October 2019 http://iberphenol.webs.uvigo.es

Universida_{de}Vigo

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Editors:

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PRESENTATION

Iberphenol is a cooperative research network in the field of polyphenols and their industrial applications, funded by POCTEP (International Cooperation Programme for Spain and Portugal, 2014-2020; Ref. 0377_IBERPHENOL_6_E).

The project was born from the University of Salamanca's idea of enhance the research that had been ongoing for several years in the working group GIP (Polyphenol Research Group of University of Salamanca). The GIP had stable collaborations in place with the rest of the members included in this consortium. With the aim of strengthening these connections and pursuing goals far more ambitious than those so far carried out, we set out to create the network Iberphenol as the Iberian Polyphenol Research Network.

The purpose of Iberphenol is to establish a network of topical research on polyphenols, encouraging the following avenues of action:

- > Fomenting the integration of R&D capabilities in this area.
- > Promoting the training and mobility of researchers between academic centres and businesses.
- Development of collaborative R&D projects (involving research centres and businesses), with the aim of producing workable results, applied mainly in the agrifood industry and in health and cosmetics.
- Encouraging the transference of technology between the public institutions and the productive framework.

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Plenary lecture

Interactions of Polyphenols with gut bacteria and implications in human health

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Epidemiological research indicates that dietary habits that supply higher daily intakes of (poly)phenols decrease the risk of chronic diseases and particularly the cardiometabolic ones. However, the demonstration of the effects of specific (poly)phenols in randomised clinical trials remains elusive as the treatments are effective for some volunteers while they do not affect others resulting a global statistically non-significant outcome. To understand this inter-individual variability, we, as well as other scientists, launched almost twenty years ago, the hypothesis that due to the limited bioavailability of these (poly)phenols they reach the colon where they interact with the resident gut microbes that are modulated by these compounds, and gut bacteria convert them into metabolites that are better absorbed and that have relevant biological effects. The gut microbiota polyphenol conversion occurs for different groups of phenolic compounds including soy isoflavones, ellagitannins, proanthocyanidins, flavanones, and lignans among others. Recent advances in the metabolic conversion of (poly)phenols by gut microbes, the microbial species involved, the biological activity of the metabolites and their relevance in the inter-individual variability in the health effects will be discussed.



Proposed section: Phenolic agro-industries Proposed format: oral

Phenolic and sensory profiles of raspberries (Kweli and Tulameen) produced under conventional *versus* organic agricultural practices

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Raspberries are among the most common berries in the world, which are consumed as fresh fruits, processed or as ingredients in various foods [1]. Raspberries are mainly from Asia, Europe and North America. In Portugal, the most commonly cultivated species is the European red raspberry (Rubusidaeus L.) [2]. The raspberry production area in Portugal increased 764 ha between 2010 and 2016 and during this period the annual production increased from 2 216 to 16 972 tons [3]. The growth in the market of organic products can be explained by the rising consumer demand for products perceived as being healthier and tastier [4]. Raspberries, have a high antioxidant potential, being a valuable source of potentially healthy compounds, associated with a high content of anthocyanins, ellagic acid derivatives and vitamin C [1]; nevertheless, the impact of organic versus conventional agricultural practices on its phytochemical composition is unknown. Therefore, this study aimed to compare the accumulation pattern of polyphenols as well as the sensory profile of two raspberries cultivars 'Kweli' and 'Tulameen' grown under organic and conventional agricultural practices in the same edaphoclimatic conditions. Determination of total soluble solids (°Brix), total acidity, total phenolic compounds, total anthocyanins, antioxidant capacity, phenolic compounds profile by HPLC and the sensory profile, of 'Kweli' and 'Tulameen' raspberry variety, produced under the two agricultural practices, was performed. Results showed that the phenolic profile analysed by HPLC indicates that in all raspberries, the major phenolic compounds were anthocyanins (namely cyanidin-sophoroside). The raspberry agricultural production practice affects significantly the content of total soluble solids, total acidity, total phenolic compounds, total anthocyanins and consequently its antioxidant capacity and sensory characteristics.

Keywords: Raspberries; Agricultural practice; Antioxidant activity; Phenolic profile; Sensory profile.

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Proposed section: Phenolic agro-industries Proposed format: oral

Influence of the growth cycle on the bioactive properties and phenolic composition of *Cynara cardunculus* L. var *altilis*

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Cynara cardunculus L. (syn. Cardoon), is an erect perennial herbaceous plant, native to the Mediterranean basin. It is characterized by a high variety and richness in compounds with health benefits. Also, its cultivation and economic impact are highly important due to the diverse industrial applications (*i.e.* vegetable rennet, biomass, bioenergy, etc.) [1, 2]. Due to its increasing consumption and commercial interest, this study purposes the analysis of phenolic compounds and bioactive properties of different cardoon parts (heads, bracts, and seeds) collected in Greece during different maturation stages. The phenolic composition was determined by HPLC-DAD-ESI/MS. The antibacterial and antifungal activities were evaluated by applying the microdilution method. The cytotoxic effects were evaluated in four human tumor cell lines using the sulforhodamine B assay, while the antiinflammatory activity was evaluated through the inhibition of NO production in macrophage cells. Finally, the antioxidant activity was measured through the TBARS and OxHLIA assays. The content in phenolic compounds decreased with the maturation process, except in the seed extract. All the tested samples exhibited antibacterial and antifungal activity, where lower MICs were observed when the plant reached maturity. Regarding the cytotoxic and anti-inflammatory activities, the earliest harvest stages revealed the highest activity, except for seeds. Moreover, with the maturation process, extracts presented higher capacity to OxHLIA and TBARS inhibition. The heterogeneity of the biological results reveals that other compounds than phenolic ones may be correlated with these bioactivities. This study proved the high biological potential of cardoon parts as also its possible use as a source of important bioactive compounds. Nevertheless, further studies are needed to understand which compounds are responsible for the observed bioactivities, as well as to find the stage of maturity that provides the best bioactive properties.

Keywords: Cardoon; Polyphenolic profile; Antioxidant activity; Cytotoxic activity; Antibacterial activity.

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Proposed section: Phenolic agro-industries Proposed format: oral

Lamiaecae family plants: a rich source of phenolic compounds

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Herbal teas, particularly infusions with both medicinal and nutritional purposes, have a long history of traditional uses among different cultures and regions [1]. The family Lamiaceae is an important medicinal plant family that is widely used in traditional medicine as a cure for various disorders [2]. In order to characterize and study the bioactivities of individual plant species of that family, three different plant species were selected: *Melissa officinalis*, L. (leaves), *Mentha spicata* L. (inflorescences), and Prunella vulgaris L. (leaves). Infusions were prepared from each plant species being further analyzed in terms of nutritional value, phenolic composition, and bioactive properties. The antioxidant activity was evaluated through two different *in vitro* assays: inhibition of lipid peroxidation in brain homogenates by thiobarbituric acid reactive substances (TBARS) and oxidative hemolysis inhibition assay (OxHLIA). The cytotoxic activity was assayed using MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung carcinoma), HeLa(cervical carcinoma) and HepG2 (hepatocellular carcinoma) human tumor cell lines, and also in a non-tumour cell line (porcine liver primary cells, PLP2). The phenolic profile was accessed through and UPLC-DAD-ESI/MS system. *M. officinalis* presented a high percentage of carbohydrate with sucrose being the predominant free sugar and no macronutrients were found in *P. vulgaris* infusion. Regarding phenolic compounds' composition, the major detected compound in the three infusions was rosmarinic acid. Regarding, the bioactive properties, P. vulgaris and M. officinalis presented the best results for TBARS and OxHLIA assays, respectively. *M. spicata* showed the best anti-inflammatory activity and cytotoxic properties, with lowest GI₅₀ values for all the tumour cell lines tested. None of the infusions revealed hepatotoxicity for non-tumour cells (PLP2). In general, M. spicata also presented the strongest antimicrobial activity. Thus, the complete characterization of these individual species confirms that they are excellent sources of bioactive molecules, especially phenolic acids.

Keywords: Lamiaceae; Infusion; Phenolic acids; Bioactivities.

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Proposed section: Phenolic agro-industries Proposed format: oral

Effect of toasting process in French oak barrels with different oxygen transmission rate (OTR): evolution of the volatile composition in aged red wine

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The oak barrel's selection for wine ageing is usually related with the oak type and also, by its grain. The amount of oxygen wine receives in the barrel is known as oxygen transfer rate (OTR) and it is a property that depends, among others, on the wood species. This oxygen transfer rate is a key factor, since it influences in the reactions occurring between the wine compounds and those released from wood during ageing. Therefore, it will determine the evolution of the process defining the final characteristics of the aged wine. Thus, the possibility to choose the barrel's OTR, would allow winemakers to control globally the ageing process of the wine.

The objective of this work was to study the volatile composition of French oak staves (*Quercus petraea*) classified according to their oxygen transfer rate (OTR) for the construction of barrels with low and high oxygenation, and the transfer of these compounds to the same red wine aged in these barrels for a one year, compared to control barrels built with unclassified staves.

For this study, staves from *Quercus petraea* Liebl. oak wood classified according to its potential OTR [1, 2] were naturally seasoned in the wood yard for 3 years before being processed and toasted (180 °C during 40 minutes) in the cooperage. The same young red wine (cv. Tinta del País) was aged for oneyear in 10 French barrels: 4 barrels with high and 4 barrels with low oxygen transmission rate [3] as well as two control barrels. Wines were sampled at 3, 6, 9 and 12 months of ageing and analysed in terms of volatile composition by GC-FID.

The results related to the wood showed that the volatile composition of the wood was different depending on whether it was wood classified as high or low OTR staves. Results indicated that for the whole aging period of the study, the concentration of most of the volatile compounds studied in wine increased progressively, being the wines from high OTR barrels those presenting a higher increase.

Keywords: Oxygen transfer rate; Oak barrel; Toasting; Volatile compounds; Wine.

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Proposed section: Phenolic agro-industries Proposed format: oral

Determination of the ellagitannins and anthocyanins in red wine during ageing in oak barrels with known oxygen transfer rate

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The ageing of wine in oak barrels is a common practice in oenology, being French oak wood (*Quercus petraea*) one of the most used in cooperage. This work presents the results obtained after ageing for one year the same red wine in French oak barrels with known oxygen transfer rate (OTR). In a first stage, 3000 fresh staves from the same batch were classified according to their OTR [1] and were used for the construction of barrels with high and low oxygenation, as well as control barrels that were manufactured by the system usually used in the cooperage and with unclassified wood from the same batch. The previous study of the barrels built showed that barrels with a high oxygenation rate transmitted 2.4 times more oxygen than barrels with a low oxygenation [2].

The objective of this work is the characterization of anthocyanins and ellagitannins present in the same red wine aged for one year in barrels with different OTR. The composition in ellagitannins and anthocyanins present in the wines of the different barrels at 6 and 12 months of ageing were analyzed by HPLC/DAD-MS in collaboration with the University of Salamanca and the Universidade do Porto respectively [3, 4]. The results obtained showed higher ellagitannin content in both wood and wines aged in low oxygen barrels for one year. As for anthocyanins, some pigments were detected earlier in the wine from the HW-OTR barrels, suggesting an effect of oxygen as a catalyst for the appearance of these compounds.

Keywords: Oxygen transfer rate; Ellagitannins; Anthocyanins; Barrels; Red wine.

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Proposed section: Phenolic agro-industries Proposed format: oral

Analysis of the relationships between the wine oxygen consumption kinetics and its chemical composition using Artificial Neural Networks

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Oxygen is a very important parameter in wine aging process. Wine oxygen consumption has been widely studied in the literature. Most of this studies measure the oxygen content in very specific times, but only few of them perform measurements along time in order to measure oxygen consumption kinetics, which is the measurement of interest in this work.

From a mathematical point of view, relating oxygen consumption kinetics with the chemical composition of the wine implies relating time series data, which is avariable length array of data, with chemical parameters that describe the wine. The strategy chosen in this work to establish these relationships is to calculate descriptive parameters from the oxygen consumption kinetics and relate them with the chemical parameters. In this way the time series data, which usually have a large and variable number of points, is transformed into a reduced and fixed number of parameters. There are several parameters that can be extracted to describe curves like oxygen consumption kinetics. Some of them are related with the oxygen magnitude (maximum, minimum, percentile values, etc.), other with the time (duration of the process, time interval between two fixed oxygen levels, etc.), and some other with the area under the oxygen consumption curve. Moreover, not only the oxygen consumption kinetic function could be considered, but also its derivative functions, from which also several parameters could be extracted. Finally, another option considered to extract parameters that describe a curve is to fit a parametric curve to the oxygen consumption curve and use the parameters of the fitting curve as the parameters to describe the oxygen consumption kinetics.

Once the parameters that describe the oxygen consumption kinetic curve are extracted, they must be related with the chemical composition of the wine. To this end, due to the big number of variables to be considered and the complex relationships among them, Artificial Neural Networks are one good solution to model these relationships because they are able to model non-linear relationships among a big number of variables.

Keywords: Oxygen consumption kinetics; Artificial neural networks; Wine science.

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Proposed section: Phenolic compounds circular economy Proposed format: oral

Valorisation of phenolic compounds from Polyvinylpolypyrrolidone (PVPP) waste used in wine fining

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PVPP is a water-insoluble synthetic polymer obtained by cross-linking of polyvinylpyrrilidone (PVP) [1, 2]. Due to its excellent adsorbent properties, selectivity, stability, inertness and non-allergenicity [2], PVPP is generally used in the production of wine and other beverages to avoid haze formation. eliminating phenolic compounds responsible for bitterness, astringency, browning and pinking [3, 4]. Phenolic compounds bind to PVPP mainly through hydrophobic interaction between PVPP pyrrolidone ring, and phenolics aromatic ring and by hydrogen-bond between the phenol groups and carbonyl group of pyrrolidone [1, 3, 4]. The structure of the polyphenol plays an important role in their interaction with PVPP [5]. PVPP creates large amounts of waste that end up on the municipal wastewater treatment plants. In 2014, the use of PVPP was estimated in 1,034 tons, and after use, PVPP contains significant amounts of adsorbed phenolics, having a significant and negative impact on the environment [6]. Thus, the main purpose of this work was to develop a simple, cheap and eco-friendly PVPP recycling process to recover used PVPP and the adsorbed wine phenolics. By using an ammoniacal solution of ethanol to desorb the wine phenolics from the used PVPP it is possible to obtain pure extracts of wine phenolics and a recycled PVPP to reuse in the wine industry with high performance. The process allows desorbing phenolics with high recoveries (average 73%). The used PVPP obtained from different wineries presents a vield of phenolics ranging from 2.82 to 10.90 g/kg of wet PVPP. Extracts were almost pure concerning the phenolics abundance (>89%), without the need for further purification. It was recovered 20 to 24 phenolics depending on the used PVPP (wine characteristics associated with grape varieties, region and winemaking technologies), being the most abundant (+)-catechin (\sim 42%), trans-caftaric acid (~18-45%), gallic acid (~23%), chlorogenic acid (~11-16%), and (-)-epicatechin (~11%). The phenolics recovered from the used PVPP can be easily used by other industries such as food, pharmaceutical or cosmetic.

Keywords: Phenolic compounds; Recycling; PVPP; Sustainability.

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Proposed section: Phenolic compounds circular economy Proposed format: oral

Optimization of the extraction of colouring agents from leaves of Ocimum basilicum var. purpurascens

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Ocimum basilicum var. *purpurascens* (red rubin basil) is a variety rich in anthocyanin compounds, commonly used by the food industry as aromatic plant [1]. The Heat Assisted Extraction (HAE) associated to the response surface methodology (RSM) is applied in order to use sustainable extraction methodologies at the industrial level. The extraction methodologies can be affected by a number of independent variables (processing time, temperature and solvent) and the application of Response Surface Methodology (RSM) is a time- and reagent-saving statistical tool increasingly used in process optimization, since one-factor-at-a-time experiments cannot predict optimal conditions and neglect interactions between variables [2].

In this work, the extraction of anthocyanins from *O. basilicum* var. *purpurascens* leaves, was optimized through HAE, by applying a RSM combining different independent variables of the process. Furthermore, the bioactive properties (antimicrobial and cytotoxic activities) of the optimized extract were evaluated. The anthocyanins' profile was determined by HPLC-DAD-ESI/MS, using 520 nm as the preference wavelength and operating in positive mode. The antimicrobial activity was determined using the microdilution method in Gram-positive and Gram-negative bacteria, and in fungi; the cytotoxicity was evaluated in four tumour cell lines (MCF-7, NCI-H460, HeLa and HepG2) and a non-tumour primary cell culture (PLP2), by applying the sulforhodamine B assay. The optimal HAE conditions for the anthocyanins' extraction were: t= 65.37 ± 3.62 min, T= 85.00 ± 1.17 °C and S= $62.50 \pm 4.24\%$ of ethanol, and originated 114.74 \pm 0.58 mg of anthocyanins/g of extract. This extract also revealed antimicrobial and cytotoxic properties in tumour cells, without any cytoxicity for the tested non-tumour cells.

In general, the study highlighted red rubin basil leaves as a promising natural matrix to extract colorant compounds, using green solvents with reduced extraction times, also providing bioactive properties.

Keywords: Natural colorants; Anthocyanins; *Ocimum basilicum* var. *purpurascens* leaves; Extraction optimization.

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Proposed section: Phenolic compounds circular economy Proposed format: oral

Fighting Antibiotic Resistance: grape stems as a new opportunity

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The continuous emergence of multidrug resistant bacteria is becoming a huge threat to human, animal and environmental health (*One health* approach). Therefore, the research for new natural antimicrobial compounds is the most promising alternative to effectively control multidrug bacterial infections. The grape stems are by-products from the winery industry are a cheap source of polyphenols with potential biological activities such as antioxidant, antibacterial and anti-inflammatory, as recently showed [1, 2]. Thus, the aim of our study was to demonstrate the effect of long-term storage on the antimicrobial activity of grape stems.

Polyphenols of three varieties of Portuguese winery by-products (Sousão, Syrah, Tinta Barroca and Tinta Barroca Cima-Corgo) were extracted with a methanol/water (70:30). The antimicrobial activity was tested by disc diffusion assay against six different bacterial strains: *Listeria monocytogenes* ATCC 15313; *Staphylococcus aureus* MJS241; *Enterococcus faecalis* MJS257; *Pseudomonas aeruginosa* ATCC 10145; *Escherichia coli* MJS260; *Klebsiella pneumoniae* MJS281. Strains were isolated from the gastrointestinal tract of humans and supplied by Centro Hospitalar de Trás-os-Montes e Alto Douro.

The antimicrobial properties, from all tested winery by-products, were not affected by 64 days of storage. All extracts (with and without storage) were able to inhibit the bacterial growth of the grampositive bacteria and gram-negative bacteria (except *E.coli* and *K.pneumoniae*). It is important to highlight that Tinta Barroca by-products showed higher efficiency than antibiotics in the inhibition of the bacterial growth of *S.aureus* and *E.faecalis*.

Keywords: Grape stems; Antibiotic resistance; Circular economy.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: oral

Extraction of phenolic compounds by high hydrostatic pressure from eight edible algae species from the North-West coast of Spain: Process modelling and optimization

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The oceans cover about the 71 % of the earth surface and this makes that over 90 % of the living biomass in the Earth is contained in the oceans that is why it is of great interest to find raw material that may be of value in said medium. In aquatic ecosystems we can find algae, which are photosynthetic plants with very heterogeneous characteristics.

Algae are not only of high ecological, but also of great economic importance. Many of these algae contain bioactive compounds all of them compounds of great interest to the industry of nutrition and pharmaceutical. A bibliographic review allowed us to conclude that the main biological activities of algae are: antioxidants, antibacterial, anti-inflammatory, antifungal / antiprotozoal, antiviral, cytotoxicity / antiproliferative, adipogenesis, MAA / UV protection, matrix metalloproteinases and blood fluidity.

In this work, high hydrostatic pressure (HHP) was applied to the extraction of phenolic compounds from eight edible algae species. The edible species used in this study were Kombu (KR, *Laminaria spp.*), Kombu real (KR, *Saccharina latissimi*), Espagueti de mar (EM, *Himanthalia elongate*), Wakame (W, *Undariapinnatifida*), Nori (N, *Porphyra spp.*), Dulse (D, *Palmaria palmate*), Alga percebe (AP, *Codium spp.*) and Lechuga de mar (LM, *Ulva spp.*).

The process was optimized by response surface methodology using a five-level central composite design combining the independent variables of processing time (t, 5-90 min), pressure (P, 10-600 MPa) and solvent (S, 0-100 % of ethanol, v/v).

The results obtained with this method allow us to conclude that high pressures are a promising emerging technology that can be used to extract phenolic compounds from algae species with several advantages like it uses a green solvent and reduced extraction times.

Keywords: Algae; Phenolic compounds; Extractions; Bioactivities; Phenolic compounds applications.

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Proposed section: Phenolic compounds circular economy Proposed format: oral

Vine & wine residues: Unlimited resources of high-value polyphenols towards Industrial Applications

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The Agro Food industry produces large amounts of agro-residues every year which represents a waste disposal problem. EU directives in the H2020 program for the management of agro-food wastes are: recycle, reuse and recover. Wine industry in Portugal is one of the most important activities contributing significantly to the national economy but several waste products are generated. However, many of the polyphenolic compounds found in original plant materials may still be recovered from pruning material, grape stems and grape pomace. The generated by-products are currently not properly taken care in environmentally and economic terms. So, a still inexistent waste management plan should be created in order to use those agricultural by-products.

This work comprises original strategies to stabilize different polyphenols (anthocyanins, portisins, tannins and stilbenes) obtained from the selected agro-residues or chemically transformed, using lignins (polymeric polyphenolic compounds) that will also be isolated from the same industrial residues as a source of coating-biopolymers for the encapsulation of the target compounds. Further stabilization using non-polyphenolic compounds coating-biopolymers (polysaccharides and whey proteins), liposome and micelles-based systems will also be tested for encapsulations and stabilization of the polyphenols in food matrices.

The interaction between each coating material and the target compounds and the encapsulation efficiency will be evaluated by different techniques that include UV-Vis Spectroscopy, Stopped Flow, HPLC-MS, NMR, STD-NMR and DLS [1].

Keywords: Anthocyanin-derived pigments; Colour stabilization; Lignin; Nanoparticles.

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Proposed section: Phenolic compounds circular economy Proposed format: oral

The circular economy of fruits by-products extracts: potential use in the food industry

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Circular economy is a relative new concept which can be defined as regenerative system that aims to reduce or eliminate waste, emission and energy leakage through several strategies such as recycling, reuse and remanufacturing [1]. Phenolic compounds are secondary metabolites, naturally produced by plants for their defence against external stimuli, such as radiation and predators [2]. Fruits by-products are considered food waste, their economic value is low so they are a valuable choice to be reused in nutraceutical, pharmaceutical and food industries [3]. Food packaging main goals are to assure food safety and to extend their shelf-life. Active food packaging can achieve these goals by directly interact with the packaged foods, for example emitting active compounds that will inhibit or delay the natural degradation of foods [4]. Antioxidant and antimicrobial compounds obtained from fruits by-products can be added to these packages, reducing the direct addition of food additives to foods. Lemon and apple+ginger (50:50, w/w) ethanolic extracts were prepared and incorporated into PLA, G-Polymer[™] and LDPE (low density polyethylene) and migration studies were carried out to evaluate the potential migration of phenolic compounds from the active packaging to a simulant of fatty foods. The antioxidant capacity results indicate that the lemon extract presented the highest content in phenolic compounds and the films incorporated with this extract presented a higher migration of phenolic compounds into the fatty food simulant. Further analysis to evaluate the effectiveness of an active film with lemon extract will be carried out to verify if this is able to inhibit the lipid oxidation of a model fatty food.

Keywords: Active packaging; Fruits by-products; Phenolic compounds; Antioxidant; Antimicrobial.

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Proposed section: Phenolic compounds and taste Proposed format: oral

Molecular basis of the modulation of astringency by biopolymers

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Wine astringency in mainly resulting from the interaction between phenolic compounds and salivary proteins. This organoleptic property can be modulated by polysaccharides since they are described to interact with flavanols, the main responsible for astringency [1]. Wine polysaccharides could be derived from grape skin (grape polysaccharides) or from yeast (mannoproteins).

The objectives of this work were to evaluate i) the usefulness of polysaccharides for astringency modulation, ii) the possible relationship between the structural features of polysaccharides and their ability to interact with flavanols and to affect the flavanol-salivary protein interactions and iii) the molecular mechanisms involved in those interactions. The structural characteristics of polysaccharides have been evaluated by means of chromatographic techniques and the potential effect of polysaccharides on astringency and their ability to influence the interactions between flavanols and salivary proteins have been studied by HPLC-DAD-MS, dynamic light scattering (DLS), isothermal titration calorimetry (ITC) and molecular dynamic simulations (MDS).

On the one hand, mannoproteins have shown to importantly affect the interaction between flavanols and salivary proteins. These polysaccharides can strongly interact not only with flavanols but also with salivary proteins, thus preventing the flavanol-salivary protein interaction, which might modulate astringency sensation. Moreover, results pointed out the formation of mannoprotein-flavanol-salivary protein ternary aggregates that may be soluble, which would reduce the protein precipitation. The mannoprotein structure seems to be determinant for their ability to influence the interaction and the mechanisms driven those interactions, since differences on the protein content of mannoproteins lead to differences on the mannoprotein effect and on the forces involved in the interaction. On the other hand, results also showed that the modulation of wine astringency by the addition of grape polysaccharides is greatly affected by the polysaccharide origin and structure. These results pointed out that the structure of these biopolymers could be key in their ability to modulate wine astringency.

Keywords: Flavanols ; Wine ; Grape polysaccharides; Mannoproteins; Salivary proteins.

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Proposed section: Phenolic compounds and taste Proposed format: oral

Molecular Taste: molecular approaches to understand food polyphenols astringency and bitterness

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Polyphenols are widespread in plant-based foodstuffs and contribute directly to their flavor, e.g. astringency and bitter taste [1]. These taste properties can be appreciated in some foodstuffs, such as beer, coffee, and red wine, but in most cases they are unwanted and efforts are taken to reduce them. As a result, an unbiased recognition and knowledge on how and which polyphenols are bitter and/or astringent is important for a focused modulation of these sensory properties.

It is well-known that bitterness is a taste perceived by activation of bitter taste receptors (TAS2Rs) [2]. Still, the mechanism for astringency onset has been a debated topic. Several mechanisms have been proposed to explain its onset, including the activation of trigeminal ganglion [3], involvement of oral pellicle [4], among others [5]. A general agreement is that the interaction/precipitation of salivary proteins (SP), namely proline-rich proteins, by some food polyphenols, mainly tannins, occurs and contributes to the overall astringency.

Along the years, our research group has used/developed molecular approaches to understand the molecular phenomena driving to the bitterness and astringency of polyphenols. The bitterness of a library of polyphenols from different classes (procyanidin dimers type B, anthocyanin, hydrolyzable tannins and phenolic acid ethyl esters) has been studied by a cell-based assay [6]. The TAS2Rs activated by these polyphenols and the half-maximum effective concentration (EC₅₀) of each agonist-TAS2Rs pair was determined. Additionally, it was observed that the presence of SP could impair the activation of TAS2Rs by some polyphenols. Regarding the astringency, several physical-chemical techniques, such as STD-NMR, ITC and fluorescence quenching have been used to study the individual interactions between different families of SP basic, glycosylated, acidic proline-rich proteins and P-B peptide with some model food tannins (e.g. procyanidin B2, procyanidin B2 3'-O-gallate and malvidin-3-glucoside, among others) [7]. Using these techniques it has been possible to determine the affinity of SP toward the polyphenols. Recently, a new oral model has been developed, allowing to study the contribution of each oral constituent (e.g. SP, mucosa pellicle and oral cells) on the binding process of different procyanidins when they occur as a mixture [submitted].

Keywords: Salivary proteins; Bitter taste receptors; Oral cells; Mucosa pellicle.

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Proposed section: Phenolic compounds and taste Proposed format: oral

Study of the supramolecular interactions between phenolic compounds and salivary proteins and their relationship with astringency

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Astringency of red wine has been widely associated with the presence of phenolic compounds, but the underlying molecular mechanisms of this process have not yet been clarified. One of the most widely accepted mechanism consist on the interaction between polyphenols and some specific salivary proteins, namely, proline-rich salivary proteins (PRPs) [1]. Moreover, in the last few years, other approaches have suggested that astringency could be engendered by the activation of specific taste receptors or even by direct interaction between tannins and oral epithelial cells [2- 4].

In this context, the present work aims to study, at molecular level, the interactions between wine phenolic compounds and salivary proteins as the main mechanism for the astringency development. To achieve this objective, we have assessed the interaction between salivary proteins and flavanols catechin and epicatechin by HPLC-DAD, ITC and molecular dynamics (MD) simulation, evaluating the existence of synergisms of astringency caused by mixtures of flavanols. Additionally, this study aims to go further into depth on the knowledge of the mechanisms involved in oral astringency, considering both salivary proteins and epithelial cells, evaluating the effect of the presence of salivary proteins on these interactions using a cell-based model of oral mucosa.

Results obtained showed the existence of a cooperative behaviour between catechin and epicatechin molecules in their binding to salivary proteins, leading into a synergistic effect on the perceived astringency. On the other hand, a direct interaction between tannins and oral epithelial cells has been pointed out, although this interaction could be inhibited when tannins are previously incubated with saliva.

Keywords: Astringency; Salivary proteins; Wine; Flavanols; Epithelial cells.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: oral

Study on model systems of the molecular mechanisms involved in the biological activity of phenolic compounds

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Dietary consumption of phenolic compounds from fruits and vegetables has been associated to health benefits, including protection against aging-related diseases, such as cardiovascular pathologies, cancer and neurodegenerative disorders [1]. However, the actual contribution of these compounds to health maintenance and the mechanism behind their biological activity are still not well established. Traditionally, the bioactivity of and phenolic compounds has been associated to their antioxidant and radical scavenging properties, but currently it is assumed that other mechanisms contribute to their overall effects in the organism and could be even more important than the radical scavenging properties [2]. In order to progress in the knowledge of the mechanisms involved in the biological effects of the dietary flavonoids, model organisms like the nematode *Caenorhabditis elegans* are increasingly used to perform studies related with genes or cellular pathways. This worm is a simple multicellular organism that constitutes an excellent model for studying molecular mechanisms of action because of its short lifespan, fast generation time, good molecular and genomic tools and well-defined genetic pathways [3, 4]. Furthermore, aging signaling pathways are conserved from yeast and worms to mammals and, among them, the insulin/IGF-1 signaling pathway (IIS) that is related to longevity and oxidative or heat shock stress response. With the aim to contribute to the knowledge of the actual mechanisms involved in the biological effects of dietary polyphenols, different studies are conducted by our group using the C. *elegans* model. In this presentation, the involvement of some key genes of the IIS conserved pathway on the effects of quercetin and epicatechin has been explored, using wild type and knockout mutant worms, gene expression analyses and transgenic animals expressing the GFP fluorescent reporter.

Keywords: C. elegans; IIS pathway; Mutant worms; Epicatechin; Quercetin.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: oral

Experimental evidence on the phenolic richness of plants from Guinea-Bissauan medicinal flora and relation with bioactivities

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The drug discovery based on plants used in Traditional Medicine remains as one of the most successful strategies on drug development. In spite of being a small West African country, Guinea-Bissau boasts a great cultural and biological diversity, and a high ethnobotanical value is attributed to its native flora. Its local flora englobes 1459 native species, 195 of which have been documented for their therapeutic properties, over half of these having a ubiquitous distribution throughout the whole country [1]. Therefore, the ethnobotanical richness of Guinea-Bissau is undeniable and the scientific validation of the knowledge acquired by the local healers could be transformed in a promising strategy to find new therapeutic natural drugs. Amongst the phytochemicals obtained from plants, phenolic compounds can be highlighted due to their several biological properties.

In this sense, ethnopharmacological evidence for the use of *Xylopia aethiopica* (Dunal) A. Rich and *Salacia senegalensis* (Lam.) DC in the management of inflammation-related diseases was screened. To achieve these purposes, the anti-inflammatory capacities of both leaf extracts were examined in different cell line models and cell-free assays. In addition, the phenolic composition of each extract was analyzed by HPLC-DAD in order to establish possible structure-activity relationships.

X. aethiopica and *S. senegalensis* leaves display significant anti-inflammatory effects, based on the capacity to interfere with conventional inflammatory targets, namely the eicosanoid-metabolizing enzyme 5-lypoxigenase and nitric oxide. The determined phenolic compounds could be partially related with the observed bioactivities. Overall, both leaf extracts, obtained using cheap and non-hazard solvents, can constitute a source of potential crude drugs or bioactives, thus valorizing the two medicinal plants.

Keywords: Phenolic compounds; Medicinal plants; Ethnopharmacology; Inflammation.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: oral

Profiting from Thai ethnomedicinal knowledge: Phenolic profiling and biological insights in the anti-inflammatory effects of Thai plants

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The use of herbal drugs in Thai healthcare services is still widespread, denoting the utility of ethnomedicinal knowledge. Nevertheless, data on the efficacy of plant-derived constituents of herbal preparations is frequently scarce [1]. While *Vitex peduncularis* and *Homalium bhamoense* are used on the amelioration of inflammatory-mediated conditions, studies addressing their anti-inflammatory potential are absent. Similarly, ethnomedicinal surveys reveal the ubiquitous use of *Ficus* spp. in Traditional Thai Medicine, but there are no reports on the biological properties of *Ficus curtipes*.

The use of *V. peduncularis* in folk medicine is partially validated, due to the significant inhibition of phospholipase A_2 and the efficient reduction of NO levels in LPS-challenged RAW 264.7 macrophages, upon exposure to an extract obtained from the stem bark [2]. While the trunk bark extract from *H. bhamoense* did not lead to noticeable effects in NO levels in the same cellular model, significant inhibitory effects towards 5-lipoxygenase and hyaluronidase were recorded, IC₅₀ values of 29.0 and 396.9 µg mL⁻¹ being determined, respectively. Robust experimental data on the anti-inflammatory properties of *F. curtipes* leaves was also obtained, being worth to highlight the interference with NO levels in LPS-stimulated RAW 264.7 cells, apparently derived from a bimodal mechanism, based on 'NO scavenging effects and modulation of iNOS. Modulatory effects upon inflammatory mediators after exposure to the three extracts appear to be partially related with their phenolic profiles. The stem bark extract obtained from *V. peduncularis* contained a high content of apigenin and luteolin derivatives, while the extract obtained from *F. curtipes* leaves was solely characterized by the occurrence of apigenin-di-glycosides. Phenolic profiling of *H. bhamoense* trunk bark extract allowed the identification and quantitation of ellagic and methyl ellagic acid, as well as a series of glycosylated derivatives.

Keywords: Ethnopharmacology; Inflammation; Polyphenols; Traditional Medicine Asia.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: oral

Evaluation of the neuroprotective and antidiabetic potential of phenolrich extracts from virgin olive oils by *in vitro* assays

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The increasing interest in the Mediterranean diet is based on the protective effects against several diseases, including neurodegenerative disorders and diabetes mellitus. Polyphenol-rich functional foods have been proposed to be unique supplementary and nutraceutical treatments for these disorders.

In this work, phenol-rich extracts from 'Brava' and 'Mansa de Figueiredo' (two cultivars recently identified from north-western Spain) extra-virgin olive oils (EVOOs) were examined, for the first time, to establish their capacity to inhibit key enzymes involved in Alzheimer's disease (AD) (acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)), major depressive disorder (MDD) and Parkinson's disease (PD) (monoamine oxidases: *h*MAO-A and *h*MAO-B respectively), and diabetes mellitus (DM) (α -glucosidase and α -amylase). In addition, a systematic phenolic characterization of the studied oils was undertaken using LC-ESI-IT-MS system. 'Brava' and 'Mansa de Figueiredo' EVOOs exhibited inhibitory activity against BuChE (IC₅₀ = 298 ± 6 - 668 ± 26 µg of dry extract/mL), *h*MAO-A (IC₅₀ = 35 ± 2 - 64 ± 4 µg of dry extract/mL) and *h*MAO-B (IC₅₀ = 223 ± 10 - 236 ± 16µg of dry extract/mL), respectively [1]. Furthermore, it is very interesting to notice that rich phenol extracts from EVOOs (IC₅₀ = 60 ± 8 - 118 ± 9µg of dry extract/mL for 'Brava' and 'Mansa de Figueiredo', respectively) were stronger inhibitors of α -glucosidase than acarbose, used as commercial inhibitor (IC₅₀=356 ± 21 µg/mL) [2]. Only 'Brava' extracts showed inhibitory activity against AChE and α -amylase being both presented the IC₂₅ value. Although central nervous system (CNS) inhibition activities were lower than those of the positive controls, our results suggest that the extracts obtained from EVOOs could represent valuable strategies

Keywords: Extra-virgin olive oil; Phenolic compounds; Neuroprotection; Diabetes mellitus.

to prevent and/or reduce events underlying CNS related- disorders and hyperglycemia.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: oral

Impact of *in vitro* gastrointestinal digestion and colonic fermentation on the phenolic composition and bioactivities of *Rosmarinus officinalis* L.

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Rosmarinus officinalis L. (Lamiaceae) is a plant native to the Mediterranean, popularly known as rosemary. In addition to its culinary use, it has also been used for therapeutic purposes since antiquity [1]. The European Union has considered rosemary extract as a safe and efficient food preservative, being rosmarinic acid (RA) its main constituent [2]. Extracts rich in RA not only have anti-inflammatory, antioxidant and hepatoprotective effects [1], but also prevent oxidation in foods without compromising their sensory acceptance. However, information on the stability of bioactive compounds in rosemary extract is scarce. The aim of this work was to investigate the potential phytochemical losses occurring throughout the sequential steps of in vitro gastrointestinal digestion and colonic fermentation of a rosemary aqueous extract. For this purpose, the crude (CE), digested (DE) and fermented (FE) extracts were characterized in terms of their phenolic profile and biological activities. Rosmarinic acid was the phytochemical that underwent the most significate transformation during digestion and fermentation, which amounted to 60% compared to the 26% degradation of the total phenolic compounds. Overall, the simulated digestion step decreased the antioxidant activity estimated by DPPH, ABTS, FRAP, ORAC and TBARS assays. Both CE and DE did not present anti-proliferative potential, however, FE exhibited a pronounced cytotoxic activity (Gl₅₀ = 116 µg/mL) against HeLa cervical carcinoma cell line. CE and DE showed to be moderate inhibitors of methicillin resistant Staphylococcus aureus (MRSA), methicillin-susceptible S. aureus (MSSA), and Listeria monocytogenes, whilst the FE acted as a moderate inhibitor of MRSA and MSSA. In view of the relevant biological activities confirmed in this study, it is possible to suggest the application of the rosemary aqueous extract as a food additive, either as a preservative and/or as a functional ingredient. However, the use of RA-rich formulations in food industry should consider the stability and bioavailability of this bioactive compound.

Keywords: Antioxidant activity; Anti-proliferative potential; Bactericidal effect; Rosmarinic acid.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: oral

Contribution of phenolic compounds for the biological activities of Acanthus mollis

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Acanthus mollis L. is a plant native to the Mediterranean region belonging to the extended family Acanthaceae. Traditionally it is used externally for wounds and burns treatment, as analoesic and antiinflammatory, and internally as diuretic, anti-inflammatory for the digestive system and urinary tract [1]. Unlike other plants of the genus Acanthus, its efficacy in the pathologies referred by folk medicine has not yet been scientifically verified. In previous work we proved the anti-inflammatory activity of an ethanol extract obtained from Acanthus mollis leaves [2]. In this study, this extract, as well as a decoction and infusion were evaluated for their antioxidant/anti-radical, anti-inflammatory and/or cytotoxicity activities. These extracts were further gualitative and guantitatively characterized using the HPLC-PDA-ESI/MSⁿ as described by Matos et al. (2018) [2] and HPLC-PDA, respectively.

Phenolic compounds and hydroxamic acids, mainly flavonoids and phenyl propanoids, were detected in the extracts in different concentrations. Based in literature data, we infer that some of these compounds could be responsible to some of the uses of the plant in traditional medicine. The isolation and biological activity research of these compounds is in course to prove their contribution for the antioxidant, antimicrobial, anti-inflammatory, anticancer and antidiabetic activities, some of which support its use in the ethnomedicine.

Keywords: Acanthus mollis; Phenolic compounds; Biological activities.

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Proposed section: Phenolic compounds and health Proposed format: Oral

Effects of Chestnut Flower Extract on an Animal Model of Prostate Cancer

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Prostate cancer is one of the most frequently tumor in men, affecting approximately, 1.3 million men worldwide [1]. Natural compounds have been used to prevent or treat several diseases. Chestnut flowers (e.g., infusions and decoctions) are reported as having health benefits, they can be used in colds, cough and intestinal problems [2]. The aim of this study was to evaluate the in vivo antitumor properties of a decoction extract obtained from chestnut flowers (CF), using an animal model of prostate cancer chemically and hormonally induced. The extract was further characterized in terms of its phenolic profile by HPLC-DAD-ESI/MSⁿ.

All the animal experiments were approved by the Institutional Animals Ethics Committee and by Portuguese national authorities (Direção Geral de Alimentação e Veterinária, approval number 021326). Forty male Wistar Unilever rats were randomly divided into four groups: control group (n=10), induced group (n=15) control+CF (n=5) and induced+CF (n=10). Animals from induced groups received a multistep induction protocol, which consisted of sequential administration of flutamide, testosterone propionate, the carcinogenic agent MNU and crystalline testosterone. The CF extract, rich in ellagitannins especially trigalloy-HHDP-glucose, was administered in drinking water (3 mg/animal/day, being the extract stable for 5 days) during 49 weeks. Animals were sacrificed at 61 weeks of age. Animals treated with CF developed less prostatic intraepithelial neoplasia than untreated animas. Also, the CF reduced the extension of inflammation. The untreated induced animals evidenced areas of inflammation of the dorsolateral prostate lobe greater than 50%, whereas animals exposed to CF extract can be used as chemopreventive agent against MNU-induced prostate cancer.

Keywords: Chemoprevention; Natural compounds; Rats; Carcinogenesis; MNU.

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Acknowledgments: This work was supported by post-graduation grant SFRH/BD/136747/2018. L. Barros also thanks the national funding by FCT, P.I., through the institutional scientific employment program-contract for her contract.

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Proposed section: Phenolic compounds and health Proposed format: oral

The marine biome and its unlimited potential: Brown seaweed phlorotannins as multimodal agents in inflammation and allergy network

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The chronic nature of several emerging diseases, along with the lack of effective preventive and curative therapies, is directing research into new sources of bioactive compounds with multimodal actions. Inflammatory processes are at the root of several biological processes and, in the last decades, a complex interplay between inflammation and allergic reactions has been acknowledged.

Phlorotannins are phloroglucinol-based phenolic compounds exclusively found in brown seaweeds (Ochrophyta, Phaeophyceae) that have been pointed out as potent modulators of several biochemical processes linked to the breakdown of homeostasis in major chronic diseases.

To harness the biotechnological potential of the Portuguese marine flora, different species of *Fucus*, the most prominent and species-rich genus within the order Fucales, were analysed for their phlorotannin composition (HPLC-DAD-ESI/MS^{*n*} and UPLC-ESI-QTOF/MS) and the *in vitro* anti-inflammatory and anti-allergic potential of their phlorotannin-targeted extracts was addressed. Besides inhibiting the activity of eicosanoid-metabolizing enzyme 5-LOX, the overproduction of the pro-inflammatory mediator NO was also efficiently surmounted by the phlorotannin extracts [1]. Phlorotannin-targeted extracts from *Fucus* spp. acted upon critical steps of the allergic response, reducing basophils' degranulation and inhibiting hyaluronidase activity [2]. Overall, it was demonstrated that the unique phlorotannin profile of the selected *Fucus* species was behind the observed bioactivities, supporting the potential of these polyphenols as valuable naturally occurring pharmacological alternatives with a large spectrum of activity.

Keywords: Phlorotannins; Fucus spp.; Inflammation; Allergy.

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Proposed section: Phenolic compounds and health Proposed format: oral

Extra Virgin Olive Oils obtained from Galician ancient varieties: the Role of the Phenolic Compounds on MCF-7 Human Breast Cancer Cells

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Breast cancer is the second most common worldwide cancer, the most frequent among women and the fifth cause of death from cancer overall (522,000 deaths) [1]. This fact makes new therapeutic strategies and chemotherapeutic candidates to be continuously explored. Currently, this search is partly focused on natural substances that could interfere in the progress of some tumors or cancers. Due to many of these natural substances are present in food, dietary intervention has become considered an important cancer chemopreventive strategy. In particular, olive oil possesses known beneficial effects on health, which are related, above all, with the content of oleic acid and bioactive compounds.

One of the current trends in the olive oil market is the production of high quality Extra Virgin Olive Oils (EVOOs) from traditional minor olive varieties with specific origin and particular and differentiated sensory, nutritional and healthy promoting characteristics, like 'Brava' and 'Mansa de Figueiredo' [2-3].

Therefore, the purpose of the present study was (i) to characterize the complete phenolic profile of monovarietal EVOOs from 'Brava' and 'Mansa de Figueiredo' using LC-MS, and (ii) to investigate the potential health effects of phenolic extracts from 'Brava' EVOO on MCF-7 breast cancer cell line by evaluating the anti-proliferative, cell cycle arresting, apoptosis activating effects through regulation of intrinsic and extrinsic apoptotic signaling as well as autophagy regulatory genes. The energetic metabolism has also been studied by using the XF24 Extracellular Flux Analyzer.

Our results show that secoiridoids from oleuropein and ligstroside range from 67% to 83% of the total phenolic compounds in both EVOOs. Phenolic extracts from 'Brava' EVOO could be cytotoxic for this model in a dose- and time- dependent manner.

Keywords: Extra virgin olive oil; Phenolic compounds; Breast cancer; Cytotoxicity; Energetic metabolism.

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Proposed section: Phenolic compounds and health Proposed format: oral

Let your food be your medicine: Polyphenols as modulators of Food Allergies

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Food allergy (FA) is a substantial and evolving public health issue, increasing over the last decade as an allergy epidemic [1]. Recent evidence is emerging suggesting a crucial role of nutrition in the development of FA [2, 3]. The lack of therapies, the severity of the FA symptoms and the increasing in prevalence promotes the need to establish prophylactic dietary habits, which could stop or control an almost epidemic disease. Consumption of dietary antioxidants found in fruits and vegetables has been related with low prevalence of FA [4]. Among dietary antioxidants, polyphenols (PHEN) have strong potential as modulating agents in FA. Several mechanisms have been already proposed nevertheless further research is needed. Used as nutraceuticals, PHEN are thought to dampen the onset of allergic inflammation, by acting on several immune cells, but concerns remain about their real employment by the organism that assumes PHEN through diet, because of their bioavailability, gut transformation and pharmacokinetics. Recent studies proved the ability of PHEN to bind food antigens, which clearly opens a new perspective to elucidate the PHEN influence in FA. A broad approach is therefore needed to fully understand the immunomodulatory process from ingestion to allergy systemic effects manifestation.

This project aims at highlighting the immunomodulatory effects of PHEN through the ability to bind to allergens. To evaluate the impact in egg, peanut and milk FA development, PHEN-allergen complexes will be characterised to further verify their behaviour during all statements that lead to a systemic response, from ingestion to transport to mucosa associated lymphoid tissue, blocking the chain reactions which lead to mediator's release. Furthermore, given that microbiota could influence the digestive and metabolic process and taking into account the dysbiosis observed in allergic people, the effect of gut microbiota in the maintenance of oral tolerance will also be observed during the digestion and absorption process.

The expected results intend to establish a new basis of knowledge helping to control an emerging public health problem, the increasing in FA prevalence. It was strived to reach the starting point of a new research field that encompass different types of expertise combining food chemistry, analytical chemistry, proteomics and biochemistry to provide some important information in the nutrition field.

Keywords: Protein-polyphenol interactions; Food allergy; Dietary Antigens; Dietary polyphenols.

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Proposed section: Phenolic compounds and health Proposed format: oral

Effects of *Ganoderma lucidum* on oxidative stress in a mice model of tubulointerstitial fibrosis

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Interstitial fibrosis is strongly associated with the progression of Chronic Kidney Disease (CKD) [1]. Oxidative stress (OS) plays a central role in the pathogenesis of renal fibrosis [2]. *Ganoderma lucidum* (GL) is a medicinal mushroom, revealing in its composition high amounts of triterpenoids and some phenolic acids, which have been associated with antioxidant activities [3]. Thus, in this study, we investigated the potential effects of GL hydroethanolic extract on OS in mice with unilateral ureteral obstruction (UUO), a well-established CKD model marked by a significant tubulointerstitial fibrosis [2].

FVB/n female mice (8 weeks old) were divided into three groups: sham group (operated-healthy control, n=8); UUO group (without treatment, n=10); UUO+GL group (treated with GL extract, administered in drinking water (2.0 mg/mouse per day), n=9). Animals were sacrificed 14 days after surgery (Directive 2010/63/EU). OS markers were assessed in liver and left kidney. Haematoxylin and eosin staining was used to assess histological changes.

Ganoderic acid H (58.13±0.03 mg/g of extract) and *p*-hydroxibenzoic acid (0.364±0.01 mg/g) found in the extracts, respectively. Reactive oxygen species (ROS) liver levels decreased in UUO+GL group compared with UUO group (p<0.05), but no significant differences (NSD) were found between the UUO groups in any other marker of OS in the liver. Left kidney catalase activity in the UUO groups decreased compared with the sham group (p<0.05). Glutathione peroxidase renal activity was lower in the UUO group compared with the sham group (p<0.05). NSD in the superoxide dismutase and glutathione-S-transferase renal activities, and in the reduced glutathione and oxidized glutathione renal levels occurred after UUO. ROS renal levels were increased in UUO groups. Furthermore, there were NSD in interstitial fibrosis and tubular dilatation scores between the UUO and UUO+GL groups. This study showed that GL extracts, at 2.0 mg/mouse *per* day, do not reversed the OS induced by UUO and consequently does not modified UUO- induced tubulointerstitial fibrosis.

Keywords: Unilateral ureteral obstruction; Medicinal mushroom; Oxidative stress; Renal fibrosis.

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Proposed section: Phenolic compounds and health Proposed format: oral

Strawberry polyphenols as possible modulators of pre-adipocytes differentiation: a possible tool to control obesity onset and progression

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Obesity is one of the major problems of the 21st century worldwide. It is characterized by an expansion of white adipose tissue mass resulting from increased adipocytes number and/or size. Excessive accumulation of mature adipocytes is associated with high triglyceride levels and with a general impairment of catabolic pathways. In addition, reactive oxygen species have been recognized as important modulators of adipogenesis and increased oxidative stress into adipocytes is considered an important pathogenic mechanism of obesity associated metabolic syndrome. Various natural bioactive compounds have demonstrated to present anti-adipogenic effects in different in vitro and in vivo experimental models. In this work, we evaluated the effect of a strawberry methanolic extract (SE) on adipogenesis and 3T3-L1 adipocytes metabolism. For such purpose confluent pre-adipocytes were differentiated using a standard protocol and were treated with different concentrations of SE. The results demonstrated that SE significantly reduced 3T3-L1 pre-adipocytes differentiation, lipid accumulation and down-regulated the mRNA expression of the adipogenic transcription factors C/REB- a and PPAR-y. Consistently, it inhibited the expression of FABP4, ACC and SREBP1. Likewise, it stimulated the AMPK, Sirt1and LKB1 in a dose-dependent manner, suggesting that the anti-adipogenic effects of strawberry extract are mediated by the activation of the AMPK pathway. SE also inhibited oxidative stress and inflammation biomarkers, increased antioxidant enzymes activities and induced phenotypic effects similar to brown adjocytes in 3T3-L1 cells by increasing oxygen consumption, uncoupled respiration and mRNA expression of UCP1 and PDK4. It also enhanced the expression of PGC-1a suggesting an increased mitochondrial biogenesis. Taken together, all these results suggest the potential anti-obesity effect of the bioactive components present in the strawberry extract.

Keywords: Strawberry; Adipogenesis; Browning; Oxidative stress.

POSTERS



Proposed section: Phenolic agro-industries Proposed format: poster

Flavonoid composition and colour characterisation of wines from D.O.P. Sierra de Salamanca made from autochthonous Rufete red grapes

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In the present and future scenario of global climate change, the ability of adaptation demonstrated during centuries by autochthonous grape varieties has increased the interest on them in most of the growing regions. Furthermore, the typicity that autochthonous grape varieties can bring to the wines can fulfil the consumers' demand of new types of wine. In addition, local authorities are also betting on their re-emergence as a way to improve the development of rural regions. In Sierra de Francia region (Salamanca, Spain) the D.O.P. "Sierra de Salamanca" has been recognised from 2010. Among the varieties approved for this D.O.P., Rufete (Vitis vinífera L.), an autochthonous red grape variety, is probably its flagship. Previous studies on the flavonoid composition of Rufete grapes [1, 2] have revealed the potential of this variety to make quality red wines. The fact that nowadays all of the wineries belonging to the D.O.P. are marketing 100% Rufete wines brings the opportunity to study different features of a huge array of 100% Rufete wines, made at different cellars with different types of ageing and, in addition, in different vintages (from 2014 to present). To be precise, the present work has studied the CIELAB colour parameters, flavanol, flavonol and anthocyanin compositions in order to establish the features common to all of the samples that might be defining the typicity of Rufete wines from a phenolic point of view. Furthermore, the present study has also evaluated how the presence of Tempranillo grapes during winemaking can modify the "typical" flavonoid composition and colour features of Rufete wines. Wines were analysed by HPLC-DAD-MSⁿ (anthocyanins and flavonols) and by HPLC-MS-MRM (flavanols) [1, 2]. Some of the flavonoid markers that were observed in previous studies on Rufete grapes [1, 2] were still present in all the Rufete wines. For a same vintage, important differences could be observed among wineries, highlighting the relevance of the winemaking and ageing processes in the final composition of the wine. Furthermore, the availability of wine samples from 2014 to present has allowed the estimation of the evolution of flavonoid composition over ageing and subsequently, the evaluation of the suitability of Rufete wines to be marketed as aged wines.

Keywords: Vitis vinifera L. cv Rufete; Wine authenticity; Biochemical markers; Colour; Flavonoids.

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Proposed section: Phenolic agro-industries Proposed format: poster

Analysis of the relationships between the wine oxygen consumption kinetics and its chemical composition for commercial red, rose and white wines using Artificial Neural Networks

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Oxygen is a key factor in wine making, since it plays an important role in the different processes taking place in the winery [1]. Measuring the oxygen content during winemaking and bottle storage has become increasingly popular due to its impact on the sensory quality and longevity of wines. Nevertheless, only a few attempts to describe the kinetics of oxygen consumption based on the chemical composition of wines have been published [2].

The aim of this work was to analyse the relationships between the oxygen consumption kinetics of different wines (red, rose and white) and their basic chemical composition, color and antioxidant capacity, to study the influence of each one on the oxygen consumption capacity.

Thus, seventy-two young commercial wines from different Spanish appellations of origin and varieties, among red, rose and white, were used. To not modify the initial composition of the wines during sampling, the bottles were opened inside a glove chamber in the absence of oxygen. After that, and to study the oxygen consumption kinetics, wines were spargled with air up to saturation (100% air sat.) [1]. The oxygen concentration was monitored in *Sensorvials* up to consumption was considered ended, when wines were again analysed for the same parameters as the initial ones.

The results obtained revealed that red wines were able to consume oxygen faster, followed by white and rose ones. The total time needed to consume this oxygen was lower in red wines and slightly higher in whites and rosés ones, among which no differences were observed. Related to color, all wines presented an increment in the color intensity after oxygen consumption, having been found in the case of white wines the highest increase values, followed by red and rosé wines.

Keywords: Antioxidant capacity; Color; Oxygen consumption rate; White; Rose and red wines.

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Proposed section: Phenolic agro-industries Proposed format: poster

Evolution of phenolic compounds and color during wine aging with different micro-oxygenation systems and oak wood alternatives

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The use of oak barrels is the traditional technique for wine aging. Nowadays more and more wineries use alternative systems to the barrel based on the addition of pieces of wood (chips, staves...) together with the addition of oxygen either by direct injection, or by using tanks made of different materials permeable to oxygen. These systems attempt to reproduce the phenomena that occur in traditional barrel ageing, such as the transfer of wood compounds and the transfer of small doses of oxygen, in order to modify and improve the characteristics of the wine, thus obtaining a more complex product [1, 2].

In the present work, the same red wine made with the Tinta de Toro variety has been aged in 7 different ageing systems for 6 months in order to evaluate the phenolic and sensorial evolution of the wine in each system and establish the influence of the system used. Six passive micro-oxygenation systems using tanks made of HPDE, PDMS or stoneware, and one ageing system with active micro-oxygenation have been evaluated together with the barrel. In all cases, French oak with similar medium toasting has been used. In both the tradition albarrel and alternative systems using staves, wood from the same cooperage and from the same batch has been used.

It has been found that the way in which oxygen is incorporated into wine determines its evolution and final properties. The results indicate that the same wine aged in different systems, apparently formulated with the same amount of wood and oxygen, acquires a different phenolic and sensory profile. Wine aged in barrels maintains a better level of individualised anthocyanins, which has a direct effect on its colour.

Keywords: Phenolics; Wine; Barrel; Oxygen; Chips.

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Proposed section: Phenolic agro-industries Proposed format: poster

Vis spectroscopy and color markers with multivariate analysis of red wine during different oxidative maturation stages

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The oxygen that the wines receive during barrel-ageing is essential to ensure their correct maturation, determining the final properties of the aged wine. Wine color is one of the characteristics most influenced by oxygen during ageing, for this, visible spectroscopy technique combined with multivariate analysis is used to defined markers to correlate both, spectroscopic data and chromatic parameters with the ageing level.

For this reason, the aim of this work was to determine the vis spectroscopy and color markers of the same red wine during different oxidative maturation stages.

For this study, a young red wine made from a red single-variety grape (cv. Tinta del País) was aged for one-year in 8 French barrels (*Quercus petraea*) with a customized oxygen input: 4 barrels with high and 4 barrels with low oxygenation. Wines were sampled at different maturation stages of barrel ageing (3, 6, 9 and 12 months) and also, at the end of each previous period, a bottled simulation ageing were carried out [1]. Between barrel and bottled aging and to simulate the oxygen input that wines receive during the bottling process, a saturation process with air up to 100% of air sat. was carried out and the oxygen consumption kinetics were monitored [2]. When the oxygen was consumed, the bottling periods simulation started. In all wines (after different maturation stages, after oxygen consumption and after bottled simulation) the vis spectrum in the range of 350–780 nm was measured.

Related to the oxygen consumption, it was observed that wines saturated with air, consume oxygen following a curve defined by each wine parameters, which are related with the oxygen that wine receive during the maturation stage. The visible spectroscopy information indicates an increase in color intensity, once the maturation period evolves. At the end of oxygen consumption for each maturation stage, wines from high OTR barrels were those with the highest chromatic intensity. After bottled simulation periods, the differences observed among wines aged in the different barrels were lower. On the other hand the vis spectrum was significantly different depending on the bottle maturation time for both wines from high and low OTR barrels.

These results show the importance of the oxygen that wines receive during barrel aging, and the effect it has on the kinetics of oxygen consumption and therefore on the life of the wine.

Keywords: Barrel; Oxidative maturation stage; Red wine; Vis spectroscopy.

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Proposed section: Phenolic compounds circular economy Proposed format: poster

Bioactive chemical compounds in food matrices and by-products

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Bioactive compounds are phytochemicals that are found in plants, food matrices or by-products that are able to modulate metabolic processes, resulting in the promotion of better health. Includes an extremely heterogeneous class of compounds (polyphenolic compounds, carotenoids, tocopherols, phytosterols, and organosulfur compounds) with different chemical structures (hydrophilic or lipophilic), distribution in nature, range of concentrations, possible site of action, effectiveness against oxidative species, and specificity and biological action. These compounds exhibit beneficial effects such as antioxidant activity, inhibition or induction of enzymes, inhibition of receptor activities, and induction and inhibition of gene expression. Several factors interfere with the bioavailability of antioxidants, e.g., the food source or the chemical interactions among the phytochemicals and biomolecules.

Bioactive compounds are distinct from nutrients because they are not essential and, currently, are not recommended daily intake values. However, it is well established that a range of compounds from plant and animal sources has a positive influence on human health. There is insufficient evidence to recommend intakes, efficacy, and safety of these substances, especially as isolate supplements, but it is generally agreed that, consumed as part of a balanced diet, the benefits are significant.

Keywords: Bioactive compounds; Phytochemicals; By-products.

Acknowledgements: This work was supported by national funds from I&D project Interact - Integrative Research in Environment, Agro-Chains and Technology (NORTE-01-0145-FEDER-000017), regarding the research line "Fostering viticulture sustainability for Douro Valley: multidisciplinary efforts from field to wine (VitalityWINE)", co-founded by the European Regional Development Fund (FEDER) through NORTE-2020 (Programa Operacional Regional do Norte 2014/2020), FEDER-Interreg España-Portugal programme for financial support through the project 0377_lberphenol_6_E and from FCT – Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2019.



Proposed section: Phenolic compounds circular economy Proposed format: poster

White wine extract derived from polyvinylpyrrolidone residue reveals beneficial effects on Alzheimer's disease and type 2 diabetes interlink

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In recent years, winemaking by-products have had an increasing impact on the circular economy of the wine sector. Traditionally, polyvinylpolypyrrolidone (PVPP) is used to treat white wine on a preventive basis against adverse effects attributable to the polyphenolic oxidation and polymerization, generating a suitable resource of polyphenols for food and pharmaceutical applications. Here, we explored the bioactivity of a selective pool of polyphenols, obtained from the PVPP residue used to absorb Douro's white wine, in the context of Alzheimer's disease and type 2 diabetes interlink. HPLC-DAD analysis showed that the PVPP-white wine extract is a mixture of polyphenols dominated by flavonoids (e.g. catechins plus proanthocyanidins), hydroxycinnamic acids (e.g. caftaric and chlorogenic acids) and hydroxybenzoic acids (e.g. gallic and protocatechuic acids). These polyphenols account for the antioxidant activity of the wine extract, being related either to superoxide anion radical-scavenging activity (EC₅₀= 153.5 μ g/mL) or to protection of cell membranes from lipid oxidation induced by Fe²⁺/ascorbate system (EC₅₀= 119.0 µg/mL). Enzymatic studies indicated that the PVPP-white wine extract exhibits inhibitory activity on α -glucosidase and aldose reductase. For both enzymes, the wine extract acts by a non-competitive mechanism, K_i values being similar or lower to the K_m values (aglucosidase: Ki= 166.9 μg/mL; Km= 172.2 μM. Aldose reductase: Ki= 127.5 μg/mL; Km=137.0 μM), suggesting potential to regulate postprandial blood glycaemic levels with benefits on type 2 diabetesrelated complications. Regarding the Alzheimer's disease framework, the PVPP-white wine extract exhibited only a modest potential to modulate the brain cholinergic networks, as revealed by its inhibitory activity on rat brain acetylcholinesterase. However, PVPP-white wine extract, in the concentration range up to 58.4 µg/mL, was able to protect the neuronal SH-SY5Y cells from glutamateinduced toxicity. These neuroprotective effects are related with its ability to modulate the cell redox state, as detected by an increase of the reduced/oxidized glutathione ratio, and to decrease cell's generation of reactive oxygen species detected by DCFH-DA probe. Thus, it was reckoned, for the first time, that this winery by-product can provide viable beneficial effects on the prevention of both Alzheimer's disease and type 2 diabetes, being a promising value-added tool for nutritional and/or therapeutic approaches.

Keywords: Wine polyphenols; Antioxidant activity; Neuroprotection; Age-related diseases.

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Proposed section: Phenolic compounds circular economy Proposed format: poster

Microalgae as raw material for the production of pigments

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Microalgae have become one of the most promising sources of bioactive compounds, so microalgae have a wide spectrum of commercial applications in cosmetics, nutraceuticals and healthcare sectors. Moreover, today people demand natural products and reject chemical additives, so find new natural pigments that have no adverse effects is important. Algae could be a possible solution due to its composition. They have carotenoids, including isoprenoids which is interesting due to it is a precursor molecule for vitamin A biosynthesis in the human body (Khanra et al., 2018; Rajesh et al., 2017). They also have astaxanthin, which is interesting because it has nutraceutical value and pharmaceutical applications. Other commercial pigment are zeaxanthin, lutein, and lycopene (Rajesh et al., 2017). Among chlorophyll, the most common type is chlorophyll a, but they may have also chlorophyll b and c (D'Alessandro & Antoniosi Filho, 2016). As for phycobilin, it is useful because their absorption properties and because they can act as fluorescent markers (D'Alessandro & Antoniosi Filho, 2016).

Keywords: Pigments; Microalgae; Carotenoids; Chlorophyll; Phycobilins.

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Proposed section: Phenolic compounds and taste Proposed format: oral or poster: poster

Influence of Grape Skin Soluble Polysaccharides Addition on the Polyphenolic Composition and Sensory Properties of Red Wines

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Phenolic maturity and sugar maturity are two important indexes for enologists since they will further influence wine quality. In the past decades, the delay between these two kind of maturity has increased because of global climate change, which leads to wines with poor guality (too astringent and poor color) due to the inadequate phenolic composition of the grapes at harvest time [1]. Several strategies have been proposed to solve this problem and, among them, the addition of polysaccharides. Polysaccharides from grape skin are able to interact with polyphenols through hydrophobic and hydrogen bonds [2]. Moreover, it has also been reported that polysaccharides from grape skin cell wall materials (CWM) could absorb phenolic compounds, thus influencing wine sensory properties [3]. However, the addition of insoluble CWM into wines may also cause some loss of pigments and wine volume [4, 5]. So far, studies about the effect of soluble polysaccharides from grape skins on wine sensory properties are scarce. For this reason, in this study, the effect of the addition of soluble polysaccharides extracted from white/red grape skins on the phenolic composition and on astringency and color properties of red wines has been assessed. Results suggest that polysaccharides from white grape skins interacted with flavanols faster and protected flavanols longer than polysaccharides from red grape skins, the latter showed ability to decrease flavanols (especially procyanidin oligomers) after two-month preservation. All polysaccharides exhibited good potential in protecting anthocyanins (especially monoglucosidic anthocyanins) in wines for long-term preservation. Polysaccharides from white grape skins only decreased wine astringency during the earlier preservation (one-week and threeweek preservation), whereas polysaccharides from red grape skins showed persistant effect on decreasing astringency. Polysaccharides addition didn't induce significant color change during preservation. This research reveals that soluble polysaccharides are potential additives in modulating wine sensory.

Keywords: Wine, Polyphenols, Polysaccharides, Astringency, Color.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: poster

Phytochemical profile, mineral content and antioxidant capacity of garlic (*Allium sativam*) and black cumin (*Nigella sativa*) extract.

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Phytochemicals are chemical compounds often referred to as secondary metabolites and dietary antioxidants, such as polyphenols, seem to exert a beneficial role in improving the human antioxidant defenses against chronic diseases. Health benefits of garlic and black cumin depend on the content of biologically active compounds. To determine the phytochemical content, mineral composition and antioxidant capacity of alcoholic extract of the dried garlic clove and black cumin seed powder, UV and HPLC-DAD-ESI/MS analysis was carried out. Alcoholic extract of dried black cumin seed and garlic clove powder showed the presence of phenolic compounds, flavonols, and phenolic acids, proteins, amino acids and vitamin C that were identified and guantified by HPLC-DAD-ESI/MS. Furthermore, tocopherols, fixed oil and saponin in dried black cumin seed and organosulfur profile in dried garlic clove were also identified and quantified. The identification of phytochemical compounds was based on the peak area, retention time-molecular weight and molecular formula. After that, we evaluated total phenolic content and total flavonoid content (measured using spectrophotometric methods), and total antioxidant capacity (measured using Ferric Reducing Antioxidant Power, Trolox Equivalent Antioxidant Capacity and the DPPH assay). Our findings suggest that alcoholic extracts of the dried garlic clove and black cumin seed powder contain important phytochemicals, proximate and mineral compounds. Therefore, we performed the in vitro studies on MFC7 breast cancer cells to assess the cell viability by treating cells with different concentrations (0-15 mg/ml) of alcoholic extracts of garlic and black cumin for different incubation times (28hr, 48hr, and 72 hr). The result showed that cell viability decreased with increasing concentration of alcoholic extract of garlic and black cumin with incubation time after 48 hrs.

Keywords: Garlic; Cumin; Polyphenols; MFC7 cells.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: poster

The involvement of phenolic-rich fractions from apple against αglucosidase enzyme inhibition

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Interesting evidences suggest that inhibitors of the α-glucosidase enzyme might delay the digestion rate of carbohydrates, resulting in less effective absorption of glucose, and, therefore, decreasing postprandial hyperglycemia. Phlorizin and phloretin, belonging to apple dihydrochalcones, have been described as inhibitors of glucose transporters and carbohydrate digestive enzymes, among others [1, 2]. Nevertheless, few investigations taking into account food matrix effect have been established.

The present study was designed firstly, to characterize the phenolic profile of a local variety of apples by LC-HRMS (LTQ Orbitrap XL) for subsequent isolation and quantification of dihydrochalcone-rich fractions through LC-preparative and LC-MS/MS (QqQ); secondly, to evaluate the antidiabetic potential of whole apple extracts, dihydrochalcone-rich fractions and pure dihydrochalcones by cell-free models against α -glucosidase enzyme inhibition.

Flavan-3-ols, flavonols, dihydrochalcones, phenolic acids and anthocyanins were quantified in the selected samples. Phlorizin was detected as the main compound of this class through the following trend leaf > peel > flesh. Strongest inhibition against α -glucosidase (IC₅₀ = 217 µg of dry extract/mL) was observed for extracts obtained from apple compared to acarbose (IC₅₀ = 323 µg/mL), commercially available inhibitor; peel extracts showed a moderate inhibition (IC₅₀ = 726 µg of dry extract/mL) while a slight inhibition against this enzyme by dihydrochalcone rich fractions was determined.

Our findings suggest that apples and its by-products are a valuable source of bioactive compounds, as potential candidates in developing medicinal preparations and nutraceutical or functional foods for diabetes mellitus management.

Keywords: α-Glucosidase; Diabetes mellitus; Dihydrochalcones; LC-HRMS; LC-MS/MS.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: poster

Phenolic composition and *in vitro* antioxidant activity of common beans using MIR and NIR spectroscopy

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The relevant nutritional composition and potential health benefits of *Phaseolus vulgaris* L. are attributed to its composition, representing an excellent source of protein, dietary fibre and carbohydrates, besides phytochemicals, namely, polyphenolic compounds [1]. The aim of this study was to evaluate the performance of analytical models developed with both mid-infrared (MIR) and near-infrared (NIR) spectral data, to assess the phytochemical composition and *in vitro* antioxidant activity, besides individual phenolic compounds determined by HPLC-DAD, of flours from common beans. Partial least squares (PLS) regression was used to develop the analytical models, which were validated with an external set of samples. In MIR, the best prediction models were developed using the first derivative of the spectra (R^2c 0.86-0.99 and R^2v 0.75-0.91), while for NIR, the use of first derivative after normalization led to the best results (R^2c 0.94-0.99 and R^2v 0.83-0.97).

Therefore, this study revealed that the spectroscopical methodologies may represent an accurate and rapid method for quantification of phytochemical composition, *in vitro* antioxidant activity, and individual phenolic compounds, of bean flours, thus, their applicability in the food industry representing an alternative to the traditional approaches.

Keywords: Phaseolus vulgaris L.; Phytochemicals; HPLC; Near-infrared; Mid-infrared.

References:

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Proposed section: Phenolic compounds and bioactive properties Proposed format: poster

Phenolic compounds extraction from Algae: Properties and Bioactivities

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Nowadays, consumers, as rulers of how the industries develop products, and aware of the damages that can be caused by the excessive consumption of synthetic additives, show their preferences for those products that contain natural ingredients. Therefore, the industry adapts to these requirements and looks for alternative sources from which to obtain novel and natural ingredients. Undoubtedly, algae represent an attractive option since many of them present in their composition metabolites that can show antioxidant, antitumor, antimicrobial and neuroprotective properties, among others [1], such as phenolic compounds. Likewise, extractive techniques continue to be developed, so that a comparison between traditional techniques like ultrasound, microwave or heat-assisted extractions [2], and emerging techniques such as supercritical fluid extraction, or extraction at high pressures, can suppose a better understanding of the advantages of these innovative techniques to get the best out of them [3]. Therefore, the objective of this review is to summarize the information available about the content presented by algae in phenolic compounds; the most effective extractive techniques to obtain said compounds; the current systems of identification, guantification, purification [4] and stabilization of such compounds, and the bioactivities that have been described on this type of secondary metabolites, so that an overview of the situation of the phenolic compounds is offered in order to determine if it is possible and/or convenient its orientation towards an industrial application.

Keywords: Algae; Phenolic compounds; Extractions; Bioactivities; Phenolic compounds applications.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: poster

Fucoxanthin Extraction from Algae - Properties and Bioactivities

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The aim of this study was to review the bibliography related to the fucoxanthin molecule that can be obtain from Algae matrices, especially from brown ones, also known as *Phaeophyceae*. Algae are a great vegetative resource that has been characterized showing a high content in proteins, carbohydrates and minerals, but more importantly, in very diverse secondary metabolites like phenolic compounds and carotenes, making them a very interesting natural source for new ingredients. Being one of them fucoxanthin, one of the major carotenoids found in algae. Its structure includes an allenic bond and functional carbonyl, hydroxyl and carboxyl moieties that can be responsible for fucoxanthin's diverse properties [1].

Fucoxanthin has gather attention lately due to its strong bioactive proprieties [2]. These results imply that fucoxanthin has great potential to be included in a nutritional supplement for prevention and treatment of obesity and a wide variety of related pathologies [3].

It is also important to verify the stability of the molecule as well as its bioavailability and toxicity in the human body, so that it can be administered in the best possible way to obtain the highest results, as it appears to have in the *in vitro* and *in vivo* models studied [4]. As such, this study intends to gather several information about the various types of extractions known to obtain fucoxanthin, as well as the various bioactivities previously studied, in order to facilitate future studies of this topic.

Keywords: Fucoxanthin; Carotenoid; Extractions; Algae; Bioactivities.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: poster

Guinea-bissau flora as source of new anti-inflammatory natural products

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Anti-inflammatory properties of plants from Guinea-Bissau are widely intended by folk medicine in the treatment of several health problems associated with inflammatory conditions and pain [1, 2]. In this context, we have reasons to consider that some of these plants may have a real therapeutic potential and deserve to be studied towards scientific validation of their activity.

The aim of this work is the screening of a group of plants known for their anti-inflammatory applications in folk medicine, in order to select the worthiest to be the targets of deeper study. To do this, hydroethanolic (50:50) extracts obtained from leaves, stem barks and fruits of sixteen different species were tested by cellular assays in RAW 264.7 murine macrophages regarding to their impact on viability and ability to inhibit nitric oxide production. Based on the results, four extracts were selected to be tested in human THP-1 derived macrophages, through the evaluation of their ability to inhibit the production of two crucial pro-inflammatory cytokines, namely TNF- α and IL-6. An enzyme-linked immunosorbent assay (ELISA) was performed to measure the levels of the cytokines on the supernatant of the cellular culture after lipopolysaccharide (LPS) stimulation. The selected extracts were prepared from the stem barks of *Parinariexcelsa* and *Cassia sieberiana* and from the leaves of *Xylopiaaethiopica* and *Neocarya macrophylla*.

The results allowed highlighting *Xylopiaaethiopica* as a promising anti-inflammatory species due to the almost total inhibition of both tested cytokinesby its leaves extract. A chemical profiling through HPLC-DAD in order to access the phenolic composition of the leaves extract of this species was then performed to try to establish an association between its bioactivity and chemical composition.

Further works will provide insights on the anti-inflammatory mechanisms of this and of the other promising species.

Keywords: Anti-inflammatory; Guinea-Bissau Xylopiaaethiopica; THP-1; HPLC-DAD.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: poster

Antifungal activity and cytotoxicity evaluation of phenolic compounds extracted from *Eucalyptus globulus* biomass

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Eucalyptus globulus is very used as herbal tea and for therapeutic purposes. In fact, there is an increasing interest in its application as a natural additive in food and pharmaceutical industry. This study was designed to evaluate the antifungal activity of phenolic extract obtained from *Eucalyptus globulus* biomass. Additionally, the cytotoxicity *in vitro* was also evaluated in several cell lines. The chemical composition of the phenolic compounds was assessed by HPLC-DAD-MSⁿ. For the antifungal activity, the minimal inhibitory and minimal lethal concentrations (MICs and MLCs) were determined against several pathogenic fungi [1]. The cytotoxicity was evaluated by the MTT assay [2]. The main constituents of phenolic extract are two phenolic acids. The phenolic extract showed antifungal activity against some dermatophytes. The non-toxic concentrations of phenolic extract in neuronal cells, keratinocytes, lung cells, macrophages and fibroblasts were disclosed. This study highlights non-toxic concentrations of phenolic extract obtained from Eucalyptus globulus biomass that can be more extensively investigated for their bioactivity and future application in pharmaceutical and/or nutraceutical industry.

Keywords: Eucalyptus globulus; Phenolic compounds; Antifungal activity; Cytotoxicity.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: poster

Pyrrolizidine alkaloids and polyphenols composition of *Echium plantagineum* L. honey: from food security to health benefits

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Since ancient times, honey has been used not only as a functional food, but also in traditional medicine worldwide. Several evidences of its health-promoting properties have render honey a subject of growing research interest, being antioxidant, anti-hypertensive, antibacterial, antifungal and anti-inflammatory activities reported [1]. The bioactivity is linked to its phenolic compounds. However, molecules with potentially harmful effects may also be present, such as pyrrolizidine alkaloids. These secondary metabolites are distributed in plants throughout the world, frequently in species relevant for human and animal consumption, resulting in several outbreaks and livestock losses, particularly in developing countries [2]. This work aimed to evaluate the security and quality of monofloral and multifloral Portuguese honeys produced from Echium plantagineum L., a species known for its content in pyrrolizidine alkaloids. The botanical origin was ascertained by palynological analysis. Chromatographic techniques were used in the search of both alkaloids and polyphenols. Alkaloid- and phenolic-targeted extracts were also assessed in cellular models for their anti-inflammatory activity (murine macrophagelike cell line RAW 264.7), cytotoxicity (adenocarcinoma gastric cell line AGS) and safety (human foetal lung fibroblasts cell line MRC-5). Several phenolic compounds were determined in the studied honey samples, none of them showing the presence of pyrrolizidine alkaloids. The honey extracts decreased the 'NO production by LPS-stimulated RAW 264.7 up to 40%. In addition, the evaluation of the interference of the extracts in the mitochondrial function of cancer and non-cancer cell lines revealed that, despite their slight activity, they present selective cytotoxicity towards cancer cells. This study reveals the safety profile of the honey samples and highlights their beneficial effects on health.

Keywords: Honey; Pyrrolizidine alkaloids; Phenolic compounds; Anti-inflammatory potential.

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Proposed section: Phenolic compounds and bioactive properties Proposed format: poster

Chemical profile and biological activities of hydroethanolic extracts of Guinea-Bissau plants for new anticancer drugs

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The chemical complexity and diversity of natural products has empowered medicine with several molecules of demonstrated therapeutic efficacy and safety. Throughout the history of medicine, many of the drugs later joining the ranks of pharmacotherapy were firstly discovered based on the traditional use of their sources in folk medicine [1, 2].

In this work we explored the therapeutic potential of Guinea-Bissau's rich flora as a strategy to identify compounds that may ultimately lead to new anti-cancer drugs. After a preliminary cytotoxicity screening of 56 vegetal materials, concerning to the effect on the viability of human lung cancer (A549) and human gastric adenocarcinoma (AGS) cell lines, Parinari excelsa Sabine (stem bark), Allophylus africanus P.Beauv. (stem bark), Zanthoxylum leprieurii Hayata (stem bark), Erythrina senegalensis DC (stem bark), Xylopia aethiopica (Dunal) A.Rich. (fruit) and Neocarya macrophylla (Sabine) Steud. (leaves) were chosen. Generally, AGS cells appeared to be more susceptible to hydroethanolic extracts. The fruit of X. aethiopica was the most cytotoxic, with an IC_{50} value of $151\mu g/mL$, followed by the stem bark of Z. leprieurii and the leaves of N. macrophylla, with IC₅₀ values of 214 and 203µg/mL, respectively. P. excelsa showed ability to activate caspase-3 and -4, A. africanus activates caspase-4 and Z. leprieurii leads to the release of calcium of endoplasmatic reticulum and all plants with alterations in mitochondrial membrane potencial. Extracts obtained from these materials were characterized by HPLC-DAD. In N. macrophylla was identified 5-O-caffeoylquinic acid and derivatives of guercetin and in A. africanus vicenin-2 and derivatives as 2"-O-rhamnosyl vitexin and derivatives. As far as we know, this is the first study describing the effects of these extracts against human gastric adenocarcinoma cells and revealing their richness in phenolic compounds.

Keywords: Drug discovery; Phenolic compounds; Cancer; Guinea-Bissau.

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Proposed section: Phenolic compounds and health Proposed format: poster

Phenolic compounds of the principal medicinal plants of Galicia (NW Spain)

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Medicinal plants have been used since ancient time in traditional medicine due to its content of natural bioactive compounds with different activities. Bioactive compounds from plants are mainly secondary metabolites, being the phenolic compounds one of the most important. Several studies have reported the various beneficial effects of these compounds such antioxidants, anti-microbial, anti-cancer or anti-inflammatory. Its wide range of bioactivities leads to different industrial applications, such as cosmetics, nutrition, etc. Galicia is a region with a great history of using plants to treat several pains and diseases, for instance, gastrointestinal problems, burns, colds, as well as neurological disorders. Nowadays, old people are still using medicinal plants, preserving the ancient knowledge. Due to the interesting properties of these plants, numerous studies have been conducted to understand the chemical compounds responsible for these properties. Some medicinal plants as the common dandelion (*Taraxacum officinale*) or the broadleaf plantain (*Plantago major*) have been demonstrated to have interesting phenolic acids and flavonoids with different bioactivities. The aim of this work is to do a review about the different phenolic compounds, its bioactivities and the essays that have been made with them, from different Galician medicinal plants.

Keywords: Phenolic compounds; Medicinal plants; Bioactivities.

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[1] X. U. Cong-Cong, W. A. N. G. Bing, P. U. Yi-Qiong, T. A. O. Jian-Sheng, T. Zhang. (2017). Chin J Nat Med, 721-731. [2] M. Mosquera Paans. (2013). A nosa botica. Plantas medicinais, Edicións do Cumio, Pontevedra.

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Proposed section: Phenolic compounds and health Proposed format: poster

Preserving the ancient knowledge: a review

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Medicinal plants have been used since ancient time in traditional medicine due to its content of bioactive compounds with a wide range of beneficial health effects. These compounds are mainly secondary metabolites in plants, being the phenolic ones the key group of compounds, as it has been reported in the last decade in several studies due to their potential uses (antioxidant, anti-microbial, anti-cancer or anti-inflammatory, among others) that lead to different industrial applications, such as cosmetics, nutrition, etc. Galicia is a region at NW of Spain that traditionally has used plants as primary medical treatment to treat several pains and diseases for instance, gastrointestinal problems, burns, colds or even neurological disorders. The Galician region presents a great natural biodiversity and a complex geography. The rural areas are disperse and in many cases the newest medical advances do not reach all the territory. Due to the delay of the medicinal dissemination, this rural areas keep using medicinal plants, preserving ancient knowledge. This information is in serious danger of disappearance, as the old population is dying and the younger ones do not use medicinal plants. It is necessary to preserve the information of this medicinal plants, because it is a deep loss of knowledge, which took hundreds of years of trial and error. Although numerous studies have been conducted to understand the chemical compounds responsible for these properties, still need the continuous search and update of databases to avoid the loss of these valuable information. Some medicinal plants are common as the dandelion (Taraxacum officinale) or the broadleaf plantain (Plantago major) and it has been well documented to present a correlation between the concentration of phenolic acids and flavonoids with their different health effects in humans. The aim of this work is to help to understand the hidden link between the content in the different phenolic composition of typical medicinal plants in the NW region of Spain (Galicia) with traditional treatments that have been applied for centuries.

Keywords: Phenolic compounds; Medicinal plants; Bioactivities; Traditional applications.

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Acknowledgment: To Alga Mar for the algae material provided (<u>www.algamar.com</u>). To MICINN for the financial support for the Ramón & Cajal researcher of M.A. Prieto. To Xunta de Galicia and University of Vigo for the financial support for the pre-doctoral researchers of A.G. Pereira, Paula G. Oliveira and C. Jiménez López. To Xunta de Galicia for the financial support by the Axudas Conecta Peme of the Project IN852A 2018/58 Neuro Food. The authors are also grateful to the Interreg España- Portugal for financial support through the project 0377_lberphenol_6_E.

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Proposed section: Phenolic compounds and health Proposed format: poster

The effects of chestnut consumption on morphometric parameters in FVB/n mice

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Chestnut (*Castanea sativa*, Miller) production is mostly concentrated in Europe and Asia. It is highly appreciated in autumn, especially in the Mediterranean region, and can be consumed raw or cooked and usedin several dishes and desserts [1]. Chestnut has a high content of antioxidants, it is rich in vitamins and carbohydrates and it is also good source of minerals and essential fatty acids [2].

The objective of this work was to evaluate the effects of chestnut consumption on mouse's morphometric parameters. Animals were kept according to European Guidelines (approval by UTAD's Ethics Committee (10/2013) and the Direção Geral de Alimentação e Veterinária (authorization number 0421/000/000/2014). Eighteen FVB/n male mice with 7 months of age were randomly divided into three experimental groups (n=6): group 1 (control), fed a normal diet without chestnut supplementation; group 2, fed a diet supplemented with 0.55% of chestnuts; and group 3, fed with a supplementation of 1.1% of chestnuts. The incorporation was based on the daily mean human chestnut consumption and double that amount. Body weight, water and food intake were recorded weekly throughout 35 days. After 35 days, all animals were sacrificed by anesthetic overdose, followed by an intracardiac puncture for blood collection and determination of microhematocrit, biochemical parameters and comet assay. Abdominal and perirenal adipose tissue was collected for weight determination. The mean body mass variation was identical between groups (p>0.005) during the experimental assay, as well as the values of food and water consumption. The results showed that chestnut reduces abdominal and perirenal adipose tissue accumulation (p>0.005). The values of microhematocrit, liver (aspartate aminotransferase, alanine aminotransferase, cholesterol) and renal (creatinine) parameters were similar between groups (p>0.005). The recorded data concerning comet assay evaluation suggests that chestnut may influence DNA damage reduction. According to our results the consumption of chestnuts had no negative impact on the animals' health and well-being.

Keywords: Chestnut; Supplementation; Liver parameters; Rodents.

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