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This issue contains the abstracts of the scientific presentations of the XXXI International Conference on Polyphenols, held in Nantes, France, 3-6 July, 2023.

Scope of the issue: Structure, reactivity & synthesis; Bioactivity, bioavailability & microbiota; Metabolomics, targeted analysis & big data; Processing, sensory properties, safety & regulatory; Biogenesis and functions in plants & ecosystems, and Biomaterials, green chemistry & circular bioeconomy.

Cover picture: The Elephant (Les Machines de l'Ile, Nantes, France), Castle of "Les Ducs de Bretagne", Nantes, France, and including the logo for the XXXI International Conference on Polyphenols.

Editorial

The Groupe Polyphénols

Groupe Polyphénols was founded in 1972 by Dr. Michel Bourzeix as an international scientific association. The objective of the group is to stimulate exchanges and collaborations between polyphenol scientists both from public laboratories and industry and working in all fields of polyphenols sciences.

https://www.groupepolyphenols.com/

The International Conferences on Polyphenols (ICPs)

A second objective is to organize, on a biannual basis, the International Conference on polyphenols (ICP).

The XXXI edition of ICP took place from 3 to 6 July 2023 in Nantes, France. It was a successfull event attracting some 340 attendees from 40 nationalities. The scientific programme included 14 plenary lectures, 87 oral communications and 175 posters. This year, the Groupe Polyphénol award was attributed to Prof. Cathie Martin (John Innes Centre, Norwich, UK) and Dr. Leonard Blaschek (Copenhagen University, Denmark) received the sixth Ragai Ibrahim prize. The next ICP conference will take place in Turku, Finland, 2025.

The publications of the Groupe Polyphénols

The scientific papers related to topics of ICP conferences are published in 'Polyphenols Communications' online and free of charge. The current electronic issue is the second of the series compiling the abstracts of the ICP2023 conference.

The Groupe Polyphénols has also edited the "Recent Advances in Polyphenols Research" (RAPR) series of books, published by Wiley. This series gathers a collection of chapters written by the best scientific experts in all the fields of polyphenol sciences, including chemistry, biogenesis, bioactivity, metabolomics, sensory, safety, and regulatory aspects, just to mention a few. The publication of RAPR Volume 8, which is related to the ICP2021 Turku (online conference only), has been released this year. All RAPR Volumes are now available to all ICP participants at 50% discount (see next page).

Finally, a Groupe Polyphenols & ICP2023 Special Issue of the Journal of Agricultural and Food Chemistry will be published after the ICP2023 Nantes conference. This issue will bring together the peer-reviewed articles of the opening lecture of the ICP, of all the plenary lectures including that of the winner of the Group Polyphénols Scientific Prize, and of a selection of oral communications. A number of other scientists active in prominent topics of polyphenols research will be invited to contribute to this Special Issue as well.

Becoming a member of the Groupe Polyphénols

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THE IMPORTANCE OF COLOUR TO POLYPHENOL RESEARCH

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Anthocyanins are the most broadly distributed pigments in nature, important for colouring flowers orange through to blue and near black for pollinator attraction, for colouring aging leaves to protect their cellular constituents from photooxidative damage, and for the health benefits they confer in brightly coloured fruits and vegetables. Their colours are the delight of gardeners and horticultural breeders who often use 'phenotypically unstable' accessions to breed new colour variants. It was these unstable variants that first caught my interest for this specific group of polyphenols. I started my research career at the John Innes Centre working on phenotypically unstable accessions of the garden snapdragon, *Antirrhinum majus*. It became clear early on that the unstable phenotype *pallida*^{recurrens} was caused by insertion of a transposable element which gave flowers an ivory background colour. Somatic excision of the transposable element caused a reversion of the phenotype to the wild type, red colour and the classic sectored appearance. The presence of the transposable element in the *pallida* locus allowed us to identify the *pallida* gene by transposon tagging and to show that it encoded dihydroflavonol 4-reductase (DFR) a key enzyme involved in anthocyanin biosynthesis. Significantly we were then able to characterise a number of mutant alleles caused by imprecise transposon excision. This showed that there were key regions in the gene sequences upstream of the *DFR* gene that controlled expression and production of DFR enzyme. This important allelic series defined at the level of DNA how the gene was regulated and the sequences recognised by regulatory proteins.

Right Wild type flower of *A. majus*, Left *pal* ^{rec} flower of *A. majus*

We were also able to generate an allelic series of promoter mutations in the *Nivea* gene encoding chalcone synthase showing similar regulatory properties. This established that the genes encoding enzymes in anthocyanin biosynthesis were under the control of common regulatory factors that induced anthocyanin production in specific patterns and in response to environmental stresses. The visual impact of the different regulatory mutants produced by imprecise transposon excision provided a clear example of how other unstable accessions (for example in corn, camellia and azalea) have been used originally by Mayan cultures and later by horticulturalists and breeders to develop new varieties.

Our pursuit of the regulators of anthocyanin biosynthesis allowed us to establish regulation as an important component of biosynthesis, a contribution beyond traditional biochemistry. The importance of regulation in determining flower colour and patterning was established by genetic comparisons of different species of Antirrhinum and studies of pollinator preferences for different morphs, available in diversity collections.

This importance was reinforced when we were able to demonstrate accumulation of anthocyanins specifically in tomato fruit under the control of two transcription factors from *A. majus*. These purple tomatoes also showed health benefits when assayed in animals and helped to establish 'isogenic foods' as the best way to determine beneficial effects in intervention studies. Despite approval taking over 15 years the purple tomatoes have been 'deregulated' by UDSA and approved for human consumption by FDA in the USA where they are gathering considerable interest from consumers.

				Ternatin								
	Spirulina	Blue No. 1	Indigo Carmine	A1	A2	A3	B1	B2	B3	B4	D1	D2
L*	74.95	86.50	72.20	70.60	71.49	67.37	69.85	65.37	71.13	66.97	71.80	69.28
a*	-29.92	-32.14	-24.40	-19.99	-22.27	-14.76	-21.02	-20.21	-22.23	-15.11	-21.29	-21.04
b*	-33.93	-17.21	-34.06	-32.36	-28.28	-31.21	-29.40	-20.66	-27.88	-29.67	-32.23	-26.29
h°	228.6	208.2	234.4	238.3	231.8	244.7	234.4	225.6	231.42	243.0	236.6	231.3
C*	45.24	36.46	41.90	38.04	36.00	34.53	36.14	28.90	35.66	33.30	38.63	33.67
Colour												

Using the same regulatory system to induce anthocyanin biosynthesis, we developed suspension cultures that produce highly purified specialised and novel anthocyanins for use in medical research. These cultures were amenable to scale up production. We also initiated research on identification and characterisation of anthocyanin acyl transferases which add side chain decorations to anthocyanins. In combination with specialised glycosyl transferases we have established the role of aromatic acylation in the formation of anthocyanic vacuolar inclusions (AVIs). AVIs are produced in species such as lisianthus to give darker regions in the centre of the tepals, a feature believed to make the flowers more attractive to pollinators.

Their formation occurs when aromatically acylated anthocyanins aggregate although aggregation is reduced by selective glycosylation. AVI formation is associated with a bluing of the pigments and in search of natural blues which could be developed to replace synthetic blue colourants we have investigated the ternatins from butterfly pea. These anthocyanins are particularly stable at room temperature due to their extensive aromatic acylation and form liquid blues at neutral pH, a feature that makes them outstanding candidates for natural blue food colourants. We are currently investigating whether increasing the contribution from specific ternatins can improve the functionality of extracts as colourants.

Finally, following the recent deregulation of the purple tomatoes in the USA we have developed fresh purple tomato snacking varieties which are proving popular with consumers in the USA.

THE WINE CASE: AN ILLUSTRATION OF POLYPHENOL RESEARCH DRIVEN BY ANALYTICAL CHEMISTRY

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One of the pioneers in polyphenol chemistry, Edwin Haslam, started his testimony paper, published in 2007, with the following sentence: « Progress in scientific research hinges on the continual discovery of new methods and techniques ». This will be illustrated using grape and wine phenolics as case study.

Indeed, phenolic compounds are essential for wine quality, responsible for the colour of red and rosé wines but also for browning of white wines and contributing to taste and mouthfeel properties, Although major grape phenolic compounds, *i.e.* anthocyanins, hydroxycinnamoyltartrates and proanthocyanidins, have been described over 50 years ago, developments in analytical chemistry have enabled much progress in the characterization of their structures and reactions during wine-making and in the understanding of structure-property relationships. Owing to HPLC coupled with diode array detection and mass spectrometry (HPLC-DAD-MS), numerous reactions products have been detected in wines or model solutions. Some of their structures have been identified and their formation process and properties have been established. However, these known compounds represent only the emerged part of the wine « polyphenol iceberg ». More recently, metabolomics strategies inspired from petrolomics have demonstrated that the complex phenolic composition of red wines results from random cascade reactions involving grape phenolics but also other wine constituents such as yeast metabolites [1].

Information on the composition of these complex mixtures can be obtained using a combination of methods, including UV-visible spectrophotometry, size exclusion chromatography, HPLC-DAD-MS analysis before and after acid-catalysed depolymerisation in the presence of a nucleophile, and NMR but their characterization is still an analytical challenge.

Untargeted metabolomics using high resolution mass spectrometry and/or NMR associated with chemometrics helps unravel links between phenolic profiles, processes and quality traits if applied to large enough and well characterized sample sets. However, additional strategies are needed for data analysis and in particular annotation of HRMS signals, because of the large number of isomers potentially showing different properties. Trapped ion mobility spectrometry (TIMS) provides a new dimension to characterize and monitor isomers [2] while molecular networking established on the basis of MS/MS spectra similarities brings further insights on structural features [3]. Finally, new approaches considering groups of compounds sharing specific reactivities and properties and competing reaction pathways governed by matrix composition and process conditions need to be developed.

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TOPIC 1 - STRUCTURE, REACTIVITY & SYNTHESIS

PL.1.01 - TOTAL SYNTHESIS OF NATURAL GLYCOSIDES BY A CATALYTIC SITE-SELECTIVE ACYLATION STRATEGY

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We have developed organocatalysts that enable to deliver acyl groups into the C(4)-OH of glucopyranosides site-selectively in the presence of the intrinsically more reactive free primary OH at the C(6) [1]. The salient feature of the catalysts was successfully applied to the total syntheses of several natural glycosides.

Multifidosides A-C [2]

At the final stage of the total synthesis, catalytic site-selective introduction of the *p*-coumaroyl group into the desired (4)-OH of the glucose core of the precursor glycoside with five free hydroxy groups has been achieved. The conventionally difficult molecular transformation could be performed in a predictable manner due to the functional group tolerance of the site-selective acylation promoted by the organocatalysts. An advantage of this strategy may involve avoiding the risks of undesired side reactions during the removal of the protecting groups at the final step of the total synthesis.

Strictinin, Tellimagrandin II, and Pterocarinin C (Ellagitannin family) [3,4]

Extremely short-step total synthesis of strictinin, tellimagrandin II, and pterocarinin C has been accomplished based on the unconventional retrosynthetic routes without using protective groups for starting glucose. The key reactions are the β-selective glycosidation of a gallic acid derivative using unprotected glucose as a glycosyl donor, and catalyst-controlled site-selective introduction of a galloyl group into the inherently less reactive hydroxy group in the resulting glucoside. The overall synthetic efficiency seems to be compatible with the biosynthetic pathway.

Punicafolin and Macaranganin (Ellagitannin family) [5]

The first total syntheses of punicafolin and macaranganin were achieved in 7 steps, respectively from naturally abundant D-glucose. The prominent features of the synthesis are i) sequential site-selective introduction of the adequate galloyl groups into the requisite hydroxy groups of D-glucose, and ii) stereodivergent construction of the 3,6-HHDP bridge from a common intermediate via the flipping process of the pyranose ring to the less stable axial-rich conformer. Because no protective groups were used for glucose throughout the process, extremely short step total syntheses were accomplished.

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PL.1.02 - OPPORTUNITIES AND CHALLENGES IN THE DEVELOPMENT OF ANTHOCYANINS AS NATURAL DYES

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Anthocyanins are polyphenolic O-glycosides widely responsible for the bright red, purple and blue colors in the plant kingdom, including a great variety of fruits and vegetables. As such, they have attracted considerable scientific and industrial interest as potential natural food colorings. However, individual anthocyanins are intrinsically reactive molecules combining electrophilic, nucleophilic and electron-donating properties [1]. This reactivity may be a source of color diversity with, for instance, the formation of new pigments upon winemaking and storage, but also a cause of great color instability involving a combination of reversible and irreversible mechanisms (e.g., water addition, autoxidation) leading to colorless products. Hence, using anthocyanin-rich plant extracts as food colorants requires a deep understanding of these color-damaging mechanisms and, no less importantly, of the color-stabilizing mechanisms developed by plants, including π-stacking interactions (self-association, copigmentation), metal binding and a combination of both. The potential of anthocyanins from deeply colored vegetables, typically acylated by hydroxycinnamic acid residues (Fig. 1), will be emphasized in that respect [2]. Moreover, food-grade biopolymers (proteins, polysaccharides) may provide suitable matrices for ready-to-use formulations of anthocyanins as food colorings.
Overall, the mechanisms of color loss and color stabilization will be discussed as a function of anthocyanin structure and environment, and some challenges still ahead will be outlined.

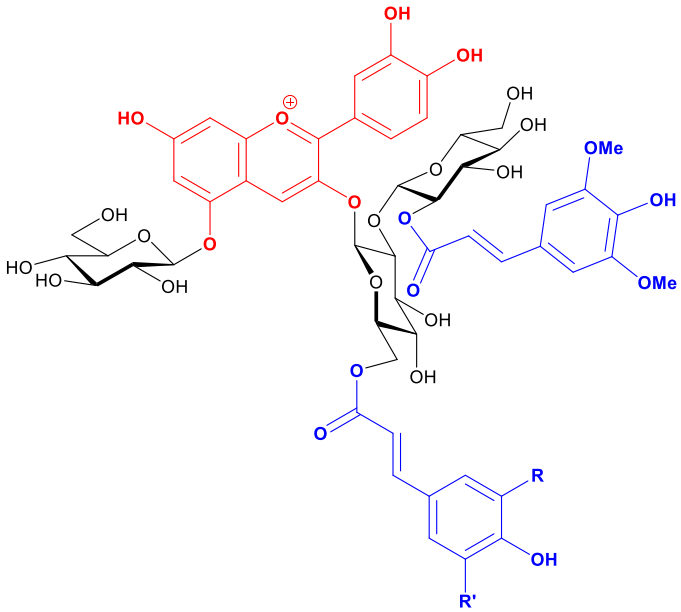

Figure 1: Red cabbage anthocyanins (R, R' = H, OMe)

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O.1.01 - ENTERING THE 4TH DIMENSION: ION MOBILITY SPECTROSCOPY IS THE NEW ANALYTICAL DIMENSION TO CHARACTERIZE AND MONITOR ISOMERIC POLYPHENOL DIMERS

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Dehydrodicatechins resulting from (epi)catechin oxidation have been investigated in different foods and natural products, but they still offer some analytical challenges due to their similar structures [1]. The purpose of this research was to develop a multidimensional method using ultra-high performance liquid chromatography coupled with trapped ion mobility spectrometry and tandem mass spectrometry (UHPLC−TIMS−QTOF−MS/MS) to improve the characterization of dehydrodicatechins first obtained in model solutions (oxidation dimers of (+)- catechin and/or (−)-epicatechin). Approximately 30 dehydrodicatechins were detected in the model solutions, including dehydrodicatechins B with β- and ε-interflavanic configurations and dehydrodicatechins A with γ-configuration. A total of 11 dehydrodicatechins B, based on (−)-epicatechin, (+)-catechin, or both, were tentatively identified in a grape seed extract. All of them were of β-configuration, except for one compound that was of ε-configuration. TIMS allowed the mobility separation of chromatographically coeluted isomers having very similar structures including dehydrodicatechins and procyanidins with similar MS/MS fragmentation patterns that cannot be distinguished by LC-MS/MS alone, which demonstrates the superiority of TIMS added to LC-MS/MS for these kinds of compounds. To the best of our knowledge, this is the first time that ion mobility spectrometry (IMS) was applied to the analysis of dehydrodicatechins. This method can be adapted for other natural products to improve structural characterization of these compounds.

Keywords: ion mobility spectrometry; flavan-3-ol oxidation products; isomeric polyphenol dimers; dehydrodicatechins ; grape seed extract

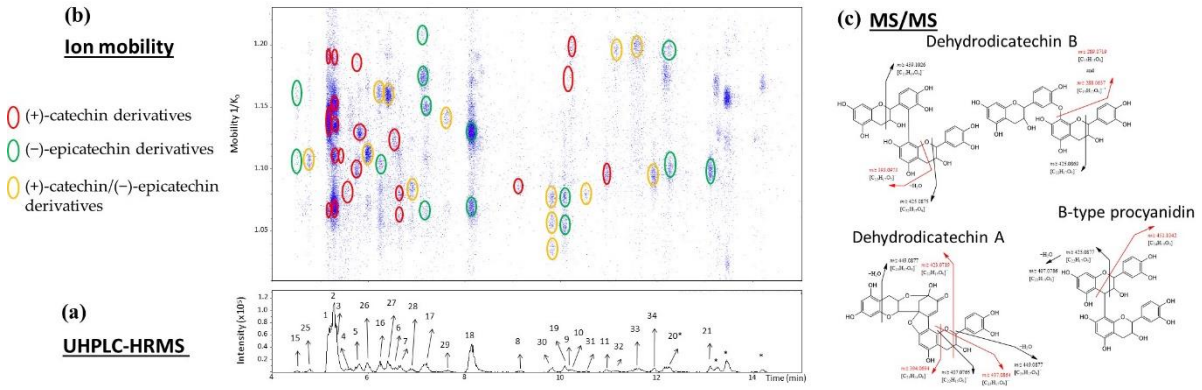

Figure 1: Multidimensional analysis of dehydrodi(epi)catechins : LC and HRMS (a), IMS (b) and MS/MS

[1] de Sousa Dias A.L., Verbaere A., Meudec E., Deshaies S., Saucier C., Cheynier V. & Sommerer N. *Molecules*, 27, 4176, https://doi.org/10.3390/molecules27134176, 2022.

O.1.02 - SYNTHESIS OF STILBENOID-BEARING AFFINITY PROBES FOR IDENTIFICATION OF TARGET PROTEINS OF RESVERATROL DERIVATIVES AND DIMERS

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Phytoalexins are a class of natural molecules produced by plants in response to external stress, including bacterial and fungal infections. Among phytoalexins, polyphenolic stilbenoids represent a widely studied group of bioactive compounds, notably known for their antibacterial and antifungal properties. For instance, monomeric stilbenoids such as resveratrol and piceatannol, as well as resveratrol dimers such as δ-, ε- and dehydro-δ-viniferins displayed relevant biological activity on foodborne pathogens [1]. However, very little is known about their mechanism of action, and their target proteins have not yet been identified.
The use of affinity-based probes constitutes a valuable tool for the identification of polyphenol-protein interactions [2]. We are currently developing two series of stilbenoid-bearing biotinylated probes to be employed for the capture and identification of target proteins in foodborne pathogens (*e.g.*, *L. monocytogenes*, *S. aureus*, *P. aeruginosa*) and in crop-threatening phytopathogenic fungi (*e.g.*, *P. oryzae* and *B. cinerea*) through chemoproteomic protocols we developed earlier [2] and bioinformatic analysis. Thus, we shall either use presynthesized biotinylated probes that will be incubated with microbial cell lysates for *in vitro* protein captures (Fig. 1a) or "clickable" alkynylated stilbenoids that will be introduced in living cells for *in cellulo* protein captures (Fig. 1b). In this latter case, after cell lysis, the alkynylated stilbenoid-tagged proteins will be combined with an azido-biotin conjugate through a copper-catalyzed alkyne-azide cycloaddition (CuAAC) [3] to enable their recovery by using a streptavidin-coated device and their identification by proteomic and bioinformatic analyses.

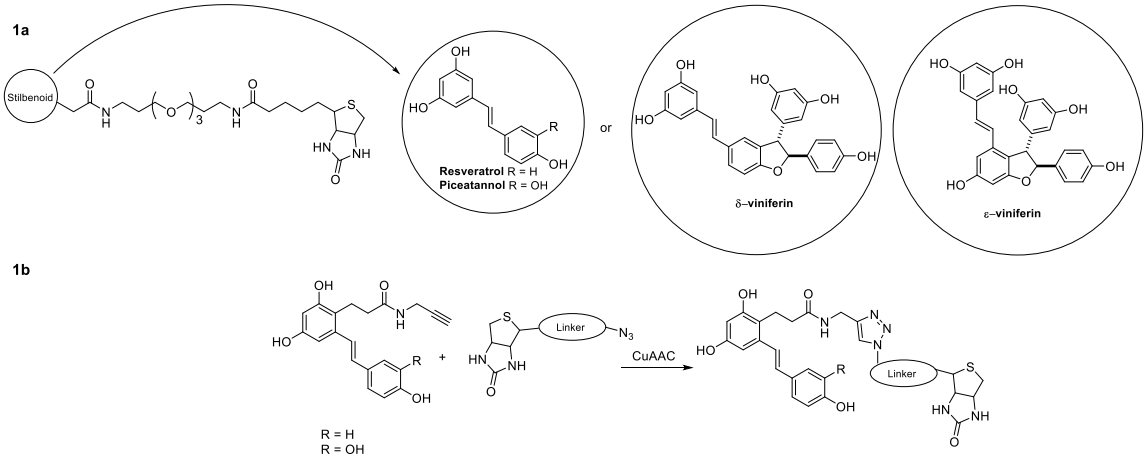

Figure 1: Structures of the proposed stilbenoid-bearing probes

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O.1.03 - WHAT DID WE LEARN THE LAST YEARS ABOUT THE SULFONATION OF THE PROANTHOCYANIDINS IN WINE

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The research outputs of the last - many - decades have established that sulfur dioxide (SO_2) and proanthocyanins, separately, are two key factors for understanding and managing wine quality. Nine years ago, it was discovered that under wine conditions these two can react producing several sulfonated monomeric and dimeric proanthocyanidins, and that such products are markers of storage at temperatures higher than the optimal [1]. Later we gained knowledge regarding the absolute conformation of the sulfonates' produced, with the C-4β addition being the favoured [2], and the concentration range of the major sulfonate products [3], namely epicatechin-4β-sulfonate and 4β-sulfo-procyanidin B2 (Figure 1). Crucial parameters for the better interpretation of any reaction are those referring to its kinetics. A study focused on the kinetics of the monomeric and dimeric flavan-3-ols sulfonation pointed out two mechanisms of reaction: a) the slowest one is the direct sulfonation of catechin or epicatechin, and b) the fastest process requires the acidic cleavage of the oligomeric and polymeric proanthocyanidins [4].
This work will give an update on the results of the last ten years regarding the sulfonation of tannins in wine.

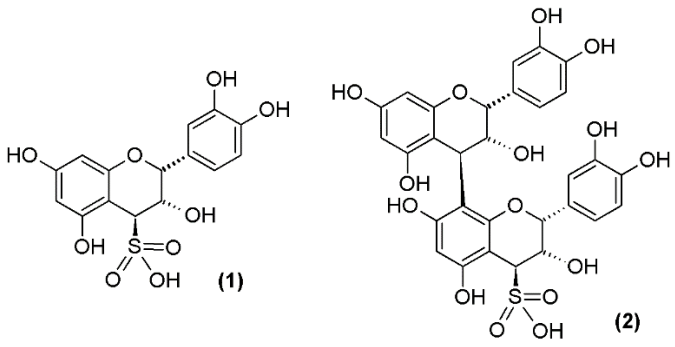

Figure 1: The structure of epicatechin-4β-sulfonate (1) and 4β-sulfoprocyanidin B2 (2)

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[3] Arapitsas P., Guella G. & Mattivi F. *Scientific Reports*, 8, 858, 2018.
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O.1.04 - MICRO- AND NANO-STRUCTURED ANTHOCYANINS – AN APPROACH TO INCREASE ANTHOCYANINS' PHYSICO-CHEMICAL STABIILITY AND BIOACCESSIBILITY

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Anthocyanins are water-soluble polyphenolic pigments, with a wide potential for industrial application as natural colorants and functional ingredients, due to their attractive colors (from bright red to purple and blue colors) and beneficial effects on human health [1]. However, anthocyanins are highly reactive molecules which causes great color instability and bioactivity loss, after extraction and transformation procedures. A possible biocompatible approach for stabilizing anthocyanins-rich plant extracts is through the micro- and nano-structuration, using natural and biopolymers such us polysaccharides as building blocks. Thus, in this work it was intended to develop anthocyanin-based polyelectrolyte nano- and micro-complexes, using λ-carrageenan (λ-Carg), a sulfated polysaccharide from seaweeds, as suitable formulations for anthocyanins industrial applications.

The molecular interaction mechanism between cyanidin-3-*O*-glucoside (model anthocyanin, Cy3Glc) and λ−carrageenan, evaluated by isothermal titration calorimetry (ITC) and ultraviolet–visible spectroscopy (UV-vis), showed the importance of electrostatic interactions in the formation of these complexes at slightly acidic pH, further stabilized by the occurrence of hydrogen bonds, and van der Waals dispersive interactions. The affinity of the interaction decreased with increasing pH value (K_a from 1.0×10^5 to 3.5×10^4 and 1.2×10^4, at pH 3.0, 3.5 and 4.0, respectively). The formation of Cy3Glc-λ-Carg complexes caused a hypsochromic and hyperchromic shifts in the Cy3Glc spectrum, suggesting the occurrence of electrostatic interactions between opposite charges, which resulted in alterations in the color of anthocyanin, from pink/red tones to more orange ones.

Complexes preparation at different ratios λ-Carg/Cy3Glc weight ratios led to the formation of soluble and insoluble complexes. At a fixed total reagents concentration, increasing the ratio resulted in a decrease in the size and surface charge (more negative). The characterization of the complexes by DLS showed that their sizes varied between 125 and 250 nm, with polydispersity indexes below 0.4 and with strongly negative surface charges, between -15 and -45 mV. The soluble complexes presented a spherical morphology (observed by cryo-SEM). In the presence of λ-Carg, the stability of anthocyanins with pH was improved (3.2 %, 6.1 %, 12.2 % and 24. 2 %, at pH 4.0, 5.0, 6.0 and 7.0, respectively), as well as their stability under *in vitro* simulated digestions (22.8%). Transport efficiency in intestinal barrier models (Caco-2), was also shown to be improved, contributing to anthocyanins enhanced bioavailability.

Acknowledgments : This work was funded by FCT through research contract (CEECIND/00029/2018/CP1545/CT0010) and research unit LAQV-REQUIMTE (UIDB/50006/2020), through national funds and cofinanced by FEDER, under the Partnership Agreement PT2020. The authors gratefully acknowledge to all entities.

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O.1.05 - DEVELOPMENT OF SULFUR/SELENIUM-CONTAINING ANTIOXIDANTS WITH MULTI-DEFENSE ACTIVITY

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The imitation of Nature's chemical principles and logics has emerged over the past decade as a major competitive strategy for the design and implementation of innovative molecular devices and systems for advanced biomedical applications. A unique source of inspiration and opportunities in this context is offered by phenols and polyphenols that are found in both plants and in animal tissue and exhibit a wide range of interesting properties, first of all as antioxidants thanks to their distinctive carbogenic diversity and the tunable redox behaviour and are exploited for a variety of applications.
Nowadays, molecular scaffold manipulation of natural phenols is a pursued approach to boost or modulate their properties. For example, the insertion of S/Se/Te containing substituents on phenols/polyphenols scaffold or the replacement of oxygen atoms with heavier chalcogens may increase/decrease their H-donor/acceptor ability by electronic and stereoelectronic effects related to the site of substitution and geometrical constrains [1].
In this frame, we report herein the preparation of sulfated derivatives of tyrosol (Tyr), one of the most representative phenolic constituents of extra virgin olive oil, and its oligomers (OligoTyr) by different approaches [2]. In particular, a mild sulfation procedure, based on the use of sulfur trioxide triethylamine complex, was developed and optimized firstly on the monomer with a view to derivatize only the alcoholic OH group of both Tyr and OligoTyr leaving the phenolic OH group, essential to the antioxidant activity, free. The sulfated polymers thus obtained proved to be more active than the corresponding monomer as antioxidants in both the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing/antioxidant power (FRAP) assays. In addition, the sulfated polymer proved to be highly efficient also as anticoagulant agent in the classical clotting times, mainly in prolonging the activated partial thromboplastin time (APTT) [2]. Being, for example, thrombosis a complex process involving multiple pathways, these sulfated phenolic polymers acting simultaneously as antioxidants and anticoagulants could be of value to prevent and treat this pathology.
Moreover, based on the reported increase of the chain breaking antioxidant activity of phenolic compounds bearing chalcogen substituents, herein we also report recent studies focused on the development of straightforward procedures for the preparation of seleno-compounds. The attention was focused in particular on the reaction of selenium tetrachloride with catechin, a widespread natural phenolic compound largely investigated due to its many beneficial properties for human health (*e.g.,* antioxidant, antinflammatory). The selenoderivative so obtained showed a superior antioxidant activity, in comparison with catechin, especially in kinetic terms and as an electron donor.
These results put the basis for the development of easy access routes to sulfur/selenoderivaties as multifunctional agents endowed not only with efficient antioxidant properties, but also with other biological properties associated with the presence of the sulfur or selenium atom, which could be exploited for example as alternatives to heparins or for the treatment or prevention of oxidative stress related pathologies.

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O.1.06 - DEVELOPMENT OF HIGH-ORDER FUNCTIONS USING TEA CATECHINS IN WATER

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When a hot tea beverage cools, turbid and brown-white particles occur and then precipitate. This phenomenon is called a “creaming-down reaction”. It was well known that tea catechins such as 2,3-*cis* gallated catechins (-)-epigallocatechin-3-*O*-gallate (EGCg) and (-)-epicatechin-3-*O*-gallate (ECg), and caffeine were abundantly contained in the precipitate of creaming-down reaction. Therefore, we attempted crystallization of the precipitate made from an aqueous solution of EGCg and caffeine, ECg and caffeine. And crystals obtained were determined to be a 2:2 complex of EGCg and caffeine (Figure. 1a), and a 2:4 complex of ECg and caffeine by X-ray crystallographic analysis [1].

These tea catechin complexes with caffeine were mainly formed by hydrophobic effect by their aromatic rings. In 2:2 complex of EGCg and caffeine, the caffeine moieties were positioned in the space surrounding the top and bottom walls of the B’ rings of EGCg moieties and right and left walls of the A and B rings of EGCg moieties. Resultly, the caffeine molecules were captured by the hydrophobic space formed with the three aromatic A, B, and B’ rings of EGCg (Figure. 1b) in the 2:2 complex, suggesting that the space had high hydrophobicity.

Based on the above findings, the high-order functions of molecular capture and chiral recognition of the tea catechins EGCg in water were investigated [2]. Complexes of precipitates formed from aqueous solution of EGCg and a variety of heterocyclic compounds were prepared, and a correlation between the chemical structures of the heterocyclic compounds and the molecular capture ability was studied.

Furthermore, the C ring of EGCg has C_2 and C_3 of two chiral carbon atoms, and then the hydrophobic space formed with the three aromatic A, B, and B’ rings of EGCg was a chiral space. Chiral recognition of compounds such as diketopiperazine Cyclo(Pro-Gly) included in the space was investigated (Figure. 1c). Also, chiral recognition of the β-adrenergic receptor blocker propranolol and the bronchodilators proxyphylline and diprophylline by EGCg were investigated.

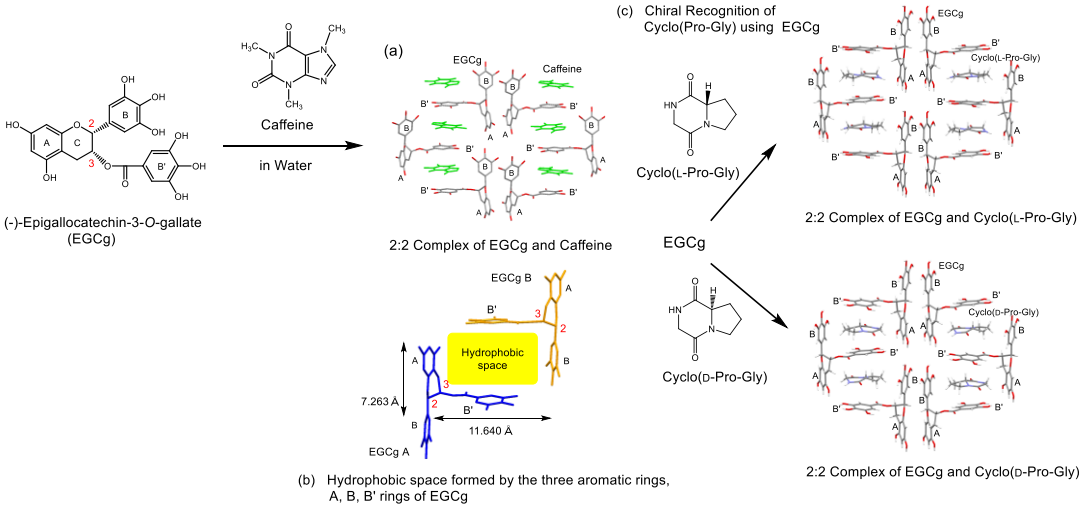

Figure 1: (-)-Epigallocatechin-3-*O*-gallate (EGCg) Complexes and Hydrophobic Space of EGCg

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OY.1.01 - A (DIS)COLOURFUL STORY ON IRON-PHENOLIC INTERACTIONS IN FORTIFIED FOOD

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Food fortified with iron can effectively reduce the global prevalence of iron deficiency. Savoury concentrates (*e.g.*, bouillon cubes) are an example of phenolic-containing food products that are promising vehicles for iron fortification, as they are widely available, frequently consumed, and affordable. However, when savoury concentrates are fortified with iron, their colour and iron's bioavailability can be compromised by the reactivity of the iron ion with phenolics [1]. For the design of iron fortified food products, it is important to understand the interplay between intrinsic (*i.e.,* pH, type of iron salt, concentration, ratio, ionic strength, taste enhancers) and extrinsic (temperature) factors on iron-phenolic complexation reactions. To this end, a three-level fractional factorial design was implemented. We concluded that the main factors affecting discolouration were the type of iron salt, pH, and temperature [2]. Besides these factors, the structural features of the phenolic itself can also affect the reactivity with iron. Therefore, we assessed the effect of the structural features of ten different flavonoids on discolouration caused by complexation, oxidation, and the formation of metal-phenolic networks [3]. Our work on iron-mediated complexation and oxidation of flavonoids showed that the presence of the *C*2–*C*3 double bond in combination with the catechol moiety and either the 4-carbonyl or 3-hydroxyl increased the intensity of discolouration, the extent of flavonoid oxidation, and the formation of metal-phenolic networks. This research gave a lot of insight into the reactivity of iron with flavonoid aglycons. However, in nature and food products, the majority of flavonoids are glycosylated. Therefore, we have also purified nine differentially (acylated) flavone glycosides and comprehensively investigated the effect of (acylated) 7-*O*-apiosylglucose substitution on the interaction with iron. The 7-*O*-apiosylglucoside can stabilise the coordination of iron to the 4-5 site, and thereby affects iron complexation. The presence of the 7-*O*-apiosylglucoside leads to an increase of absorbance in the visible light region of the spectra and discolouration for flavones possessing solely the 4-5 binding site or a decrease in absorbance and colour for flavones with an additional 3′-4′ site. In this (dis)colourful story, we shed light on the effect of intrinsic and extrinsic factors, as well as the structural features of flavonoids, on iron-phenolic interactions. These results provide in depth insight in iron-phenolic mediated food discolouration and facilitate the design of iron-fortified foods.

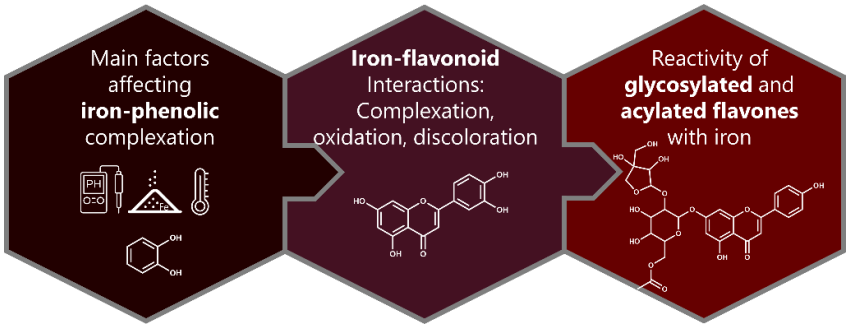

Figure 1: Unravelling discolouration due to iron-phenolic interactions

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OY.1.02 - ENZYMATIC OXIDATIVE COUPLING OF BARLEY PHENOLAMIDES

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Hydroxycinnamoylagmatines are secondary metabolites from barley (*Hordeum vulgare*) that protect the plant against environmental and biological threats. These compounds belong to the group of phenolamides and are composed of a hydroxycinnamic acid moiety linked to an agmatine group by an amide bond. Coupling products of phenolamides, called lignanamides or neolignanamides, have been reported to possess a large variety of bioactivities and are usually more bioactive than their monomeric precursors. For hydroxycinnamoylagmatines the coupling products are primarily reported to possess antifungal activity [1]. Additionally, the content of these dimeric compounds is increased upon biotic stress, indicating that these compounds are barley defence metabolites. However, the formation of these dimers and their broader bioactivities are currently poorly understood.

To better understand the link between the coupling of hydroxycinnamoylagmatines and the potential increase in bioactivity, it is important to first gain a better understanding of the oxidative coupling process. As no commercial standards of these compounds are available, a facile synthesis protocol was developed first [2]. Then, three hydroxycinnamoylagmatines, namely coumaroylagmatine, feruloylagmatine, and sinapoylagmatine were each incubated with horseradish peroxidase. The coupling reactivity of these monomers was in line with the order of their peak potentials as measured by cyclic voltammetry. Structure elucidation of fourteen *in vitro* coupling products by NMR and MS revealed that the three main linkage types, the 4-*O*-7′/3-8′, 2-7′/8-8′, and 8-8′/9-*N*-7′ linkages, were identical to those naturally present in *Hordeum* species. Furthermore, we identified two linkage types, the 8-8′ and 4-*O*-8′ linkages, that were not previously reported for hydroxycinnamoylagmatine dimers. These results show that oxidative coupling by horseradish peroxidase can be used for biomimetic formation of natural antifungal (neo)lignanamides from barley [3]. In our on-going work, we utilize this knowledge and the newly established methods to investigate oxidative coupling in more complex model systems of hydroxycinnamoylagmatines and in barley-derived extracts.

References

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[3] van Zadelhoff A., Meijvogel L., Seelen A-M., de Bruijn W.J.C., Vincken J.-P., *Journal of Agricultural and Food Chemistry*, 70, 16241–16252, 2022.

OY.1.03 - CHEMICAL STRUCTURE ELUCIDATION OF NOVEL PIGMENTS PRODUCED FROM THERMAL STRESS OF HYDROXYPHENYL PYRANOANTHOCYANINS

Voss D.M.*[a], Tang F.[a], Riedl K.[abc], Miyagusuku-Cruzado G.[a], Yao S.[a], Rodriguez-Saona L.E.[a], Hatzakis E.[a], Giusti M.M.[a]

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Hydroxyphenyl-pyranoanthocyanins (PACNs) are vibrantly colored anthocyanin-derived pigments found in redwine which can form by the reaction of anthocyanins with a hydroxycinnamic acid cofactor (Figure 1) [1]. Interest in using PACNs as food and cosmetic colorants is growing in part due to their exceptional color stability to heat. Specifically, 10-catechyl-PACNs retained up to 60% of their color after 15 hours at 90°C [2]. Interestingly, a unique colored compound formed during heating, contributing to this remarkable color stability [2]. Our objective was to identify the chemical structure of this novel, colored 10-catechyl-PACN degradation compound and to observe if other hydroxyphenyl-PACNs formed similar colored compounds.

10-Catechyl-PACNs were formed from cyanidin-glycosides from elderberry and caffeic acid, semi purified, and then heated for 22 hours at 90°C. This favored formation of the colored degradation compound which was then isolated with semi-preparatory HPLC. For structure elucidation, HPLC, QTOF MS/MS, IR, 1D and 2D NMR (1H, COSY, HMBC, HSQC), and spectrophotometry were used. Three other hydroxyphenyl-PACNs (10-p- hydroxyphenyl, 10-guaiacyl, and 10-syringyl) were similarly formed with different cofactors, isolated, and heated at 90°C with degradation monitored by HPLC-MS-MS.

The colored degradation product from 10-catechyl-PACNs was identified as a 4-carboxy-3-deoxyanthocyanin with a catechol unit (Figure 1). The exact mass was 315.0502, consistent with a chemical formula of C16H11O7+. NMR results were used to determine structure connectivity, and IR showed key discriminating bands to support the presence of aromatic rings (~1540–1520 cm-1) and the carboxylic acid moiety (~1400–1150 cm-1). The compound produced a yellow color at pH 1 (λvis-max = 480 nm) which transitioned to magenta by pH 9 (λvis-max = 546 nm); this is consistent behavior with a flavylium system. The additional hydroxyphenyl-PACNs each formed a unique, color-producing compound. The compound from 10-phenyl-PACNs produced the lowest λvis-max (465 nm) and m/z (299) and that from 10-syringyl-PACN had the highest λvis-max (491 nm) and m/z (359), matching the spectral and mass patterns observed among the parent PACNs. This suggested that these four degradation compounds share the 4-carboxy-3- deoxyanthocyanin backbone and retain their unique substitutions. These findings revealed the formation of a novel class of pigmented phenolic compounds-4-carboxy-3- deoxyanthocyanins.

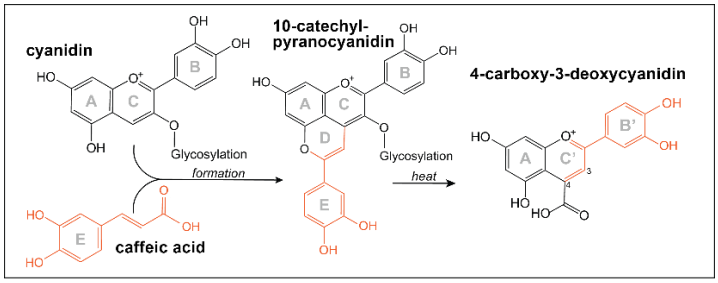

Figure 1: Formation of 10- catechyl-pyranocyanidins and the 4-carboxy-3- deoxycyanidin elucidated in this experiment. Rings B' and C' correspond to rings E and D of the precursor compound, respectively.

[1] de Freitas V. & Mateus N., *Analytical and Bioanalytical Chemistry*, 401, 1463–1473, 2011.
[2] Voss D.M., Miyagusuku-Cruzado G. & Giusti M.M., *npj Science of Food*, 16,1–9, 2022.

OY.1.04 - EFFECTS OF PHYSICOCHEMICAL TREATMENTS ON WHEY PROTEINS AND PROANTHOCYANIDINS COMPLEXATION

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Polyphenols have high nutraceutical potential. However, they are also vulnerable to their physicochemical environment. Oxygen, pH, and light can affect their structure, frequently in an irreversible way. During digestion, polyphenols are exposed to physicochemical conditions that can lead to degradation and impair their bioaccessibility. The affinity of polyphenols for specific proteins can be taken to profit to protect them during digestion. This research aims to improve the formation of complexes between proanthocyanidins (PACs) isolated from *Aronia melanocarpa* and different types of proteins. Whey protein (WPI), hemp protein (HPI) and pea protein isolate (PPI) were tested. A multifactorial statistical approach was used to assess the effect of pH, ionic force, and high-pressure homogenization (HPH) on the complex's formation. The impact of these factors on the infrared spectra, complexation ratio, and excitation/emission fluorescent spectra on complex formation were evaluated in a 3-way response surface. The hypothesis is that the HPH treatments would allow partial protein unfolding and the exposition of hydrophobic interaction sites inside their hydrophobic pockets, improving interactions with PACs. Chemometrics analyses allowed a broad screening of several environmental conditions and HPH pressure on complex formation. These PACs-protein complexes will then be tested in a follow-up study for their ability to improve PACs delivery in an *in vitro* digestion model to maximize their bioaccessibility. These preliminary investigations highlight the complexation behavior of PACs with proteins and give some evidence to explain their bioavailability regarding their environmental chemical conditions.

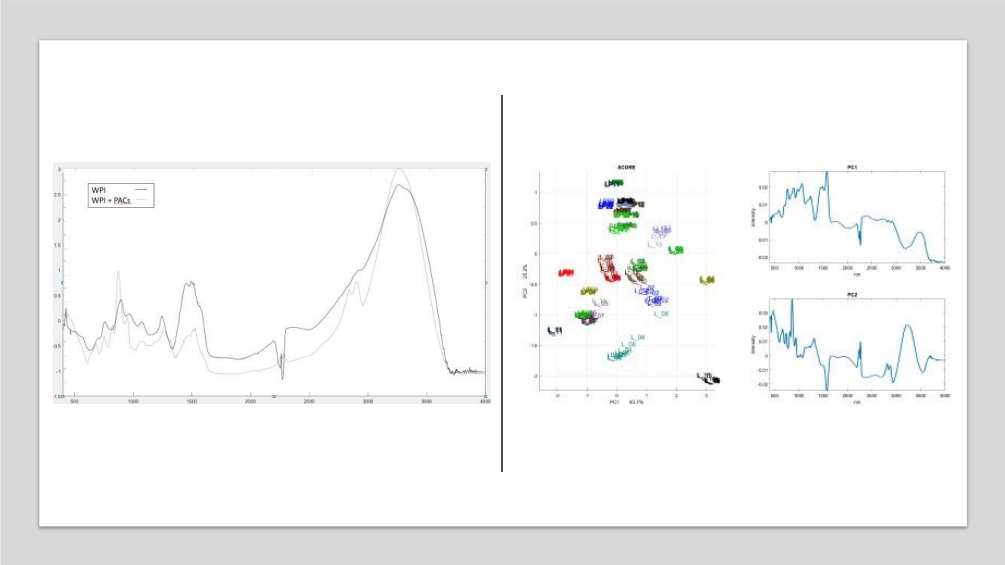

Figure 1: (Left) FTIR spectra of WPI and WPI-PACs, (Right) PCA of all of the environmental treatments tested on WPI alone as well as on complexes.

OY.1.05 - THE EFFECTS OF COCOA FLAVANOLS ON UPPER AND LOWER LIMBS DURING UNINTERRUPTED SITTING: INSIGHTS INTO MACRO- AND MICROVASCULAR FUNCTION

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Prolonged sitting affects endothelial function in peripheral conduit arteries. Nutritional strategies might play an important role in counteracting these negative effects. Consumption of cocoa flavanols enhances peripheral vascular function, as measured in the brachial artery via flow-mediated dilation (FMD); however, whether this positive outcome is also present in the superficial femoral artery is not known. The objective of this study is to investigate whether acute ingestion of cocoa flavanols can prevent sitting-induced impairments in macrovascular and microvascular function in both the upper and lower body vasculature in young healthy adults.

In a randomized, double-blind (the two cocoa beverages remain blinded as 523, and 947), cross-over trial, 13 young healthy men (age: 23 ± 3 years) completed two, 2-hour sitting conditions with consumption of either a high or low flavanol cocoa beverage. FMD and shear rate of the femoral and brachial artery, microvascular function (i.e., reperfusion slope) of the calf (assessed via near-infrared spectroscopy), and blood pressure, were collected before and after the 2-hour sitting intervention.

In these initial 13 participants (recruitment target n=36; complete set of data will be available at the time of the presentation), sitting significantly reduced femoral FMD (pooled data: 4% vs. 2.7%, p=0.011). However, this detrimental decline in vascular function was prevented in response to only one of the two cocoa beverages (i.e., 523). Reperfusion slope was not affected by sitting; however, our preliminary data suggests that cocoa beverage 523 may have a positive impact on reperfusion slope (although not statistically significant). In regard to brachial FMD, no significant changes were observed neither in response to sitting nor cocoa ingestion. Furthermore, these preliminary data suggest that sitting may have a negative impact on brachial FMD (pooled data: 5.5% vs. 4.8%, p=0.28; interaction: p=0.09), but this is only observed following cocoa beverage 947. In addition, positive shear rate declined in both the femoral (p<0.01) and brachial artery (p<0.01) following sitting, although this was not differentially affected by flavanol content (interaction: p>0.1). No significant changes in blood pressure were observed neither in response to sitting nor cocoa ingestion. Finally, the impact of individual's cardiorespiratory fitness on vascular function in response to cocoa ingestion and sitting will be available at the time of the presentation.

Preliminary observations highlight the potential impact of cocoa consumption in ameliorating macrovascular and microvascular function following uninterrupted prolonged sitting, specifically in the lower limbs. Hence, cocoa consumption may be effective in attenuating sitting-induced vascular dysfunction in lower limb conduit arteries.

OY.1.06 - A TERROIR COMPREHENSIVE STUDY ON THE QUALITY OF RED WINES: TOWARDS AN EXHAUSTIVE CHARACTERIZATION OF POLYPHENOLIC COMPOUNDS

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Phenolic compounds play a significant role on the organoleptic properties of wines such as color, astringency, bitterness and also the stability through oxidative processes. These properties depend on intrinsic factors, as grape variety, and extrinsic ones, as soil, climate and winemaking techniques. Among *terroir* parameters, climatic conditions, in particular the amount of insolation, temperatures and water balance, were found to have a major influence on the concentration of polyphenols compounds [1]. Corbières appellation is a renowned red grape varieties viticultural region in South France. This work evaluated the effect of five terroirs of this region on the polyphenolic compositions of Syrah, Grenache, Carignan and Mourvèdre mono-varietal wines produced at an experimental scale in the same vintage. Grapes arrived from representative vineyards of each terroir, were harvested at similar technological maturity and a standard vinification process was used. A general and targeted study approach on the wines' polyphenols profile was adopted. Among phenolic compounds, a focus on anthocyanins and tannins was performed, as they determine the color and astringency of red wines. Wine pigments and derived pigments were assessed through spectrophotometric measurements. Anthocyanidins were analysed through a double approach: UPLC-ESI-MS/MS, by calculating their concentration from the EIC (Extract Ion Chromatogram), and MRM analysis. After wine sample pre-treatment, tannins total concentration and their fractions were evaluated. The "apparent" average degrees of polymerisation (aDP) were calculated after chemical depolymerization through UHPLC-ESI-MS. However, in wines, many reactions take place from the beginning of the wine making process to its consumption. Within the tannin structures, the new covalent bonds created by oxidation are resistant to depolymerization conditions and oxidation markers (dimers and trimers) are then obtained [2]. These structural modifications distort to a greater or lesser extent the determination of the aDP depending on the oxidation state of the tannins. Thanks to the detection and identification of these oxidation markers through UHPLC-ESI-MS/MS, a more accurate study on the tannin composition differences among the five terroirs was possible [3]. Through a QDA sensorial analysis a link was traced between the tannin composition of wines and the astringency perception of wines.

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OY.1.07 - SYNTHESIS OF CARBOXY-FLAVANONE DERIVATIVES AND INVESTIGATION OF THEIR ANTI-INFLAMMATORY ACTIVITY: STRUCTURE-ACTIVITY RELATIONSHIP STUDY

Sinyeue C.*[a,b], Matsui M. [c], Ouary C. [a], Lasalo M.[c], Machuraux L.[c], Robertson M.[d], Oelgemöller M.[d,e], Brégier F.[b], Chaleix V.[b], Sol V.[b], Lebouvier N.[a]

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Flavonoids are naturally occurring polyphenols with broad known pharmacological properties. Various synthetic 2,3-dihydroflavanone derivatives were synthesized to optimize their anti-inflammatory potential [1]. These compounds were prepared through cyclization of the corresponding 2'-hydroxychalcone derivatives, the later accessible by Claisen-Schmidt condensation. Nitric oxide (NO) is an important inflammatory mediator. Thus, the inhibitory activity of the flavanone derivatives on NO production in LPS-induced RAW264.7 macrophages were evaluated *in vitro* using the Griess test after cytotoxicity assessment. Among the most active compounds, 2-carboxy-5,7-dimethoxy-flavanone and 2-carboxyflavanone showed IC_{50} values of 0.906 and 1.830 g/mL, respectively, while the reference molecule, pinocembrin, achieved an IC_{50} value of 203.60 g/mL as expected [1]. Carboxy-flavanone thus has a greater capacity to inhibit NO production compared to the original molecule pinocembrin. The incorporation of a highly polar, acidic, and electron-accepting carboxyl-group was therefore investigated to potentially enhance the biological potential of flavanones. Following this approach, additional compounds bearing carboxy-groups on different positions of the B-ring were synthesized and subjected to a structure-activity relationship (SAR) study on their anti-inflammatory activities (Figure 1). This SAR-study subsequently identified novel bioactive flavanones as promising candidates for the development of new immunoregulatory agents. This study was funded by the Ministry of Foreign Affairs and International Development (MAEDI) and the Economic, Social and Cultural Cooperation Fund for the Pacific.

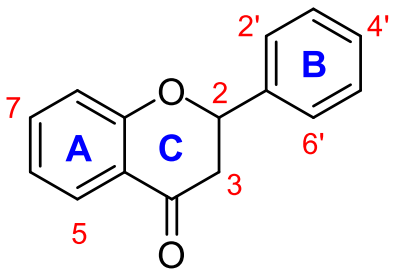

Figure 1: General structure of flavanone

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OY.1.08 - RED COLOR STABILIZATION OF ANTHOCYANINS WITH LIGNOSULFONATES FROM THE PULP INDUSTRY

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Natural colorants are gradually replacing synthetic dyes due to consumers' awareness and demand for natural products. Anthocyanins are water-soluble pigments found in Nature and are among the most used natural red colorants in the Food Industry. Anthocyanins can confer a wide range of colours (red, violet & blue) to flowers, fruits, and some legumes. Yet, the application of anthocyanin-based colorants into different food matrices can be challenging as these compounds are sensitive to pH, temperature, oxygen, and light involved in different processing, formulation, and storage conditions. Industries are investing in the development of new processes to decrease anthocyanin's colour loss and to produce more stable and appealing colours [1]. This work presents the red colour stabilization of anthocyanins with a lignosulfonate obtained from softwood sulfite liquor (SSL,11.3% w/w_{liquor}). Lignin is the second most abundant biopolymer in nature and is stable, nontoxic, inexpensive, and extremely biodegradable. Lignosulfonate was obtained after ultrafiltration and freeze-drying of the softwood sulfite liquor composed of a mixture of 90 % of spruce (softwood material) and 10 % of beech (hardwood biomass). The sulfonation degree was determined based on the electrostatic interaction between a cationic surfactant (CTAB) and the anionic lignosulfonate. Moreover, the surface charge of lignosulfonates was evaluated by measuring the potential zeta at different pH values. The results showed an increase in the negative surface charge along with the pH due to the ionization of sulfonic and phenolic hydroxyl groups. Knowing that the negatively charged groups of lignosulfonates can interact with the positive charge of the flavylium cation of anthocyanins, this interaction was studied in a pH range between 0 and 4. The results showed that the association constant increased with the pH between pH 0-2, due to the increase in the amount of negatively charged groups in the lignosulfonate structure that interact with the flavylium cation form of the anthocyanin. Bearing this, the results obtained so far show that this biopolymer can be used to stabilize the red colour of anthocyanins to be incorporated into different matrices.

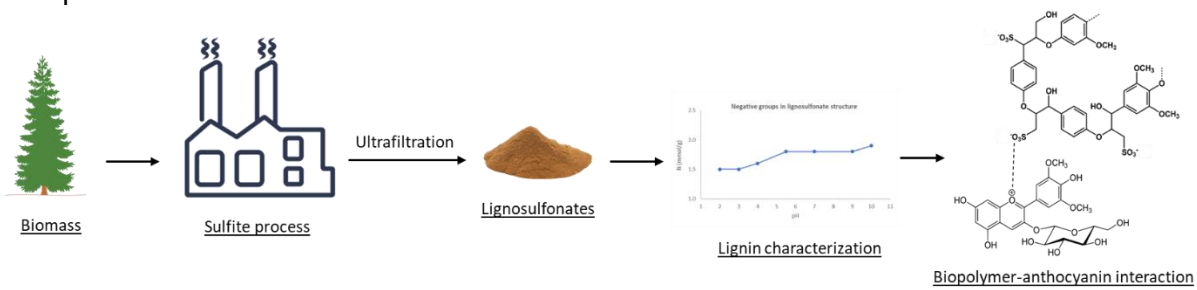

Figure 1: Isolation and characterization of lignosulfonate for interaction with anthocyanins

[1] Khoo H. E., Azlan A., Tang S. T. & Lim S. M., *Food Nutr. Res.*, 61, 1361779, https://foodandnutritionresearch.net/index.php/fnr/article/view/1257, 2017.

Acknowledgements: We thank to FCT from a doctoral grant from (SFRH/BD/146549/2019). The authors also thank AgriFood XXI I&D&I project (NORTE-01-0145-FEDER-000041) cofinanced by European Regional Development Fund (ERDF), through the NORTE 2020 (Programa Operacional Regional do Norte 2014/2020). This work was financially supported by the LAQV-REQUIMTE through the national funds from UIDB/50006/2020 and UIDP/50006/2020.

OY.1.09 - OXIDATIVE COUPLING OF RESVERATROL AND PICEID IN WINES

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Stilbenes are well-known bioactive polyphenols produced by grapevine. Among these compounds, resveratrol and its glucoside, the piceid, (Fig. 1) are naturally present in wines [1]. Resveratrol is recognized for these numerous biological properties. In addition, resveratrol can be subjected to oxidative coupling due to its phenolic structure. This reaction induces the formation of various derivatives including oligomers in wine [2]. In this study, we have investigated the resveratrol and piceid oligomerization by oxidative coupling in wines.

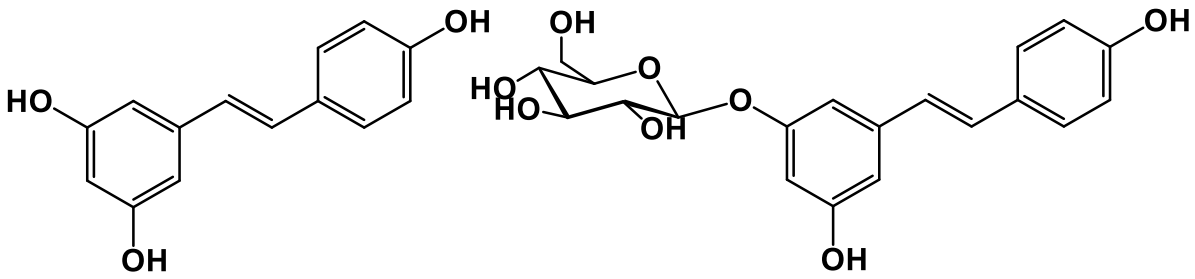

Figure 1: Resveratrol and piceid structures

First, the transformation of resveratrol and piceid in hydroalcoholic solution by oxidative coupling in presence of metals will be presented. The compounds formed were characterized by NMR and mass spectrometry.
Secondly, the formation in wines of these compounds was monitored using a UHPLC-Q-Exactive Plus mass spectrometer. The main results will be presented and discussed.
Finally, the biological properties of these compounds were evaluated. Purified products were tested on different cell line models. The results will be presented and compared with those obtained with resveratrol.

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P.1.01 - NOVEL DI-*C*-GLYCOSYLFLAVONES FROM NARTECIUM OSSIFRAGUM

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Narthecium ossifragum is a perineal flowering plant which is a common plant species in Scandinavia and the North of Europe. Flowering occurs between July and August and has been related to poisoning in domesticated animals around these months of the year for the last four hundred years.
Because of its high defence mechanisms, our research group analysed another growth stage finding several aromatic compounds in the fruits of *N. ossifragum* [1]. This incentive analysed the flowers finding five novel di-C-glycosylflavones and one well-known di-C-glycosylflavone.
Partition with organic solvents was done to frozen plant material ending in the aqueous phase. This aqueous phase was further purified with column chromatography and preparative HPLC. Several spectroscopy methods were used for the structure elucidation, and all the compounds were cytotoxicity tested towards mammalian cell lines [2].
The UV spectra showed a UV maximum absorption at 346-347 nm and 269-271 nm, and with a combination of 1D and 2D NMR spectroscopy and HRMS was possible to identify the known chrysoeriol 6,8-di-*C*-β-glucopyranoside (6). In the same way, the five novel compounds chrysoeriol 6-*C*-β-arabinofuranosyl-8-*C*-β-glucopyranoside (1), chrysoeriol 6-*C*-β-arabinopyranosyl-8-*C*-β-glucopyranoside (2), chrysoeriol 6-*C*-β-xylopyranosyl-8-*C*-β-galactopyranoside (3), chrysoeriol 6-*C*-β-galactopyranosyl-8-C-β-glucopyranoside (4), chrysoeriol 6-C-β-glucopyranosyl-8-*C*-β-galactopyranoside (5). All structures can be seen in figure 1.
Compounds 4-6 exhibit double sets of signals on their ^{1}H and ^{13}C NMR spectra, which indicates two conformational isomers created by rotational hindrance at the C (sp^3) – C (sp^2) glucosyl-flavone linkage in each of these 6,8-di-C-substituted flavones. During the isolation of these aromatic compounds using preparative HPLC, compound 1 was isolated at two different retention times from the same injected sample, demonstrating a Wessely-Moser isomerisation rearrangement [2].

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P.1.03 - DITERPENE AND BIFLAVONE DERIVATIVES FROM THUJA KORAIENSIS AND THEIR CYTOTOXICITIES AGAINST A549 CELLS

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In the screening of cytotoxicity of the Korean rare endemic plants, the extract of *Thuja koraiensis* Nakai showed potent cytotoxicity against the adenocarcinomic human alveolar basal epithelial A549 cell line. The CH_2Cl_2 fraction possessing a potent cytotoxic effect was subjected to a series of column chromatography to yield six major compounds. Through extensive spectroscopic data analysis in combination with quantum chemical calculations and competing enantioselective acylation, their structures were elucidated to be five diterpenes, including three new abietanes, a new labdane along with a known labdane, and a biflavonoid. A new abietane diterpene (**3**) and 7,7''-di-*O*-methylamentoflavone (**6**) were found to have cytotoxic effects on A549 cells by MTT cell viability and lactate dehydrogenase (LDH) assays.

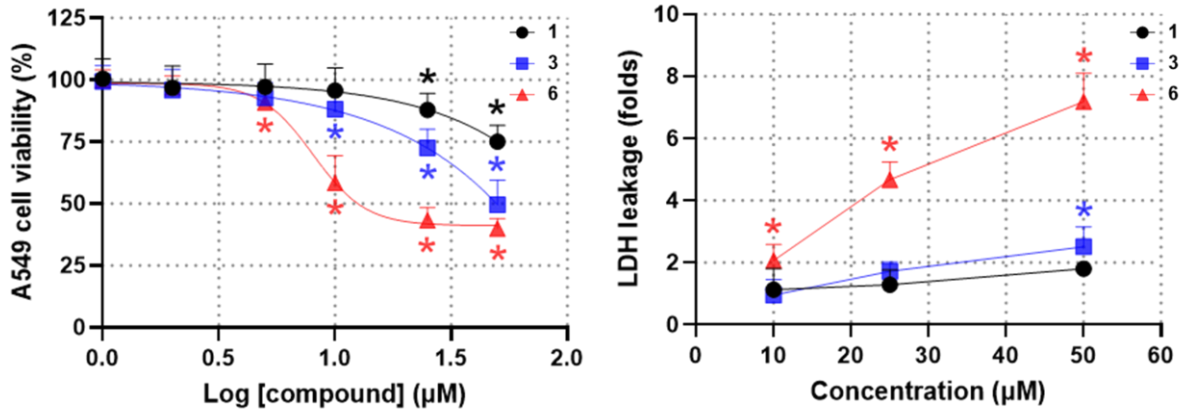

Figure 1: Cytotoxicities and LDH leakage of 1, 3 and 6 on A549 cells

P.1.04 - VALORISATION OF OLIVE WASTE – AN INVESTIGATION OF THE STABILITY AND COLOUR FORMATION OF THE OLIVE BIOPHENOL HYDROXYTYROSOL

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During olive oil production, 80% of the olive ends up as the by-product olive pomace, a viscous residue that is harmful to the environment if left untreated, due to its high content of organic substances. Olive pomace contains a large amount of biophenols, with 98% of the phenolic compounds present in the olive fruit remaining in the pomace after oil extraction, with hydroxytyrosol as the most abundant [1]. Given the increasing demand for natural plant-derived food ingredients instead of synthetic ones, olive pomace is a promising source of antioxidants that can be valorised for added value use in food products. For successful valorisation of such plant-derived side streams, it is crucial to understand the stability of the main functional compounds across varying food treatments and matrices. Also important is their colour stability across varying parameters.

To this end, the degradation and concurrent colour formation of hydroxytyrosol in real and simulated tap water systems at pH 7.5 was studied. Simulated tap waters consisted of $NaHCO_3$, with and without various metal cations (Ca, Mn, Co, Ni, Cu and Zn). Colour formation was monitored with UV/Vis spectroscopy and UPLC-MS/MS over 5 days, showing the degradation of HT and formation of autoxidation products. Selected oxidation products were identified, including a known red chromophore that is a potential contributor to the observed red colour formation. The results have implications for the stability and colour behaviour of olive pomace-derived antioxidants applied to neutral or mildly alkaline environments, and allow for more effective valorisation of olive pomace.

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P.1.05 - RADICAL CONVERSION OF B-TYPE PROCYANIDINS TO A-TYPES ON AN ANALYTICAL SCALE USING DESIGN OF EXPERIMENTS

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Since several years, proanthocyanidins are in the focus of research because of their structural diversity and high antioxidant activity. B-type procyanidins, which consist of (epi)catechin units, are found in various fruits and vegetables such as grapes, strawberries and cocoa beans. A-types, which have an ether bridge as well as an interflavan bond, are less common in nature. They occur primarily in cranberries, peanuts, avocados and plums [1]. Because of their low abundance a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical intramolecular oxidative transformation is known for their synthesis [2].

The aim of this study is to verify the feasibility of synthesizing A-type from various B-type procyanidins and to optimize the reaction in favor of a preparative scale synthesis followed by structure-activity studies. The precursor B-type procyanidins, were semisynthetically obtained from *Aronia melanocarpa* pomace, a waste product from the production of Aronia juice and willow bark, which is a waste product of the wood industry [3]. The raw material was pre-purified with *n*-hexane for the removal of lipophilic compounds. Procyanidins were extracted with 70% aqueous acetone, followed by a pre-fractionation leading to an enhanced content of polymers by ethanol/*n*-hexane precipitation (Figure 1 A). The nucleophiles (+)-catechin and (–)-epicatechin were added to the polymeric-enriched precipitate to achieve a nucleophilic attack for the synthesis of dimeric B-types. The separation of procyanidins was performed by countercurrent chromatography (CCC) followed by an isolation using preparative high performance liquid chromatography (HPLC). The optimization of the radical oxidation of B-types to their corresponding A-types was carried out using design of experiments (DoE; Figure 1 B). Three factors were optimized: time, temperature and educt ratio of B-type/DPPH radical.

In conclusion, different B-type procyanidins can be synthesized into the corresponding A-types by DPPH radical oxidation. Each conversion has various reaction products with different conversion rates.

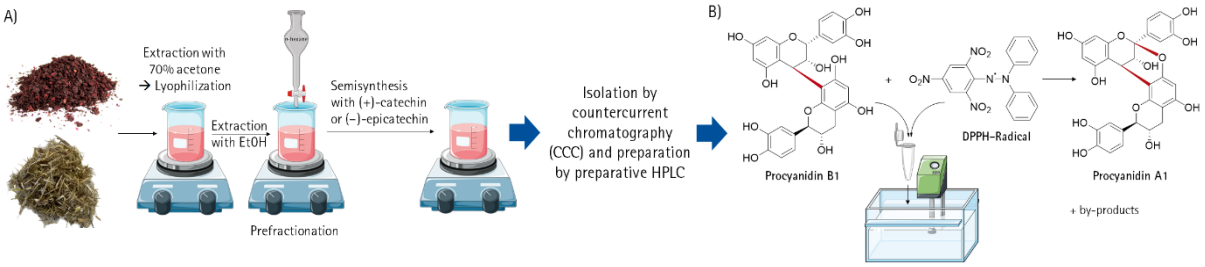

Figure 1: Schematic illustration of B-type isolation (A) and the conversion into the A-type (B) modified according to Servier Medical Art (https://smart.servier.com/).

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P.1.06 - DEVELOPMENT OF UNIFIED SYNTHETIC METHOD OF *C*-GLYCOSIDIC ELLAGITANNINS

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Casuarinin and its 1-OH epimer, stachyurin, are *C*-glycosidic ellagitannins isolated from *Casuarina stricta* [1]. These compounds possess various biological activities, including antioxidant, antimicrobial, and antitumor activities. Recently, Yamamoto and Kawamoto *et al.* revealed that casuarinin activates muscle satellite cells, and *C*-glycosidic ellagitannins potentially improve sarcopenia [2]. Casuarinin and stachyurin contain two (*S*)-hexahydroxydiphenoyl (HHDP) groups: one between the 2-oxygen position (O-2) and O-3 and the other between O-4 and O-6 of the open-chain glucose, and a galloyl group at O-5. The C-1 linked to the electron-rich 2,3-(*S*)-*O*-HHDP group is a unique structure of *C*-glycosidic ellagitannins. For example, two analogues occur naturally: stenophyllanin A with a catechin unit at C-1 and alienaninn B dimerized through C-1. Because previous studies of C-glycosidic bond formations provided a mixture of α- and β-isomers, the steric control of the *C*-glycosidic bond is a significant challenge.
We developed a unified synthetic pathway utilizing the *C*-glycosylation with complete stereoselectivity [3]. As shown in figure 1, treatment of oximes **1** delivered from D-glucose under acidic conditions selectively formed the *C*-glycosidic bond to afford **2** alone in 93% yield. Subsequent introduction of the benzyloxy group at C-1 of **2** yielded only β-isomer **3**. Removal of the protecting group of **2** and **3** achieved total syntheses of casuarinin and stachyurin, respectively. The result enabled the comprehensive synthesis of *C*-glycosidic ellagitannins, which can contribute to structure–activity relationship studies for the applied research on *C*-glycosidic ellagitannins.

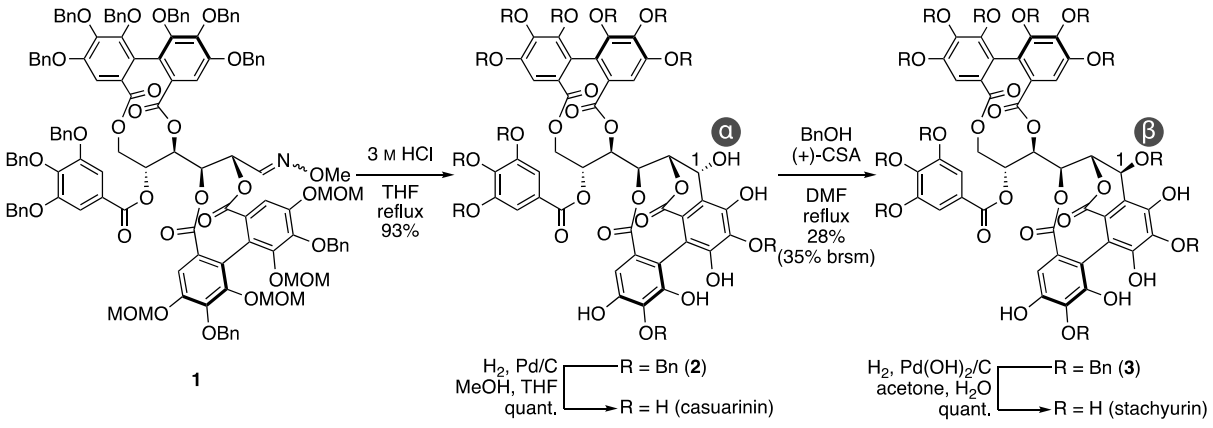

Figure 1: Unified synthetic method of *C*-glycosidic ellagitannins

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P.1.07 - CASHEW ALLERGEN-POLYPHENOL CONJUGATE AS A POTENTIAL THERAPEUTIC AGENT FOR ALLERGY IMMUNOTHERAPY

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Food allergies are serious health burdens. The current proposed management through OIT (oral immunotherapy) is a promising treatment option for food allergies, yet there are significant side effects associated with using allergenic food sources in the therapy. Plant-derived polyphenols with well-recognised antioxidant and anti-inflammatory profiles have been demonstrated to exhibit potential immunomodulatory effects. While high-affinity interaction between protein and polyphenol to form complex results in conformational changes that render them hypoallergenic, this interaction can be influenced by the environmental conditions, and such protein structural changes (if not controlled well) can have significant implications in the allergen digestibility and modulate the process of sensitization [1]. Hence, we propose allergen-polyphenol conjugate as an alternative agent functionalised with immunomodulating polyphenols. The objectives of this study were to synthesize cashew allergen-resveratrol (RES) conjugates and investigate their structural modification and associated immunological properties.

Covalent modification of cashew allergens with RES was achieved by the reduction of free amino and thiol groups. The structural analyses confirmed irreversible structure modification via coupling with RES, but still retained adequate protein solubility. Moreover, the conjugate showed increased free radical-scavenging activity, hence, improving the conjugate's overall antioxidant capacity. The conjugate displayed reduced immunoreactivity with cashew-specific IgG and serum IgE via disrupting the conformational epitopes and some linear epitope binding regions of the allergenic molecules. In conclusion, the conjugation of RES to cashew allergens could be a promising strategy to generate therapeutic agents with reduced allergenicity and increased antioxidant/anti-inflammatory properties.

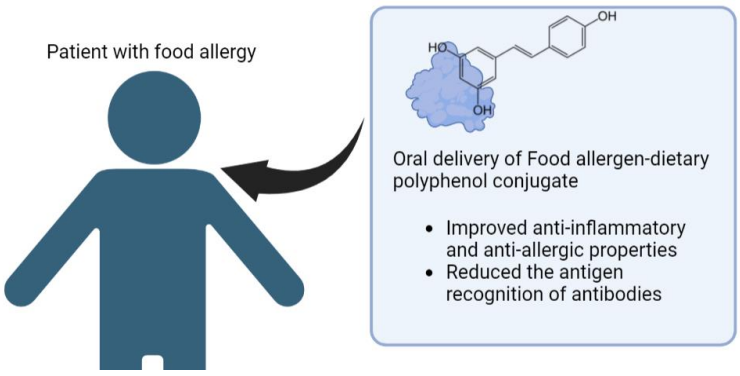

Figure 1: Schematic illustration of the polyphenol conjugate as a preventive approach against food allergy.

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P.1.08 - SINGLET OXYGEN QUENCHING BY RESVERATROL DIMERS

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We investigated the singlet oxygen quenching ability of several dimers of trans-resveratrol: We measured the total rate constants of singlet oxygen removal (kT) by the resveratrol dimers (+)-ampelopsin A, (-)-trans-epsilon-viniferin, and (+/-)-pallidol using direct time-resolved measurements of singlet oxygen luminescence lifetimes (Figure 1). These compounds have been reported to have significant antioxidant ability, including photoprotective activity. Our studies of resveratrol derivatives showed that methylation of resveratrol's hydroxy groups decreases the k_T values, and a protic solvent system results in higher k_T values, except for the completely methylated derivative. The k_T values are relatively low, mostly between 1-5 x 10^6 $M^{-1}sec^{-1}$ which is about an order of magnitude lower than the value for a-tocopherol (Vitamin E) [1]. The k_T values of resveratrol dimers were between 5 x 10^6 to 1 x 10^7 $M^{-1}sec^{-1}$ and the lack of product formation was confirmed by irradiating a sample of the dimer and Rose Bengal for up to 10 hrs.

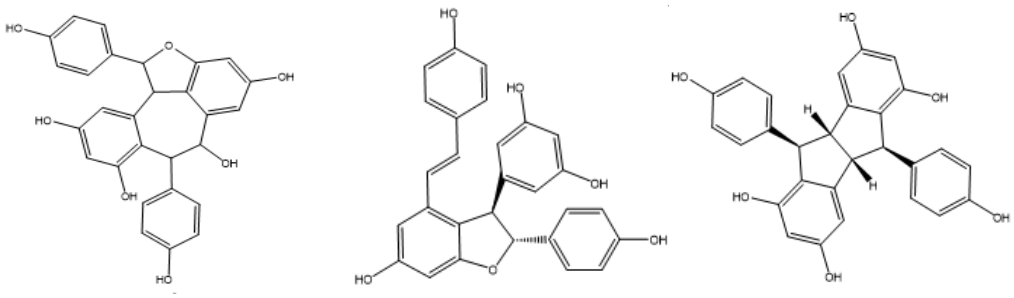

Figure 1: The structures of the three resveratrol dimers : ampelopsin A, e-viniferin and pallidol (left to right).

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P.1.09 - NUCLEAR MAGNETIC RESONANCE (NMR) MEASUREMENTS AND *IN SILICO* MODELING OF THE INTERACTION BETWEEN B-LACTOGLOBULIN AND CHLOROGENIC ACID TO REDUCE ALLERGENIC EFFECTS

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During an allergic reaction, antibodies from the immune system react with the epitopes of exogeneous substances like milk proteins. β-Lactoglobulin (β-LG) is the most common milk allergen and its epitopes are characterized by the protein folding and thus, secondary structure as well as quaternary structure like dimer formation on the surface of the protein. A new approach to reduce the allergenicity of β-LG is the chemical modification of its epitopes by phenolic compounds. The polarity and chemical properties (quinone formation) of the phenolic compounds as well as environmental factors (pH) affect the interaction of β-LG and phenolic compounds (non-covalent and covalent). Thus, the number and stability of the reaction products of β-LG and phenolic compounds is diverse. Chlorogenic acid tends to to form quinones in basic environment [1] and therefore, apt to modifying the β-LG epitopes.
Hence, the aim of this work is the investigation of the interactions (non-covalent and covalent) between β-LG and phenolic compounds, such as chlorogenic acid, in a model system using pH values 6 and 9 to verify modifications of epitopes induced by phenolic compounds for decreasing the allergenicity. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) was used for the experimental investigation of the possible non-covalent and covalent interactions between β-LG and the phenolic compound chlorogenic acid. For the *in silico* modelling molecular dynamics simulations were performed using the software *Amber* and *Gromacs*. The interactions between atoms are described using molecular mechanics, and the motion of the atoms is predicted using the Newton law of force. Simulations can show the formation of non-covalent interactions between phenolic compounds and β-LG.
^{1}H-NMR results showed a degradation of chlorogenic acid and β-LG in aqueous solution over a 36 h period, which could indicate a reaction of both components. The bioinformatics simulations showed the dynamics of β-LG in water. From the root mean square fluctuation, it could be derived that Glu^{89} is more flexible in the monomer at pH 9 than in the dimer at pH 6. The explanation can be the *Tanford* transition and the hydrogen bond between Glu^{89} and Ser^{116} in the dimer [2], which shows the influence of pH on protein folding.
Next steps are to increase the chlorogenic acid's concentration in the mixture, because at higher concentrations interactions may result in more distinct changes in the ^{1}H-NMR-spectra and the modelling of the protein. Reaction temperature will be increased to body temperature (37°C) to simulate body environment. Moreover, interactions of β-LG with other phenolic compounds like *p*-coumaric acid, caffeic acid and phlorizin will be measured as well as the digestion with trypsin for a comparison with the NMR data.
These results may contribute to the understanding of structural modifications of β-LG using phenolic compounds like chlorogenic acid to show non-covalent and covalent interactions and reaction products to modify β-LG epitopes to reduce allergenic effect.

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P.1.10 - POLYPHENOL OXIDATION, A WAY TO MODULATE POLYPHENOL-PROTEIN INTERACTIONS?

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As an alternative to meat, legumes, which are also rich in proteins, represent a promising alternative to fulfil both agri-environmental issues and feeding the planet. The faba bean (*Vicia faba*) is one of the most grown legume crops in France. Despite its protein richness, several locks remain to its use and consumption. The main issue is the high number of secondary metabolites that alter organoleptic and techno-functional properties of faba bean derived products.

Numerous polyphenols such as flavan-3-ols monomers (catechin, epicatechin and galloyl derivatives) and oligomers (procyanidins) were detected in faba bean methanolic extract [1]. Procyanidins are responsible for the astringency due to their high reactivity and strong interaction with proteins with which they will form conjugates and complexes (*i.e.,* tanning properties) and thus restrict protein bioavailability. Alkaline conditions often used to purify proteins from legumes lead to autoxidation of polyphenols to form quinones and other reactive intermediates that strongly increase the overall reactivity of polyphenols with proteins.

If it is known that multiple factors (temperature, pH and conformation/type) determine polyphenol-proteins interactions through both non-covalent (H-bonding, electrostatic interaction, hydrophobic interaction) and covalent bond [2], little is known about the relevance of the polyphenolic pool oxidative status on the protein-polyphenols interactions determinism. Here, faba bean polyphenols are obtained through ethanolic, methanolic, acetonic or mixed solvents and their profile are elucidated using UPLC-DAD-MS. Then, major compounds will be submitted to controlled oxidation and put with globulins (the main faba bean proteins [3]) to elucidate the polyphenol oxidation relevance in the polyphenol-protein interactions.

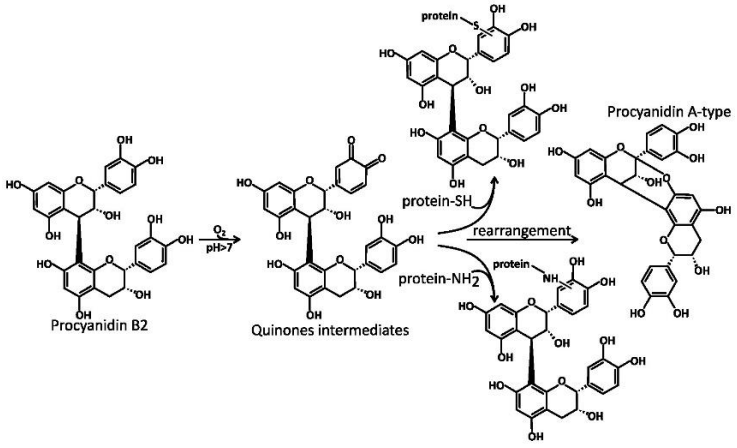

Figure 1: Possible reactions between procyanidin B2 and proteins under alkaline conditions.

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P.1.11 - SILVER NANOPARTICLES DECORATED WITH POLYPHENOLIC CARBOSILANE DENDRONS: AANTIBACTERIAL & ANTIXIDANT PROPIERTIES

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Nanotechnology is an emerging science based on the synthesis and application of nanostructures or nanomaterials that are typically in the range of 1-100 nm. Therefore, the development of different nanomaterials, such as nanoparticles (NPs) or dendritic macromolecules, as antioxidant delivery agents has received special interest in recent years.

Our research group has demonstrated an extensive experience in the use of a great variety of funcionalized nanoparticles in biomedicine, particularly using dendronized nanoparticles as vehicles for the transport of biomolecules or as therapeutic agents *per se*, against infectious diseases, cancer or neurodegenerative diseases. As one of these examples, we have shown that AgNPs decorated with cationic carbosilane dendritic systems present interesting antibacterial properties [1]. In this context, heterofunctionalization of metallic nanoparticles, MNPs, with natural antioxidants like polyphenols allows to overcome the major disadvantages of polyphenolic compounds, promoting the stability of antioxidant polyphenols in physiological conditions, improving their bioavailability and controlling their release [2]. Among these antioxidants polyphenols, we are interested in caffeic acid (CA) and its derivatives because it has been shown not only antioxidant but also antibacterial, anti-inflammatory, and anticancer activity [3]. For that reason, we have stablished as goal for this work, the heterofunctionalization of the surface of silver nanoparticles with caffeic acid and cationic carbosilane dendrons or PEG looking a cooperative effect between the antioxidant properties of polyphenols and antibacterial properties of cationic carbosilane dendrons, with the water solubility or biocompatibility that can confer PEG to the nanosystem.

Herein we report a new family of AgNPs capped with caffeic acid modified with a PEG chain (PEG-CA), to promote its solubility in physiological medium and hetero-functionalized AgNPs with PEG-CA and cationic carbosilane dendrons. In both cases, we studied their antioxidant and antibacterial properties.

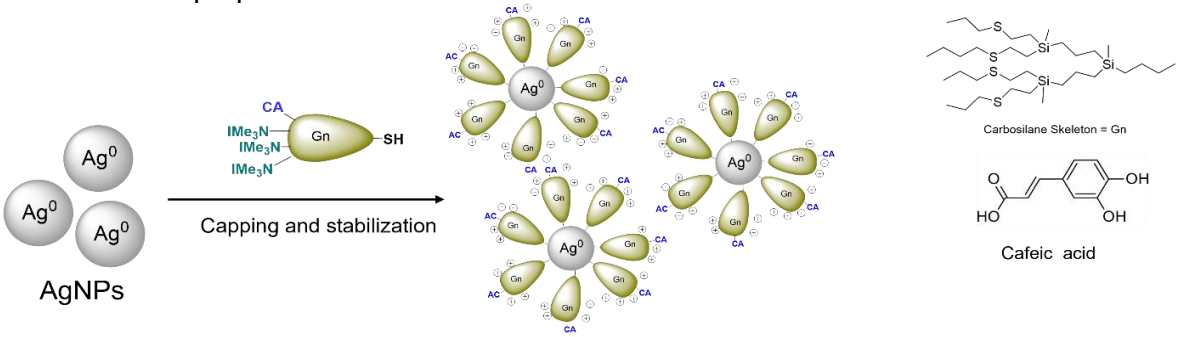

Figure 1: Synthesis of silver nanoparticles decorated with polyphenolic carbosilane dendrons

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P.1.12 - HETEROFUNCTIONALIZED POLYPHENOLIC CARBOSILANE DENDRIMERS DECORATED WITH CAFFEIC ACID: BOLOGICAL ACTIVITY

Lozano R.[a], Pena-Gonzalez C.E.[a], Grodzicka M.[b], Michlewska S.[c], , Bryszewska M.[b], Ionov M.[b], de la Mata F.J.[a,d], Ortega P.*[a,d]

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In the last decade, the nanotechnology has been improved by the development and discovery of a wide range of novel nanoparticles. One of them are dendrimers, monodispersed spheroid or globular macromolecular structures monodisperse, with a perfectly branched tree-like structure with repeated branches.

Carbosilane dendrimers present a lipophilic scaffold allows a greater biopermeability through the cell membranes than most of the dendritic systems described in the literature, which are mainly hydrophilic. The attachment of ionic groups in the periphery enables their solubility in water by compensating the lipophilic character of the skeleton, providing amphiphilic properties [1]. The possibility of modifying the functional groups at the periphery of the dendritic skeleton due to its multivalent surface demonstrated that conjugation of polyphenols with cationic carbosilane dendrimers could be a promising way of harnessing the potential of these powerful antioxidants, significantly increasing their bioavailability. Therefore, a new watersoluble family of carbosilane dendrimers functionalized with caffeic acid and surface ammonium groups has been synthesized. The most important outcome is that these dendritic systems have low toxicity and are highly effective against oxidative stress in vitro. Their antioxidant property could help to diminish oxidative haemolysis, lipid peroxidation, and ROS levels via their ability to scavenge free radicals. These findings are confirmed by various biological tests including in vitro studies on human fibroblasts, erythrocytes and A549 cells [2].

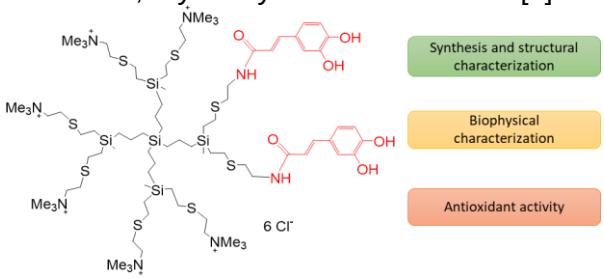

Figure 1: Heterofunctionalized polyphenolic carbosilane dendrimers decorated with caffeic acid

[1] de la Mata F.J., Gomez R., Cano J., Sanchez-Nieves J., Ortega P. & Garcia Gallego S., *WIREs Nanomedicine and Nanobiotechnology*, e1871, https://doi.org/10.1002/wnan.1871, 2022.
[2] Grodzicka M., Pena-Gonzalez C.E., Ortega P., Michlewska S., Lozano R., Bryszewska M., de la Mata F.J. & Ionov M., *Sustainable Materials and Technologies*, 33, e00497, https://doi.org/10.1016/j.susmat.2022.e00497, 2022.

P.1.14 - GREEN SYNTHESIS OF PHENOLIC PHYTOALEXINS AS NATURAL PESTICIDES

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Agriculture is facing some important challenges such as population growth, climate changes, and infections caused by plant pathogens and pests. The latter are becoming increasingly threatening due to the development of resistance to the common pesticides. Thus, new strategies to recover an equilibrium and increase the harvest production are urgently required. An important source of inspiration for the development of new antimicrobial agents is nature: as a response to pest infections, plants activate enzyme cascades resulting in the production of molecules called secondary metabolites active as antimicrobials. As part of our ongoing research program, we focused on the study of hydroxycinnamic acid dimers. Under biotic or abiotic stress conditions, hydroxycinnamic acids undergo dimerization reactions to give new natural compounds, endowed with increased structural complexity. These compounds, due to their highly defined tridimensional orientation, generally show a much more specific, and yet efficient, interaction with the biological target. The synthesis of these dimers was approached using green conditions and chemoenzymatic reactions. Further functionalization of the obtained compounds resulted in the production of a collection of optimized nature-derived analogues. The obtained compounds have been tested to evaluate the growth inhibition activity against a panel of plant pathogens (Figure 1).

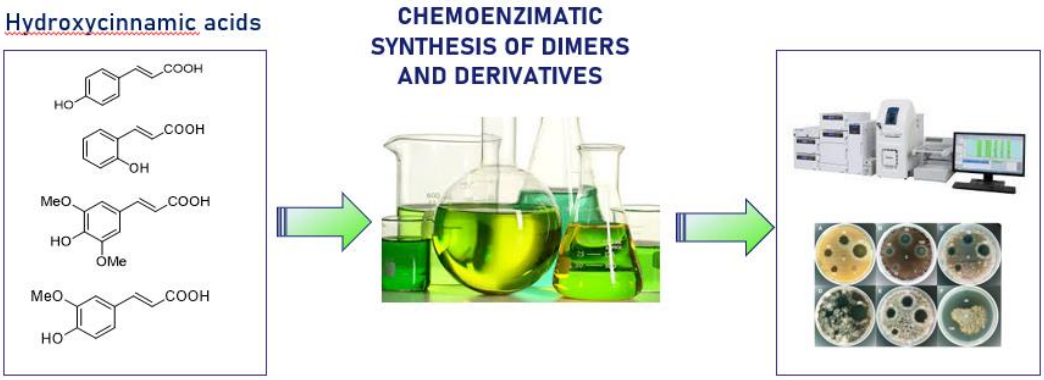

Figure 1: Reaserch design and methodologies

P.1.15 - HEMISYNTHESIS, NMR AND UHPLC-Q-ORBITRAP /MS² IDENTIFICATION OF CATECHIN OXIDATION PRODUCTS IN RED WINES AND GRAPE SEEDS

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(+)-Catechin—laccase oxidation dimeric standards were hemi-synthesized using laccase from *Trametes versicolor* in a water-ethanol solution at pH 3.6. Eight fractions corresponding to eight potential oxidation dimeric products were detected. The fractions profiles were compared with profiles obtained with two other oxidoreductases: polyphenoloxidase extracted from grapes and laccase from *Botrytis cinerea.* The profiles were very similar, although some minor differences suggested possible dissimilarities in the reactivity of these enzymes. Five fractions were then isolated and analyzed by 1D and 2D NMR spectroscopy. The addition of traces of cadmium nitrate in the samples solubilized in acetone-d_6 led to fully resolved NMR signals of phenolic protons, allowing the unambiguous structural determination of six reaction products, one of the fractions containing two enantiomers [1].
These products were then analyzed in grape seed extracts and red wines (UHPLC-Q-Orbitrap MS). The different dimers had different fragmentation patterns according to their interflavan linkage position. Oxidation dimeric compounds had a specific fragment ion at *m/z* 393, missing for B-Type dimers fragmentations. A fragment ion at *m/z* 291 occurred and was specific for oxidation dimeric compounds with a C-O-C linkage. Higher level oxidation products had abundant specific fragments: *m/z* 425, 397 and 245. These fragmentations were useful to identify them in complex samples such as grape seed extracts and wines. Three grape varieties at three ripening stages were selected and the corresponding seed extracts were obtained. The analyses revealed an increasing trend for the oxidation markers during grape ripening. On the opposite, the analysis of Syrah wines (2018, 2014, 2010) showed a decreasing trend of these molecules during wine ageing which might be due to further oxidation [2].

[1] Deshaies S., Le Guernevé C., Suc L., Mouls L., Garcia F. & Saucier, C. *Molecules*, 26, 6165, https://doi.org/10.3390/molecules26206165, 2021.
[2] Deshaies S., Sommerer N., Garcia F., Mouls L. & Saucier, C. *Food Chemistry*, 382, 132505, https://doi.org/10.1016/j.foodchem.2022.132505, 2022.

P.1.16 - TOWARD THE TOTAL SYNTHESIS OF VESCALAGIN, A BIOACTIVE NATURAL PRODUCT FROM THE *C*-GLUCOSIDIC ELLAGITANNIN FAMILY

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The *C*-glucosidic ellagitannins are natural polyphenols resulting from the specialized metabolism of certain plants [1]. (–)-Vescalagin, an emblematic member of this class of ellagitannins (Figure 1), is a water-soluble compound that can notably be extracted from oak (*Quercus*) or chestnut (*Castanea*) heartwood [2].

From a structural point of view, vescalagin features two atropoisomerically-defined bi- and teraryl units made up of galloyl units: a 4,6-hexahydroxydiphenoyl unit (HHDP) and a 2,3,5-nonahydroxyterphenoyl unit (NHTP), which are esterified onto a D-glucose core. Its characteristic structural element is the presence of a *C*-arylglucosidic bond linking the NHTP unit to the carbon-1 center of the glucose core.

Vescalagin is known to express various biological activities. For example, it acts as a preferential catalytic inhibitor of the α-isoform of the human DNA topoisomerase II, an enzyme targeted by anti-cancer drugs [2a,b]. Vescalagin also acts as an anti-actin agent capable of disrupting filamentous actin in cells [3a]. We later found that vescalagin expresses an actin-dependent inhibition of bone resorption by osteoclastic cells, making it a valuable candidate for the treatment of osteoporosis [3b]. These therapeutically-relevant biological effects and the complex chemical structure of vescalagin led us to engage in efforts towards its total synthesis, as well as that of analogues thereof, in the aim of further investigating its biological properties and its development as a pharmaceutical drug candidate.

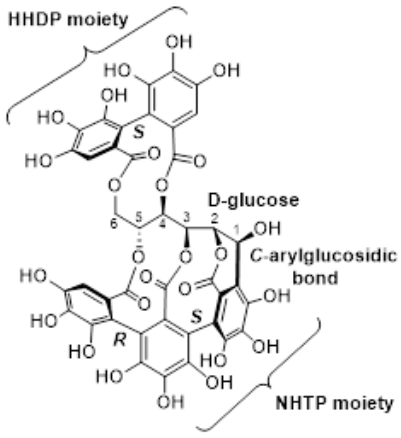

Figure 1: Structure of (–)-vescalagin

[1] Quideau S., Deffieux D., Douat-Casassus C. & Pouységu L., *Angew. Chem. Int. Ed.*, 50, 586-621, 2011.

[2] a) Quideau S., Jourdes M., Lefeuvre D., Montaudon D., Saucier C., Glories Y., Pardon P. & Pourquier P., *Chem. Eur. J.*, 11, 6503-6513, 2005. b) Auzanneau C., Montaudon D., Jacquet R., Puyo S., Pouységu L., Deffieux D., Elkaoukabi-Chaibi A., De Giorgi F., Ichas F., Quideau S. & Pourquier P., *Mol. Pharmacol.*, 82, 134-141, 2012.

[3] a) Quideau S., Douat-Casassus C., Delannoy López D.M., Di Primo C., Chassaing S., Jacquet R., Saltel F. & Genot E., *Angew. Chem. Int. Ed.*, 50, 5099-5104, 2011. b) Georgess D., Spuul P., Le Clainche C., Le Nihouannen D., Fremaux I., Dakhli T., Delannoy López D.M., Deffieux D., Jurdic P., Quideau S. & Génot E., *Eur. J. Cell Biol.*, 97, 533-545, 2018.

P.1.17 - CHEMICAL VERIFICATION OF AXIAL CHIRALITY GENERATION IN ELLAGITANNINS

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Over 1,000 ellagitannins, which are a class of natural polyphenols, have been isolated. Ellagitannins possess a fundamental structure consisting of D-glucose esterized with a hexahydroxydiphenoyl (HHDP) group containing a C–C bond formed by coupling two galloyl groups. The position where the HHDP group bridges on D-glucose determines which side is formed with its axial chirality. The axial chirality is predominantly *S*-type in ellagitannins where the HHDP group is located at 4-oxygen position (O-4) and O-6 on D-glucose. Among the more than 1,000 known ellagitannin structures, only four natural products with *R*-axial chirality of the 4,6-*O*-HHDP group are known to exist. The biosynthetic pathway for these 4,6-*O*-(*R*)-HHDP structures has been entirely unknown.

We investigated chemically-generated HHDP-group axial chirality in ellagitannins [1]. To verify the formation mechanism of the HHDP group axial chirality, we employed our 2nd generation method [2]. The chemical oxidation reaction resulted in proceeding with biaryl bond formation of the 4,6-*O*-HHDP group, followed by isomerization of the axial chirality from *R*- to *S*-configuration (Figure 1). This study firstly indicated the atrop isomerization from “mismatch” to “match” chirality on the sugar original chiral environment in ellagitannins. The HHDP group potentially isomerizes to the thermodynamically “match” chirality, especially (*S*)-HHDP group on O-4 and O-6 of D-glucose in ellagitannins. Although this study revealed the 4,6-*O*-HHDP group isomerization process under the chemical oxidation condition, the biosynthetic reaction could also include the isomerization process. Furthermore, the phenomenon elucidated in this study is expected to be similar to that involved in producing the HHDP moiety at other positions in natural ellagitannins.

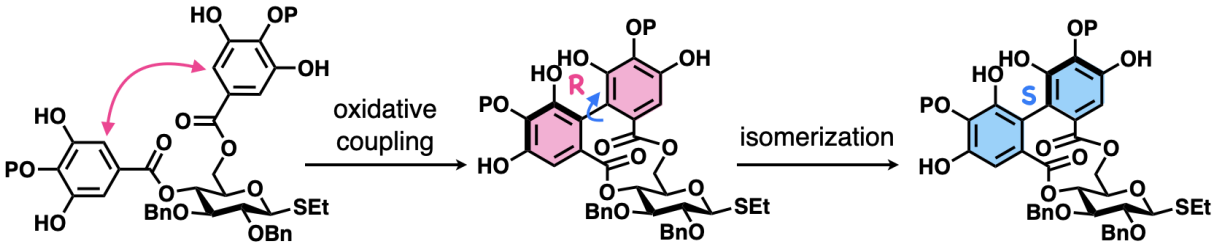

Figure 1: Reaction mechanism of HHDP group construction in ellagitannins.

[1] Wakamori S., Osada R., Matsumoto S., Kusuki R. & Murakami K., *Org. Lett.*, 24, 8130–8135, 2022.
[2] Matsumoto S., Wakamori S., Nishii K., Tanaka T. & Yamada H., *Synlett*, 31, 1389–1393, 2020.

P.1.18 - NEW HOST-GUEST SYSTEMS BASED ON MACROCYCLIC-PYRANOFLAVYLIUM COMPLEXES FOR BIOGENIC AMINES SENSING

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Biogenic amines (BAs) are biologically active nitrogen-containing compounds, formed in the normal metabolism of animals, plants, and micro-organisms. The presence of these biomolecules in food products is undesirable and their ingestion in significant amounts can cause headaches, respiratory distress, heart palpitations, and several allergenic disorders. Host-guest chemistry is a subfield of supramolecular chemistry focused on molecular recognition (noncovalent binding) of small molecules or ions (guests) by larger receptors (hosts) to form a host-guest complex with applications in sensing and drug delivery [1]. The main goal of this work was to design new host-guest systems based on the complexation of water-soluble sulfonated-based receptor hosts (calix[n]arenes and captisol) with positively charged guests such as the pH-sensitive flavylium-based dyes [2]. The response of the systems towards BAs detection was carried out by UV-Vis, fluorescence, and ^{1}H NMR spectroscopic techniques [3]. The host-guest systems were optimized in terms of dye concentration, host-guest molecular ratio, and working pH which was set taken in account the maximum differences between the pK_a of the free dye (6.72) to that of the complexes (*e.g.,* p$K_{a\ SC8\text{-}dye}$ 8.45). Overall, the host-flavylium cation complexes were able to detect putrescine/tyramine with concomitant release of the neutral quinoidal base species of dye to the bulk, recording a colorimetric variation from yellow to pink-red (Figure 1). The BAs sensing was also followed by fluorescence, showing to be host structurally dependent: a fluorescence enhancement for calix[n]arene:dye complexes were observed whereas a fluorescence quenching for the captisol:dye complex was registered. The ^{1}H NMR data confirmed the two distinct interaction mechanism modes for the different systems studied.

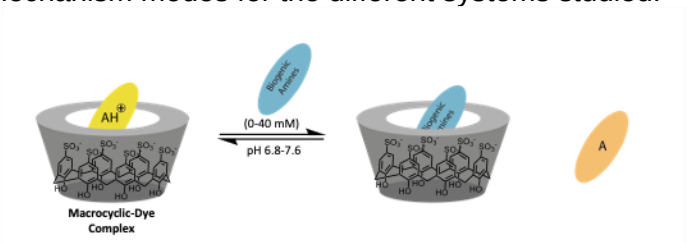

Figure 1: Strategy developed for sensing/capture BAs using molecular switch-type systems.

[1] Qu D.-H., Wang Q.-C., Zhang Q.-W.*et al.*, *Chem. Rev.*, 115, 7543–7588, 2015.
[2] Cruz L., Basílio N., Mateus N. *et al.*, *Chem. Rev.*, 122, 1416–1481, 2022.
[3] Romero M.A., Mateus P., Matos B. *et al.*, *J. Org. Chem.*, 84, 10852–10859, 2019.

Funding
This work was supported by the Associate Laboratory for Sustainable Chemistry, Clean Processes and Technologies LAQV and financed by national funds from UIDB/50006/2020.

Acknowledgements
Ana Pires and Vânia Gomes acknowledge the PhD grants from FCT (BD2021.08670.BD and SFRH/BD/136556/2018, respectively). Luís Cruz acknowledges the FCT research contract (DL 57/2016/CP1334/CT0008).

P.1.19 - CONDENSATION OF LIGNEOUS MOTIFS WITH AROMATIC NUCLEOPHILES

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Lignocellulosic biomass represents a major concern for the emancipation of fossil resources used in chemistry. Especially, lignins are an abundant source of phenolic platform molecules. However, lignins are often degraded in fractionation and extraction conditions. The degradation is due to condensation reactions between ligneous motifs. The leaving of the hydroxyl group linked to the benzylic carbon after protonation leads to the formation of benzylic carbocation which will react with aromatic ring from another motif (Figure 1 bottom).
Implementation of a competitive nucleophile should prevent unwanted rearrangements reactions. Our research team has established a method of nucleophilic substitution with metallole derivatives in acidic conditions on model molecules [1] (Figure 1 top). Applied to native biomass, the process has to integrate the complexity of the raw material, regarding to the arrangement in space and to the chemical composition.
The first aim of our project is to study the reactivity of the ligneous motifs with regard to the influence of the substitution of the aromatic ring (G, H or S) when applying condensation reaction with various nucleophiles. The second aim is to evaluate how the other components of lignocellulosic biomass impact the reactivity, notably with hemicelluloses.
To carry out the project, ligneous models with one or two beta-O-4 bonds were synthesized to study their reactivities, in an experimental way and by using quantum chemistry (DFT, DFTB, COSMO-RS). The next step is to add hemicelluloses oligomers in the reaction mixture, before the application on organosolv lignins, and, at the end, on native biomass. Specific analytical methods have to be developed for the study of these complex ligneous structures. For that, gas chromatography coupled with pyrolysis and mass spectrometry will be used.

When a furan derivative is used as competitive nucleophile (i.e., sylvan or menthofurane), the first results suggest a better reactivity of benzylic carbon bonded with guaiacyl (G) ring than with syringyl (S) ring.

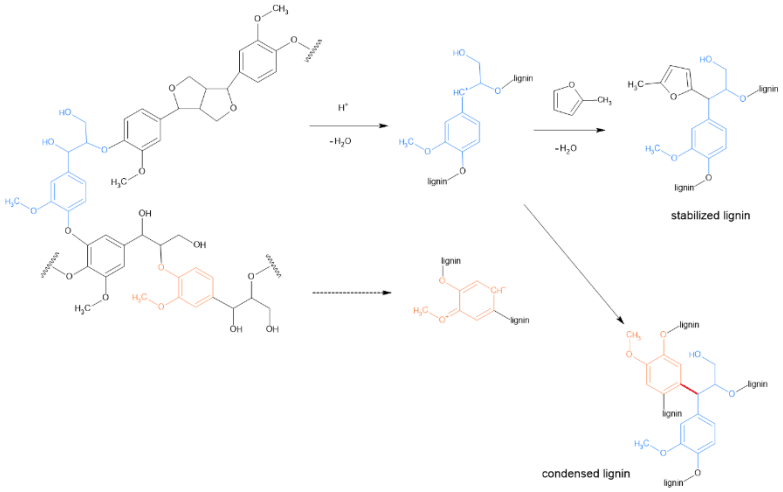

Figure 1: Scheme of condensation and substitution reactions

[1] Roumeas L., Billerach G., Dubreucq, E. & Fulcrand H., Chemically stable lignin derivative and method for preparing same, EP18778493, 2018.

P.1.20 – UNVEILING THE COOPERATIVE SELF-ASSEMBLY MECHANISM OF A PYRANOFLAVYLIUM DYE

Borges A.[a], de Freitas V.[a], Oliveira J.*[a], Basílio N.[b]

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Over the years, the mechanisms behind supramolecular polymerization have been a target of study as their complexity depends on different non-covalent interactions namely π-π stacking, hydrogen bonding, dipole moment, and van der Walls interactions that altogether govern the size and structure of the aggregates [1]. In the most frequently observed mechanism, known as the isodesmic model, the addition of monomers to oligomers or to other monomers to obtain a polymer Is independent of the length of the polymer. Therefore, every monomer addition is ruled by a single value of K, resulting in highly polydisperse self-assembled aggregates. On the other hand, cooperative self-assembly processes follow a two-step mechanism which consists in a nucleation step, where a critical size aggregate is formed, followed by an energetically more favorable elongation step that accounts for the association of monomers to this nucleus. Supramolecular cooperative mechanisms are observed in a great number of important biological processes namely the polymerization of actin and islet amyloid polypeptide [2]. In this work, the cooperative self-assembly mechanism of a pyranoflavylium dye is reported for the first time. At pH 2, pyranoflavylium dye presents a very intense purple color that with time turns into a salmon one (Figure 1). Low pH and temperatures and high dye and salt concentrations influence the kinetics of aggregation, especially the nucleation process step.

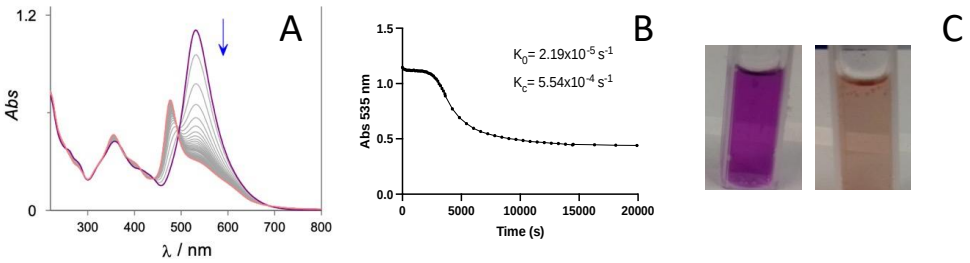

Figure 1: A, UV-Visible spectra of pyranoflavylium dye in ethanol: water (1:9) at pH 2 over time; **B,** Fitting of the data obtained from A and respective kinetic constants**; C,** Colour of the solutions after preparation (left) and aggregation (right).

[1] Dhiman S., Sarkar A. & George S.J., *RSC advances*, 8, 18913-18925, 2018.
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This research is supported by the AgriFood XXI I&D&I project (NORTE-01-0145-FEDER-000041) co-financed by European Regional Development Fund (ERDF), through the NORTE 2020 (Programa Operacional Regional do Norte 2014/2020). A.B. gratefully acknowledges her doctoral grant from the Associated Laboratory for Sustainable Chemistry, Clean Processes, and Technologies LAQV (UI/BD/151270/2021). J.O and N.B. would like to thank their contracts (2022.00042.CEECIND and CEECIND/00466/2017, respectively).

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P.1.21– POLYPHENOLS OR MELANOIDINS: AN ANALYTICAL PARADOX

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Melanoidins or Maillard Reaction Products (MRPs) are produced by post-harvest heat processing of whole and selected plant materials, fruit juices, their extracts & concentrates (sometimes referred to as "molasses"). Melanoidin formation is therefore driven by the presence of precursors such as naturally-occurring sugars, carbohydrates, poly- and oligo-saccharides, proteins, peptides and amino acids.

Technically speaking, MRPs are not naturally occurring**,** as they are formed from natural precursor compounds exposed to heat, pH and concentration gradients. This results in the incorporation of other naturally occurring plant phytochemicals or secondary metabolites, such as phenolic acids and flavonoids, into complex low and high molecular weight polymers, called melanoidins [1].

As polyphenols, flavonoids and melanoidins reportedly share some similar biological properties (*in vitro* antioxidant, anti-inflammation, metal chelation, enzyme inhibition, anti-microbial properties, etc), it is difficult to delineate which of these groups of compounds are the main contributors to mechanism of action in samples containing both polyphenols and melanoidins. In addition, analytical procedures using colorimetric reagents to track/measure *in vitro* antioxidant capacity such as DPPH, ABTS and FRAP add to the uncertainty since both polyphenols and melanoidins react [2].

Furthermore, commonly used *in vitro* colorimetric procedures, such as the Folin Ciocalteu (FC) reagent and the aluminium chloride / keto/ hydroxyl group reagent ($AlCl_3$) for polyphenols are not specific, due to interference not only from melanoidins but also from many other compounds. This results in a significant over-estimation of the level of "total" polyphenols in most processed plant extracts.

Accurate and quantitative measurement of polyphenols and flavonoids in plants must be done using $LCMS^n$ techniques against pure known individual reference compounds. Melanoidins, generated during processing of sugarcane into sugarcane juice, for example, significantly result in the overestimation of total polyphenols using the FC reagent even when using a reference standard of gallic acid equivalents, or the $AlCl_3$ reagent for total flavonoids (TF) using catechin equivalents. This means that TPP and TF data reported from many plant-derived sources that have undergone some post-harvest heating exposure are likely to report overestimated TPP and TF results [3], explaining why LCMS-based analyses often do not match up to total polyphenol measurements.

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P.1.22– A-TYPE PROCYANIDINS: FORMATION KINETICS, REGIOSELECTIVITY AND INTERACTION WITH PEPTIDE

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Oxygen plays a key role in wine chemistry and especially in the evolution of the non-volatile matrix. Condensed tannins composition and structure, as well as their polymerisation are directly impacted by oxidation. Condensed tannins play an important role in red wine and exhibit a wide diversity in their structure and properties such as wine colour evolution (i.e. formation of polymeric pigment with anthocyanin), texture (i.e. astringency) and taste (i.e. bitterness). The condensed tannins matrix evolution affects directly the red wine sensory properties. During wine oxidative evolution, new tannins linkages can be created like A-type linkage composed by an additional intramolecular bond form of the B-type procyanidins [1]. Their hemisynthesis have been reported in the literature [2] but A-type procyanidins properties are still under investigation. The aim of this study was to study the different properties of the A-type procyanidins. On one hand, the impact of the pH on the regioselectivity of the A-type linkage on an oligomeric procyanidins chain have been studied. Then, the interaction of the obtained A-type procyanidins tetramer with some peptides and proteins have been explored to determine the sensory properties of the A-type procyanidins. During the first part, the regioselectivity of the A-type procyanidins formation have been followed by UPLC-UV-QTOF. A correlation between the pH and the A-link formation in the oligomeric structure have been determined. An HRMS-MS study as well as NMR characterisation was used to determine the position of the A-type linkage along the oligomeric procyanidin chain. In a second part, the interaction between the A-type tetramer procyanidins and a peptide have been evaluated and compared to the B-type procyanidins complex. Different interaction intensities have been measured due to the structure difference between the A-type procyanidins due to the position of the A-type linkage. These results have a direct impact on the sensory properties of the A and B-type procyanidins. Moreover, different sensory analyses have been performed in order to compare A-type and B-type procyanidins.

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P.1.23 - POLYPHENOLS WITH ANTIGLYCATION PROPERTIES IN *TRAPA* PERICARP EXTRACT

Iwaoka Y.*[a], Hayakawa C.[a], Suzuki S.[a], Kato N.[a], Kawabe S.[a], Ganeko N.[a], Uemura T.[b], Ito H.[a]

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Trapa bispinosa belonging to the family Lythraceae, an annual aquatic plant, originally distributed in Southeast Asia, and the cultivation is widely extended to Southern Europe, Africa, and Asia. The pericarp extract of *T. bispinosa* has been reported to inhibit the α-glucosidase and glycation reactions. The components having these properties of this source remain still unknown. In this study, we investigated the isolation and characterization of polyphenols from the pericarps of *T. bispinosa*, and evaluation the antiglycation effects of the isolated compounds. In addition, polyphenols in *T. bispinosa* showing antiglycation property were quantified by high-performance liquid chromatography coupled to ultraviolet detection and electrospray ionization mass spectrometry (LC/UV/ESIMS) analysis.

The 70% aqueous acetone extract of *T. bispinosa* pericarps was evaporated and subjected to column chromatography over Toyopearl HW-40, to yield 13 known polyphenols including gallotannins and ellagitannins. To search for compounds with antiglycation activity also from the relative plant, the 70% aqueous acetone homogenate of pericarps of *T. japonica* was similarly subjected to column chromatography, to give a new ellagitannin along with three known tannins. The known compounds were identified by direct comparison with authentic specimens or comparison of their physicochemical data with those reported in the literature. The structure of the new compound named trabisnin was determined as 6-*O*-brevifolincarboxyloyl-1,2,3-tri-*O*-galloyl-β-D-glucose, based on 1D- and 2D-NMR, MS spectral data and chemical evidencI.

The isolated compounds from the pericarps of *T. bispinosa* were evaluated for the inhibitory effect of advanced glycation end products (AGEs) formation generated by the glycation reaction Iween HSA and glucose or fructose, and the AGE cross-link cleaving effects. Gallotannins and ellagitannins showed more significant inhibitory effect on AGE formation than those of low molecular polyphenols such as gallic acid. Furthermore, gallic acid showed most potent AGE-derived crosslink cleaving activity among the tested polyphenols.

A total of thirty pIlyphenols in *T. bispinosa* pericarps were comprehensively identified by LC/UV/ESIMS analysis. The contents of tannins and the related polyphenols were also analyzed using LC/UV/ESIMS, indicating that gallic acid, gallotannins, and ellagitannins showing antiglycation effects were contained the major level in the pericarps of *T. bispinosa* [1].

These findings suggested the *T. bispinosa* polyphenols are good source for antiglycation effects, which could be applied as functional foods or nutritional supplements to improve human health benefits.

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P.1.24 - STUDY OF THE EVOLUTION OF TANNINS DURING WINE AGEING BY MASS SPECTROMETRY MONITORING OF OXIDATION MARKERS RELEASED AFTER CHEMICAL DEPOLYMERIZATION

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Among the many compounds in wine, condensed tannins play an important role in the organoleptic properties of the products; they are partly responsible for astringency, bitterness and also contribute to the color. This research work aims to study the oxidation state of these bio-heteropolymers which is an important lock in the analysis of processed products in order to better control their quality. Indeed, their identification remains at present a challenge because of the large heterogeneity of their degrees of polymerization (DP) based on 4 monomers (epicatechin, catechin, epigallocatechin, epicatechin-3-*O*-gallate) thus multiplying the number of oxidation products. Due to the difficulties of separation by liquid chromatography and detection by mass spectrometry of tannins with high DP [1], tannins were analyzed by UHPLC-ESI-MS after chemical depolymerization. This pre-treatment of the samples allows the cleavage of the interflavanic bonds linking the constituent monomers of the tannins and gives access to the average DP and the proportions of the different monomers. However, in wines, many reactions take place from the beginning of the wine making process to its consumption. Within the tannin structures, the new covalent bonds created by oxidation are resistant to depolymerization conditions and oxidation markers (dimers and trimers) are then obtained. These structural modifications distort to a greater or lesser extent the estimation of the average DP depending on the oxidation state of the tannins. Faced with the complexity and the large number of oxidation products generated, over the last ten years a study conducted on model solutions has allowed the identification of more than one hundred oxidation markers [2,3].
Thanks to the detection and identification of these oxidation markers, an in-depth study of the tannin fraction of wines has recently allowed us to understand their evolutions during ageing. Three Syrah wines (2018, 2014 and 2010 vintages) were analyzed. An accelerated oxidation of the 2018 vintage sample was also performed in order to evaluate the impact of this oxidation compared to the natural evolution and evaluate the ability of this oxidation to imitate natural evolution. The monitoring of 6 types of extension, extension/terminal and terminal markers at two oxidation levels was investigated. An evolution of the tannin oxidation state during ageing by the increase of the markers of the second oxidation level over the vintages was observed. In the 2018 oxidized wine sample, the first oxidation level markers are similar to the 2014 vintage but the second oxidation level markers are higher than other vintages, indicating a more advanced state of tannin oxidation. This study showed for the first time that it was possible to follow the oxidative evolution of wine tannins by monitoring some relevant dimeric tannin oxidation markers generated after chemical depolymerization.

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[2] Mouls L. & Fulcrand H., *Journal of Mass Spectrometry,* 47, 1450-1457, 2012.
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P.1.25 - RATIONAL DESIGN MODIFIES REGIOSPECIFICITY OF CATECHOL O-METHYLTRANSFERASE: FROM VANILLOID TO ISOVANILLOID MOTIFS IN FLAVONOIDS

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A rational redesign was performed on the substrate pocket of phenylpropanoid-flavonoid O-methyltransferase (PFOMT) from *Mesembryanthemum crystallinum* [1]. This enzyme specifically methylates the 3'-position (meta-position) in catechol moieties of flavonoids to guaiacol moieties. The aim was to generate variants with the opposite, 4'- (para-) regioselectivity and improved catalytic efficiency. Amino acids which occur with high frequency in cation-independent class I OMTs possessing a different substrate spectrum or regiospecificity than PFOMT were introduced into the substrate pocket at non-conserved positions. By employing this approach, a double variant (Y51R/N202W) was constructed using a newly developed colorimetric assay based on the complexation of catechol moieties with the representative substrate caffeic acid by ferric ions. This variant effectively modified the para-position in flavanone and flavanonol substrates, enabling the synthesis of the sweetener molecule hesperetin and other rare plant flavonoids with an isovanilloid motif.

[1] Dippe, M., Davari, M. D., Weigel, B., Heinke, R., Vogt, T., & Wessjohann, L. A., *ChemCatChem*, 14, e202200511, https://doi.org/10.1002/cctc.202200511, 2022

P.1.26 - STRUCTURAL CHARACTERIZATION OF POLYPHENOLS IN PERSIMMON FRUIT

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Persimmon fruits (*Diospyros kaki Thunb*.) is an edible and medicinal plant of the genus of persimmon in the Persicaceae family. Polyphenols are the main biological components in persimmon fruit including both low-molecular-mass compounds such as flavonols, flavanols and phenolic acids [1], and high-molecular-mass compounds known as polymeric proanthocyanidins [2]. In this study, by UPLC-Q-Exactive-Orbitrap/MS, total of 50 low-molecular-mass polyphenols in persimmon fruits were identified, including 28 flavonols, 10 phenolic acids, 8 flavanols, 2 other compounds, 1 flavanone and 1 dihydroflavonol, among which 44 compounds were quantified by UPLC-QqQ-MS/MS. For structural characterization of polymeric polyphenols in persimmon fruit, tiopronin, as a thiol-containing nucleophile, was introduced for depolymerizing polymeric polyphenols into low molecular weight fragments. Then the degradation fragments were qualitatively and quantitatively analyzed by UPLC-Q-Exactive-Orbitrap/MS and UPLC-QqQ-MS/MS. The results indicated that the polymeric polyphenols in persimmon fruits were consisted of, in addition to (+)-catechin, (–)-epicatechin, gallocatechin, (–)-epigallocatechin, gallocatechin-3-*O*-gallate, (–)-epigallocatechin-3-*O*-gallate, (+)-catechin-3-*O*-gallate and (–)-epicatechin-3-*O*-gallate as proanthocyanidin constitutive extension or terminal units (Figure 1), also anthocyanins, flavonols and phenolic acids exclusively as terminal units. For the first time, the phenolic composition of both oligomers and polymers in persimmon fruits were comprehensively analysed.

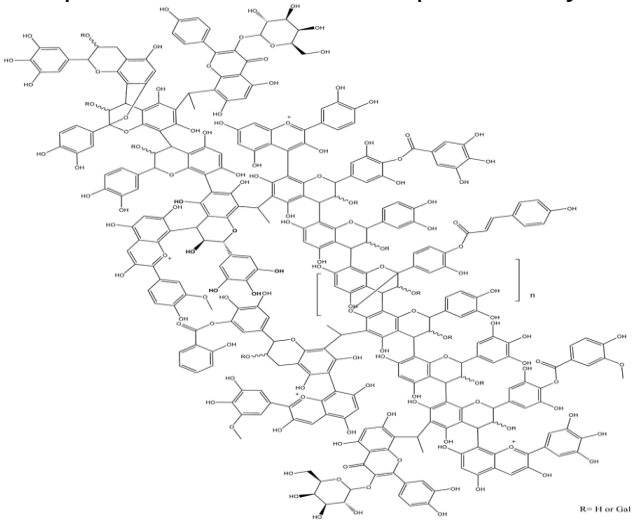

Figure 1: Postulated structure of polymeric polyphenols in persimmon fruits

[1] Heras, R.M.L., Quifer-Rada P., Andrés A. & Lamuela-Raventós R., *Journal of Functional Foods*, 23, 370-377, 2016.
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TOPIC 2 - BIOACTIVITY, BIOAVAILABILITY & MICROBIOTA

PL.2.01 - CROSSTALK BETWEEN PHENOLIC COMPOUNDS AND BIOLOGICAL RHYTHMS

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(Poly)phenols are important plant food compounds that exert health effects. Proanthocyanins (PACs) are a class of polyphenols composed of flavanol polymers and their gallate derivatives. Our group recently demonstrated that their bioavailability and bioactivity depend on biological rhythms [1]. Nevertheless, the interaction between phenolic compounds and biological rhythms seems to be bidirectional (Figure 1), as some of them have demonstrated their activity as modulators of circadian and seasonal rhythms [2]. In this regard, our group demonstrated that PACs can modulate central and peripheral biological rhythms, both in healthy animals under jet-lag conditions and in obese rats. The interaction of phenolic compounds with the clock system has recently been postulated to be a potential mechanism for their beneficial effects [2].

Moreover, the results of phenol-rich fruits administered to rats exposed to different photoperiods to simulate the specific seasons showed that fruit seasonality and their geographical origin determine a distinctive phenolic hallmark from the environment that influences their health effects depending on the photoperiod conditions [3].

In conclusion, there is a bidirectional interaction between dietary phenolic compounds and biological rhythms, and the time of the day and season in which they are consumed influences their health effects, which, in turn, could be mediated by the interaction of these compounds with the clock system, acting as synchronizers.

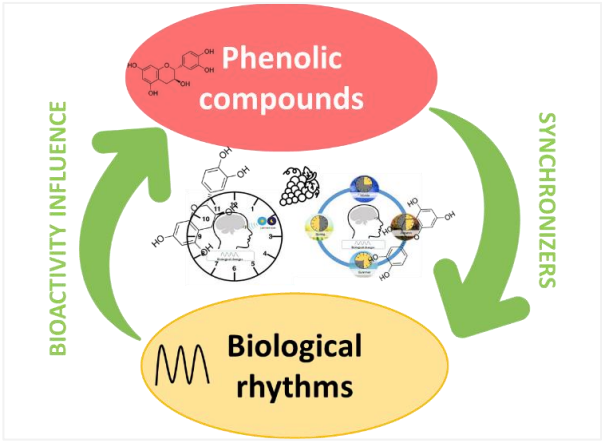

Figure 1: Bidirectional interaction between phenolic compounds and biological rhythms.

[1] Arola-Arnal A., Cruz-Carrión Á., Torres-Fuentes C., Ávila-Román J., Aragonès G., Mulero M., Bravo F.I., Muguerza B., Arola L. & Suárez M., *Nutrients*, 11, 2602, https://doi.org/10.3390/nu11112602, 2019.

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[3] Cruz-Carrión Á., de Azua M. J. R., Bravo F. I., Muguerza B., Suárez M. & Arola-Arnal A., *Frontiers for young minds*, 10, 754655, https://kids.frontiersin.org/articles/10.3389/frym.2022.754655, 2022.

PL.2.02 - POTENTIAL OF POLYPHENOL-RICH PRODUCTS TO PREVENT AND/OR IMPROVE ENDOTHELIAL DYSFUNCTION AND SENESCENCE: FOCUS ON ANTHOCYANINS

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Endothelial dysfunction is now well established as a pivotal early event in the development of major cardiovascular diseases including hypertension, atherosclerosis, diabetes and also ageing. The alteration of the endothelial function is often triggered by an imbalance between the endothelial formation of vasoprotective factors including nitric oxide (NO) and endothelium-dependent hyperpolarization (EDH), and an increased level of oxidative stress involving several pro-oxidant enzymes such as NADPH oxidase and, often also, the appearance of cyclooxygenase-derived vasoconstrictors [1]. In addition, endothelial senescence has been reported to be an early trigger of endothelial dysfunction [2].
Pre-clinical studies have indicated that polyphenol-rich food, including anthocyanin-rich products, can activate pathways promoting an increased formation of vasoprotective factors including NO and EDH, and can prevent the induction of endothelial senescence and dysfunction in endothelial cells and isolated blood vessels [3a,b]. Similarly, intake of anthocyanin-rich products has been associated with the prevention and/or the improvement of an established endothelial dysfunction in several experimental models of cardiovascular diseases, including physiological ageing [3a,b]. Moreover, clinical data indicate that polyphenol-rich and anthocyanin-rich products can improve the endothelial function and the vascular health in Humans with cardiovascular diseases [3b,c].
This presentation will discuss both experimental and clinical evidences indicating that several polyphenol-rich foods and natural products, and especially anthocyanin-rich products, are able to promote endothelial and vascular health, as well as the underlying mechanisms.

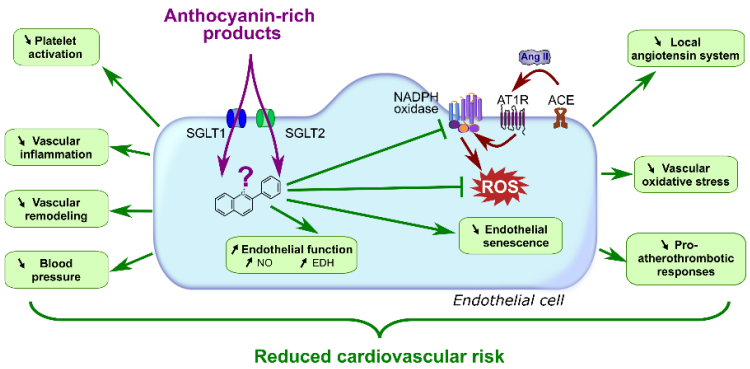

Figure 1: Anthocyanins and anthocyanin-rich products improve the endothelial function by several mechanisms leading to decreased cardiovascular risk

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O.2.01 - WHEN (POLY)PHENOLS MEET LIPIDS: IMPLICATIONS OF PLASMA (NUTRI)METABOLOMICS ON ENDOTHELIAL MEMBRANE BIOPHYSICS

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Processed and ready-to-eat foods are routinely consumed resulting in a sharp rise of sugar intake in people's daily diets. The incidence of diabetes on the worldwide population has tripled in the past 5 decades but even more alarming is the number of undiagnosed people and the increasing incidence of diabetes among children and young adults likely to escalate the socio-economic burden of diabetes in the near future. Epidemiological and intervention studies have shown that the ingestion of (poly)phenol-rich foods lead to an overall improvement of fasting glucose levels in healthy individuals as well as in diabetic patients, thus reducing the risk of type-2 diabetes onset and associated complications.

Research in our group has shown that the cargo of (poly)phenol microbial metabolites in circulation in patients with type-2 diabetes mellitus (T2DM) is greatly reduced when compared to normoglycemic donors. In spite of the reduced bioavailability, *in vitro* studies on cultured endothelial cells grown under glucotoxic conditions have shown that incubation with (poly)phenol microbial metabolites, at physiologically relevant concentration and residence times, were able to decrease the release of inflammatory IL-6 cytokines thus ameliorating the glucose-induced inflammatory response [1].

In view of the extracellular biophysical polyphenol-lipid interaction able to trigger an intracellular biological event, very little is known on how bioactive (poly)phenol compounds and their metabolites permeate the endothelial barrier to interact with membrane proteins and exert the well-recognised signalling response. In this work, we present our findings on *in vitro* membrane and cell models designed to investigate: 1) the lipid remodelling of plasma membranes in endothelial cells during hyperglycemia [2], 2) the impact of lipid remodelling on the lipid bilayer organisation [3], and 3) the role of (poly)phenols on the modulation of the membrane's biophysical properties and how this is reflected on the activity of membrane proteins. Findings from this study will improve our understanding on the permeability and transport of bioactive (poly)phenols across cholesterol-rich endothelial membranes in diet-related diseases.

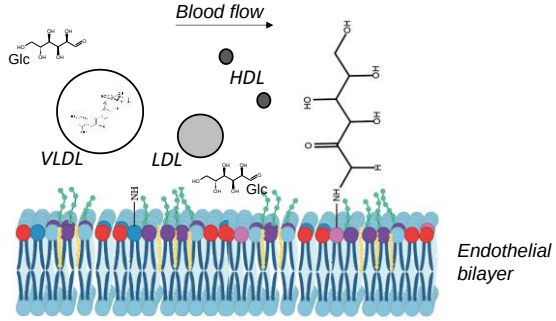

Figure 1: Schematic representation of lipoprotein-transported (poly)phenol metabolites interaction with endothelial lipid bilayer in hyperglycemic conditions.

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O.2.02 - INTERACTIONS WITH TWO MEMBRANE MODELS OF LEAF EXTRACTS OF *C. FLAMMULA* AS UNDERLYING MECHANISMS OF ANTI-INFLAMMATORY ACTIVITIES

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Inflammation is a normal physiological defense mechanism triggered by injury to the cell. However, it should not be aggravated by the release of neutrophils lysosomal content that might lead to cell lysis [1]. *Clematis flammula* is widespread in the Mediterranean basin. In Algeria, the leaves of this plant are traditionally used in the treatment of rheumatism. Five extracts of *C. flammula* leaves generated by a selective extraction procedure were tested on two membrane models: liposomes and erythrocytes (representing lysosomal model), in order to assess their anti-inflammatory potential. Moreover, the characterization of these interactions was carried out by HPLC-MS analysis. The tests on the erythrocytes included hemolysis (toxicity) and anti-hemolysis activities. *In vitro* tests of anti-trypsin and inhibition of denaturation potentials were also performed. Extracts were equally tested on liposomes to assess lysis and leakage potentials. Anti-bacterial tests were conducted. Results showed that *C. flammula* ethyl acetate (EA) extract was the most potent against *S. aureus* and *E. coli* (MIC of 78 and 1250 µg/mL respectively), presumably related to its high lytic activity of liposomes (71.22 ± 3.62 %). It equally exhibited the highest inhibition of protein d©naturation (IC50 = 580 ± 15.2 µg/mL), probably attributed to its high polyphenol content (192.64 ± 1.51 mg GAE/g E). On the other hand, t©chloroform (C) fraction revealed strong anti-inflammatory activity with the highest anti-hemolytic and anti-trypsin activities (IC50 = 7.23 ± 0.42 and 9.68 ± 0.08 µg/mL, respectively). HPLC-MS analysis revealed the presence of flavonoids mainly flavones and flavonols (isorhamnetin) (Figure 1).

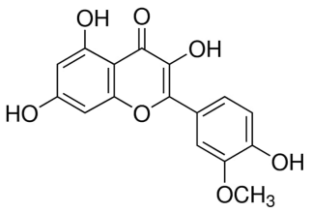

Figure 1: Isorhamnetin identified in ethyl acetate fraction of *C. flammula*

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O.2.03 - PRODUCTION OF ESCULETIN FROM 5-*O*-CAFFEOYLQUINIC ACID BY A GUT BACTERIUM AND ITS IMPACT ON THEIR GUT BARRIER INTEGRITY

Balaj G.[a], Bousarghin L.[b], Tamanai-Shacoori Z.[b], Gautier T.[b], Sauvager A.[a], Nebija D.[d], David-Le Gall S.[b], Guyot S.[c], Tomasi S.*[a], Abasq M.L.*[a]

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Polyphenols, provided by the consumption of fruits and vegetables, have a positive effect on human health due to their antioxidant properties and their ability to modulate the redox signaling pathways. These compounds are biotransformed by the gut microbiota into low molecular weight metabolites with higher bioavaibility in systemic than the parent polyphenol, which may lead to a higher bioactivity [1]. Thus, we have studied the biotransformation of 5-*O*-caffeoylquinic acid (5-CQA), a dietary polyphenol by *Lactobacillus reuteri, Bacteroides fragilis and Bifidobacterium longum*, belonging to the dominant bacterial phyla of the gut microbiota. The biotransformed extracts were analysed by LC-MS/MS and the data were subject to MzMine 2.53 and GNPS treatment for subsequent molecular networking analysis. *L. reuteri* was only able to bioconverse 5-CQA into several metabolites, including caffeic acid (CA) and a known natural compound esculetin [2]. This study put in the spotlight, for the first time, an interesting oxidative pathway carried out by gut bacteria. Considering that 5-CQA reach the colon at µM concentration and its microbial metabolites such as CA and esculetin could be close to the gut epithelium when produced, we have investigated whether these compounds could have a direct impact on epithelial barrier integrity using a quadruple *in vitro* model [3]. Different measurements were evaluated in presence of the tested polyphenols: cell viability, transepithelial electrical resistance (TEER) and gene expression such as *occludin* and *ZO-1*. Esculetin exhibited finally a beneficial effect on this integrity compared to the parent polyphenol (5-CQA) and one of the other biotransformed metabolites (CA) (Figure 1).

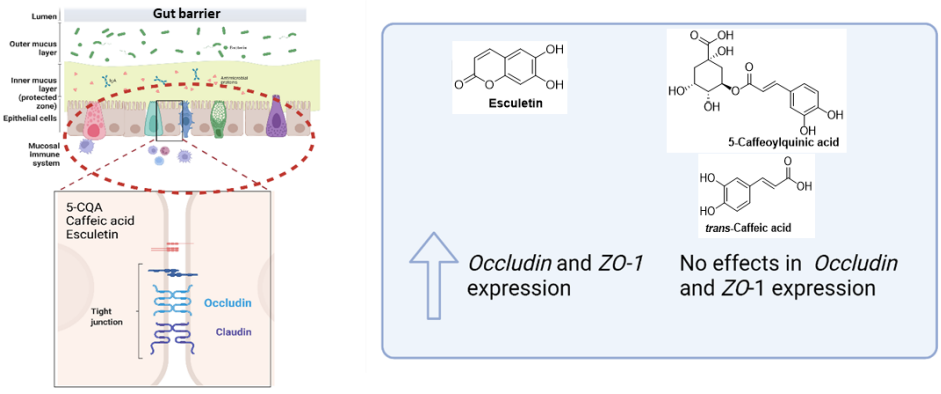

Figure 1: Impact of 5-CQA, CA and esculetin on the gut barrier integrity

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O.2.04 - POLYPHENOL-RICH CRANBERRY JUICE FOR SIX WEEKS AFFECTED UV-INDUCED ERYTHEMA, SKIN CONDITIONS, AND INFLAMMATION IN WOMEN IN A RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED, CROSS-OVER TRIAL

Christman L.[a], De Benedetto A.[b], Johnson E.[c], Khoo C.[c], Gu L.*[a]

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UV irradiation, chronic inflammation, and oxidative stress contribute to skin aging. This study aimed to determine whether cranberry juice affects UV-induced erythema and again-related skin conditions in healthy women using a randomized, double-blinded, placebo-controlled, cross-over design. The research was approved by IRB and registered on Clinicialtrials.gov. Twenty-two healthy women (n=11 aged 25-39, n=11 aged 40-65) with Fitzpatrick skin types 2 and 3 completed this study. Participants were advised to avoid *Vaccinium* products, several polyphenol-rich foods or dietary supplements, and probiotic supplements during the study. After a 7-day run-in period, participants consumed 16 oz of 100% cranberry juice (132 mg procyanidins) or a placebo (0 mg procyanidins) daily for six weeks. Following a 21-day wash-out period, participants switch to the opposite beverage for additional 6 weeks. Skin conditions, including transepidermal water loss, hydration, elasticity, UV-induced skin erythema, and others, were measured at baseline and after six weeks. Inflammatory and oxidative stress biomarkers in the blood were assessed using ELISA and colorimetric methods. A repeated-measures mixed model was used to determine the statistical significance of the measurements. After drinking cranberry juice for six weeks, transepidermal water loss on the face decreased significantly from 16.63 to 14.61 in women under 40, and from 21.66 to 18.33 in women over 40 ($p \leq 0.05$) compared to baseline. Cranberry juice consumption significantly decreased gross elasticity, net elasticity, and wrinkles on the face only in women over 40 ($p \leq 0.05$) but not in women under 40 compared to baseline. Similarly, on the forearm, consumption of cranberry juice caused a significant decrease in gross elasticity and net elasticity and an increase in biological elasticity in women over 40 ($p \leq 0.05$) but not in women under 40 compared to baseline. Only in women under 40 was a significant decrease in skin erythema after UVB exposure observed ($p \leq 0.05$). However, no differences in skin condition were observed in comparison to the placebo. There was no significant change in skin melanin, erythema, color, hydration, pH, smoothness, roughness, or scaliness. In the blood, the levels of glutathione peroxidase increased, while IL-17 and TNF-α decreased in women over 40 ($p \leq 0.05$) compared to baseline. Superoxide dismutase increased and TNF- α decreased in women under 40 ($p \leq 0.05$) compared to baseline. In both age groups, the levels of AGEs were significantly lower after cranberry juice consumption compared to after placebo juice consumption ($p \leq 0.05$). This study showed that 6 weeks of cranberry juice consumption resulted in decreased sensitivity to UVB exposure and improvement of certain skin properties associated with aging, such as elasticity, wrinkles, and TEWL compared to baseline but not to placebo. When participants were stratified by age, these results were found to be more significant in participants ≥40 years old. The effects on skin conditions may be explained by the decrease in inflammation and oxidative stress. (This research was funded in part by Ocean Spray Cranberries, inc.).

O.2.05 - FOOD PHENOLICS, IMMUNITY AND INFLAMMATION: PAVING THE WAY TOWARDS NOVEL FUNCTIONAL INTERVENTIONS IN CELIAC DISEASE

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Latest researches estimate that approximately 1.4% of the world's population has celiac disease (CD). CD is a T-cell mediated enteropathy triggered by ingestion of gluten proteins from wheat, barley, and rye by genetically predisposed individuals carrying the HLA-DQ2/DQ8 haplotype. As with many other autoimmune conditions, CD has emerged as a major public health problem, whose incidence is considerably increasing over time (around 7.5% each year) so that nowadays it has an extensive epidemiological distribution, affecting almost all countries and ethnicities [1]. Currently, the mainstay of treatment for CD depends on a strict life-long adherence to a gluten-free diet (GFD). Nonetheless, doubts still exist as to whether gluten exclusion completely restores the intestinal mucosa of CD patients or whether consequences of the previous strong immune response persist despite adherence to GFD.
Thoroughly researched for their health care potential in the prevention of several chronic diseases such as diabetes, cardiometabolic and neurodegenerative disorders, in the last few decades, little was still known regarding the biological significance and mechanisms of action of polyphenols in Celiac Disease (CD), for which the immunomodulatory function behind their therapeutical potential has never been explored before [2].
In view of such unknowns, herein, DQ8 transgenic mice and gluten-specific intestinal T-cell lines generated from biopsy specimens of HLA-DQ2 CD patients, were used to exploit the health-promoting properties and applicability of green tea catechins and grape seed procyanidins as an alternative strategy to block gluten toxicity, based on their ability to 1) ameliorate some of the most characteristic histological changes of gliadin-treated DQ8 mice, including villus flattening, crypt hyperplasia, and infiltration of intraepithelial lymphocytes, 2) to increase the intestinal nucleophilic tone of DQ8 mice by orchestrating an adaptive antioxidant response characterized by enhanced GSR enzyme activity and GSH content and 3) to modulate, in a dose-dependent manner, IFN- γ production by CD $CD4^+$ T cell lines [3]. Taken together, this work constitutes a highly relevant breakthrough as it provides the very first fundamental basis concerning the significance of natural polyphenols to be used in, for instance, the development of innovative functional approaches aimed at CD individuals.

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O.2.06 - METABOLITES OF FLAVONOIDS: A PATHWAY FOR DEVELOPMENT OF NOVEL ANTIPLATELET DRUGS

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Epidemiological studies suggested that higher intake of flavonoids from the diet decreases the incidence of cardiovascular diseases including ischemic stroke. Antiplatelet activity can contribute to this phenomenon. As parent flavonoids have a low bioavailability, their metabolites could be responsible for the observed effect. The aims of this study were 1) to test if parent flavonoids and their known metabolites can at biologically achievable concentrations affect platelet aggregation, 2) to define the mechanism of action, 3) to confirm the results in a real population sample, and 4) to establish structure-activity relationship.
A series of 29 flavonoids, 18 isoflavonoids, 29 their phenolic metabolites and 22 structural congeners were tested in human blood *ex vivo*. Different methods were employed (standard turbidimetry, impedance aggregometry, ELISA detection of prostanoids). A group of 53 generally healthy persons were enrolled for confirmation of the effect and comparison with clinically used acetylsalicylic acid (ASA).
Isoflavonoids were in general more potent than flavonoids. The most active parent compound, tectorigenin, reached even the activity of ASA. It acted as an antagonist at thromboxane receptors. Only 4 out of 29 metabolites appeared to have a clinically relevant effect, with 4-methylcatechol (4MC) being the most active of them by far. It inhibited platelet aggregation with an IC_{50} of approximately 3 µM, which is about one order of magnitude lower than that of ASA. A cross-sectional study confirmed the superior effect of 4MC over ASA on both collagen and arachidonic acid triggered platelet aggregation. Its mechanism of action seems to be based mainly on inhibition of thromboxane synthase-cyclooxygenase 1 coupling. Derivation of 4MC structure showed that catecholic ring is not necessary for the effect.
Conclusion: 4MC, a common metabolite of many flavonoids, is a strong antiplatelet compound with higher potency than ASA. Chemical modification of 4MC could produce strong antiplatelet drugs.

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O.2.07 - RESOLUBILISATION OF TANNIN–PROTEIN COMPLEXES

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Tannins can be distinguished from other natural polyphenols by their distinct property to interact and form insoluble precipitates with proteins and other biological macromolecules in aqueous solutions. Tannin–protein interactions are important for numerous bioactivities that can benefit e.g. human and animal nutrition and health via various applications. Our previous studies showed that the interactions between hydrolysable tannins (HTs) and proteins are highly HT-specific regarding the protein precipitation capacity as well as the stoichiometry of the precipitation reaction [1, 2].

In addition to complex formation, the stability of the formed complexes is important for the realised bioactivity. For example, in the double benefit hypothesis of tannins for ruminants, tannin–protein complexes stable enough to survive the pH conditions of the ruminant digestive system are first formed in the earlier parts of the digestive tract and resolubilised later in the small intestine. There, the rumen-escape protein is absorbed, and bioactivity of the liberated tannins is realised e.g., via targeting small intestine nematodes. Unfortunately, little is known about the reversibility of HT–protein complexation at different pHs. To unravel this, we investigated the resolubilisation of HT–protein complexes with 17 structurally varying HTs and model protein bovine serum albumin (BSA), utilising turbidimetry, UHPLC-DAD and UHPLC-DAD-MS.

The results showed that the stabilities of the formed complexes and liberation of the tannins from the complexes (Figure 1) by change of pH depended highly on the exact HT structure. In general, the insoluble complexes formed between good protein precipitators and BSA were less susceptible for the changes in pH. Also, HT–BSA complexes were more sensitive to increasing the pH above the isoelectric point of the protein (IP) than when moving towards lower pHs.

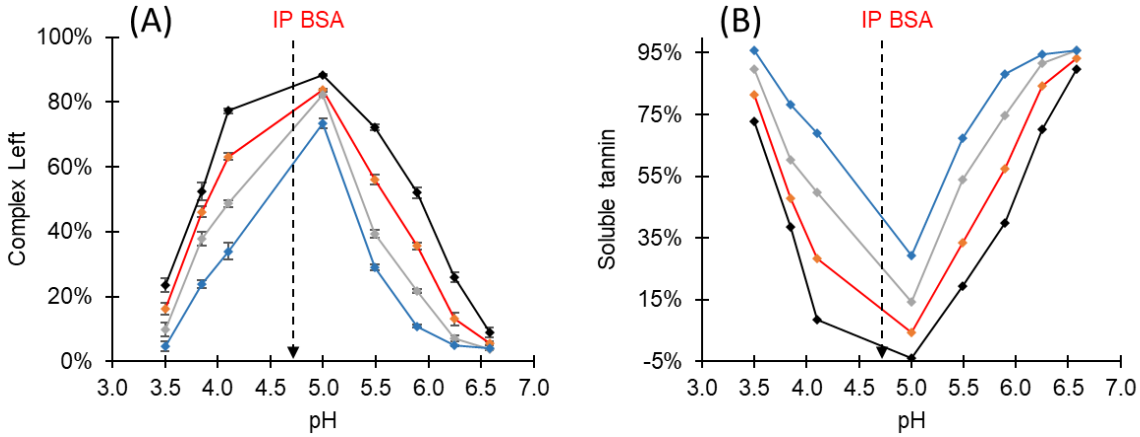

Figure 1: The amount of HT-BSA complex left (A) and the amount of soluble tannin after pH adjustment of the reaction solution. The PGG:BSA ratios in the initial complexation reaction at pH 5 were 5 :1 (black line), 4 :1 (orange line), 3 :1 (grey line) and 2 :1 (blue line).

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O.2.08 - POLYPHENOLS IN SUGARCANE: POTENTIAL FOR MODULATING GLYCAEMIC RESPONSE

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A diet high in carbohydrates leads to elevated postprandial glucose spikes in the blood, a major risk factor for metabolic diseases, such as type 2 diabetes. Consumption of certain polyphenols is associated with a reduction in the risk of type 2 diabetes, via mechanisms that regulate digestion, inflammation and metabolism.

Multiple publications have reported polyphenol analysis of the various parts of the sugarcane plant, and of the many products derived from processing. Although some studies have used $LCMS^n$ with standards, many have only identified polyphenols following hydrolysis and using HPLC with diode array detection. The latter gives only a tentative identification of aglycone forms. Critical analysis of the literature allowed us to define polyphenol profiles in various parts of the plant and in processed products. We identified apigenin, luteolin, tricin, ferulic acid, caffeic acid and chlorogenic acid as the most recorded polyphenols in both sugarcane plant parts and products including leaves, rind, culm, node, juice, syrup, sugar, bagasse, molasse and non-centrifugal sugarcane products. However, the techniques used to extract and analyse the composition were crucial. Acid hydrolysis has been used by some researchers to remove the sugars attached to polyphenols. This step simplifies analysis because glycosylation patterns can be complex, and often such complex standards are not available as reference compounds [1]. Various extraction techniques, such as solvent extraction and resin-based extraction, have frequently been combined by researchers with analytical tools such as HPLC or LCMS. Apigenin, for example, appears to survive sugarcane processing well, as it has been found in almost all plant parts and products. A careful examination of the methodology of each recorded instance of apigenin is required to confirm the accuracy of this data. Despite conducting a detailed analysis of sugarcane composition, we found some methods, such as total polyphenolic content using the Folin-Ciocalteu assay, to overestimate the amount of total polyphenols due to its ability to react with a variety of other compounds [2].

We have examined a polyphenol-rich sugarcane extract as a by-product of sugarcane molasses. This extract can inhibit glucose transporters in Caco-2 human intestinal cells and can restore insulin production in insulin-dysfunctional pancreatic beta-cells [3]. We hypothesise that it can reduce the glycaemic response *in vivo* additionally through the inhibition of carbohydrate digestion. We demonstrate that the polyphenol-rich sugarcane extract can inhibit human sucrase-isomaltase in a dose-dependent manner. Acarbose, a prescription drug for type 2 diabetes, was used as a positive control. We are currently exploring the ability of polyphenol-rich sugarcane extracts to lower postprandial glycaemia in humans when consumed together with a high-carbohydrate meal.

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OY.2.01 - A SILVERSKIN EXTRACT RICH IN CHLOROGENIC ACIDS PROMOTES THE GROWTH OF *LACTOBACILLUS PARACASEI*

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Silverskin, a by-product of green coffee roasting, is rich in polyphenols, especially chlorogenic acids [1]. These bioactive compounds are poorly absorbed by upper gastrointestinal tract and the small intestine where they can be metabolized by probiotic bacteria. These bacteria exert a positive effect on human health being associated with a lower risk of disease [2]. Thus, the aim of this work was to produce a silverskin extract and verify its impact on the growth of *Lactobacillus paracasei*.

First, a silverskin extract was produced using the Multi-frequency Multimode Modulated ultrasonic vibration technique. The silverskin extract was freeze-dried and chlorogenic acids quantified by RP-HPLC-DAD and monitored at 320 nm [3]. The freeze-dried silverskin extract (FSE) was incorporated into glucose-free MRS broth at different concentrations (0.5 to 4%) along with 10% *L. paracasei* inoculum (0.3 McFarland). Inulin, glucose and glucose-free MRS broth were used as reference prebiotic, positive control and negative control, respectively. After 48 h of incubation, counting was performed on MRS agar plates after successive decimal dilutions and pH was measured (in triplicate).

The results show that the FSE is rich in chlorogenic acids, especially 5-caffeoylquinic acid (2,15 mg/g). The medium enriched with FSE stimulated the growth of *L. paracasei* in a dose-dependent manner (ΔUFC/ml = 2.30 to 3.00 x 10^8). After incubation, the pH of the samples decreased with increasing concentration of FSE, showing that the probiotic bacterium is able to metabolize the FSE into acidic metabolites, a typical behaviour of these bacteria.

In conclusion, the silverskin extract showed to be suitable for prebiotic applications. Being major compounds, chlorogenic acids seems to contribute to this potential prebiotic effect. However, studies testing these compounds individually will be essential to prove their impact on the gut microbiota.

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OY.2.02 - INHIBITION OF GLUT2 GENE EXPRESSION BY COFFEE AND SILVERSKIN EXTRACTS

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Moderate coffee consumption has been associated with a decreasing risk of type 2 diabetes, mainly due to its richness in chlorogenic acids (CGA) [1]. Coffee silverskin (CS) is the major by-product of coffee roasting, and coffee companies are increasingly committed to valuing this by-product and integrating it into a circular economy approach [2]. Considering that silverskin bioactive compounds are similar to those of coffee [1], its potential on the prevention of type 2 diabetes was explored and compared with that of roasted coffee (RC). For that, extracts of RC beans and CS were prepared by aqueous ultrasound-assisted extraction and freeze-dried. CGA and caffeine contents were analysed by RP-HPLC-DAD and the effects on the uptake of radioactive analogous of glucose and fructose (^{3}H-deoxy-D-glucose (^{3}H-DG) and ^{14}C-fructose (^{14}C-FRU), respectively) by human intestinal epithelial (Caco-2) cells were evaluated. In addition, the influence of the extracts on the expression of sugar transporter genes was also investigated and compared by RT-qPCR [1]. CS extract presented the lowest amounts of CGA and caffeine, but it promoted similar inhibitory effects to those observed with RC extracts (being even higher than RC extract in the case of ^{3}H-DG uptake). Furthermore, the extracts very markedly reduced the mRNA expression levels of GLUT2 (in about 64-72%, p<0.05), a transporter that has been appointed in several studies as the most important pathway for the intestinal absorption of sugars when high concentrations reach the intestinal lumen [1]. In addition, a decrease in SGLT1 mRNA levels was also found for all extracts (~30-38%, not statistically significant for CS extract), but no reductions in GLUT5 mRNA levels were found with none of the extracts. These results highlight that CS can be an interesting alternative to coffee, since it showed to be as efficient as RC in reducing the absorption of glucose and fructose at the intestinal level and the mRNA expression levels of a very important intestinal sugar transporter (GLUT2). Hence, this preliminary study opens the doors to the valorization of this coffee by-product, which might contribute not only to the health and wellness of the population but also to the sustainability of the coffee chain.

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Funding: This work was funded by the project PTDC/SAU-NUT/2165/2021- COBY4HEALTH- Can coffee by-products decrease the risk of metabolic syndrome? A comprehensive approach to reduce waste and valorize health benefits, funded by Fundação para a Ciência e Tecnologia (FCT)/ Ministério da Ciência, Tecnologia e Ensino Superior (MCTES), Portugal.

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OY.2.03 - GLYCOSYLATED DIHYDROCHALCONES WITH A CHLORINE ATOM OBTAINED BY BIOTRANSFORMATION AND THEIR POTENTIAL BIOACTIVITY

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Among a great variety of flavonoids, derivatives with a chlorine atom exhibit great antimicrobial, anti-inflammatory, and immunomodulatory potential [1]. Flavonoids in plants usually occur as glycosides and in this form are more stable, water-soluble, and bioavailable than their aglycone forms. The pharmacological application of flavonoids is still limited by their low concentration in plants and low yield in the chemical synthesis of flavonoid glycosides [2].
The presented study's main objective was to obtain glycosylated flavonoids with a chlorine atom using combined chemical and biotechnological methods and to assess their biological activity and physicochemical properties with computer-aided simulations. In the first step of the study, 2'-hydroxychalcones with a chlorine atom at different positions of ring B were synthesized. Then, enzymatic glycosylation of the synthesized compounds was performed in cultures of selected entomopathogenic filamentous fungi strain *Isaria fumosorosea* KCH J2. As a result, four new flavonoid derivatives were obtained. The predictions made with SwissADME online tool showed their improved water solubility, bioavailability, and high gastrointestinal absorption. The Way2Drug Pass Online tool simulations showed that these compounds may act, to name a few, as cholesterol antagonists, membrane integrity agonists, and anticancerogenic and antimicrobial agents.
The conducted studies indicate that entomopathogenic filamentous fungi strain *I. fumosorosea* KCH J2 is able to glycosylate chlorochalcones. The obtained glycosylated dihydrochalcones with a chlorine atom have not been previously reported in the literature and show interesting potential biological activities that require further study.

Acknowledgements
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OY.2.05 - PHARMACOKINETICS OF NARINGENIN SULFATES IN HUMAN INTESTINAL CELLS

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Naringenin is a flavanone with a wide range of pharmacological properties, in particular anti-inflammatory action. In an *in vitro* Caco-2 cell-based model mimetizing the intestinal absorption process, naringenin proved to be able to penetrate the barrier formed by these cells [1]. However, flavanones are often metabolized during absorption, originating sulfates and glucuronides derivatives [2]. So far, it is unknown the role of sulfur groups in naringenin's pharmacodynamic and pharmacokinetic features [3]. To contribute to this discussion, we have mapped the chemical space of 124 known sulfated flavonoids from literature and calculated a number of physico-chemical properties for these molecules. Two compounds, naringenin 4'-*O*-sulfate and naringenin 7-*O*-sulfate, occupy a chemical space quite unique when compared with the remaining sulfated molecules (Figure 1). We have synthesized such molecules and assessed their potential toxicity and permeability in human cells. For evaluating the potential toxicity of these molecules, viability and membrane integrity studies were conducted using the non-cancer cell lines HaCaT and MRC-5, which pointed to the absence of toxicity of naringenin 4'-*O*-sulfate up to 50 µM and of naringenin 7-*O*-sulfate up to 100 µM. Regarding the permeability of these molecules, a transwell-based Caco-2 model was chosen in tandem with HPLC-DAD to assess and quantify the transport of compounds from the apical to the basolateral side and compare them with the parent molecules.
This study presents a new perspective on naringenin's safety and pharmacokinetic properties, as well as new insights on the role of sulfation to said properties.

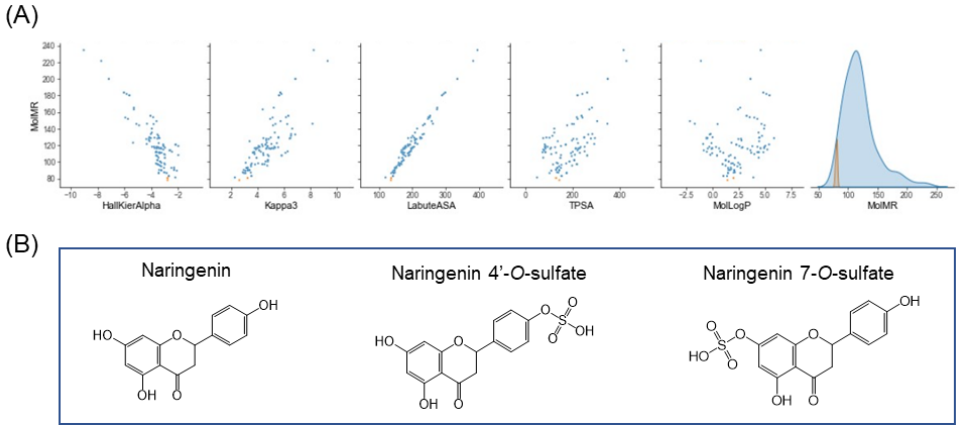

Figure 1: (A) Analysis of flavonoid sulfates (blue), including naringenin 4'-*O*-sulfate and naringenin 7-*O*-sulfate (orange) using different molecular descriptors**. HallKierAlpha**: Hall-Kier alpha value; **Kappa3**: molecular shape index for three bonded fragment; **LabuteASA**: Labute's Approximate Surface Area; **TPSA**: topological polar surface area ; **MolLogP**: Wildman-Crippen LogP value; **MolMR**:Wildman-Crippen MR value. (B) Chemical structure of naringenin and naringenin sulfates.

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OY.2.06 - RELATIONSHIP BETWEEN THE STRUCTURE OF THE FLAVONE *C*-GLYCOSIDES OF FLAX (*LINUM USITATISSIMUM* L.) AND THEIR BIOLOGICAL ACTIVITIES

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Antibiotics are one of the most important discoveries that have saved and is saving millions of lives in the world, but the emergence of resistant bacteria induces little or not effective for these synthetic products. In some situations, the appearance of resistance prevents the treatment of infections caused by bacteria. The discovery of alternative antimicrobial agents has become urgently required.

Many products of plant origin, in particular the class of flavonoids, exhibit antibacterial activity often associated with a high level of antioxidant power [1]. Despite the many studies on this issue, the relationship between structure and function is currently poorly understood. To fill this gap, the biological activity of 12 flavone *C*-glycosides structurally close was studied. The structural variability of these compounds (derived from apigenin and luteolin) depends on the position and nature of the sugars, the number of hydroxyl groups and the presence of a methyl group [2].

Thus, orientin, isoorientin, vitexin, isovitexin, swertisin, swertiajaponin, carlinoside, schaftoside, lucenin-1, lucenin-2, vicenin-1 and vicenin-2 were extracted from the aerial part of winter flax (*Linum usitatissimum*). The hydroalcoholic extract was purified by preparative HPLC and by the drowning-out crystallization method. Then, the control of the purity (greater than 99%) and the confirmation of the chemical structures were carried out by NMR and LC/MS. Antioxidant activity was tested by methods such as CUPRAC, ORAC, DPPH and FRAP and antimicrobial potential was assessed using common foodborne pathogens such as *P. aeruginosa*, *E. coli*, *L. monocytogenes*, *L. innocua*, *S. arizonae*, *E. faecalis*, *S. aureus*, *B. subtilis*.

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OY.2.07 - PREDICTING NON-COVALENT INTERACTIONS BETWEEN POLYPHENOLS IN BIOLOGICAL MEMBRANES THROUGH MOLECULAR DYNAMICS

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There have been many evidences about noncovalent interactions between polyphenols, especially in water. Noncovalent association between anthocyanins and other polyphenols is known as co-pigmentation. This association is mainly driven by π-π stacking interactions and it participates in the stabilization of the anthocyanin colour in plants and derived beverages like red wine [1]. Food and cosmetic industries have gained much interest in this process to develop new strategies in pigment/dye stabilisation. More surprisingly, this noncovalent association has also been described in lipid bilayer membranes in a joint fluorescence quenching and in-silico study [2]. This phenomenon is key to rationalize synergism between antioxidants, including polyphenols, vitamin E (α-tocopherol) or vitamin C. This effect has gained much interest in food industry as it allows developing efficient antioxidant cocktails, reducing concentration of active agents and subsequently potential toxicity.
This study aims at benchmarking the performance of Molecular Dynamics (MD) simulation to infer the formation of such noncovalent complexes with two prototypical antioxidants (quercetin and vitamin E), in a pure 1,2-dipalmitoylphosphatidylcholine (DOPC) lipid bilayer (Figure 1).
Quercetin and vitamin E association was studied through free MD simulations of µs-time scale. Experimentally, the quenching of vitamin E fluorescence by quercetin was found to be mainly ruled by a sphere-of-action quenching model and to a lower extent (20%) by the formation of transient π-π stacking complexes. The fitting between experimental data and the theoretical model, picturing these two quenching mechanisms, exhibited a R^2 value of 0.99.
MD simulations revealed very accurate results by reproducing quenching intensity with a mean absolute error of 0.39. Also, the ratio of π-π stacking complex was evaluated at 18%, close to the experimental 20%. The predictive ability of MD simulations at capturing this noncovalent association is at stake and under evaluation with other pairs of π-conjugated antioxidant polyphenols. More complex in-silico membranes are also under study, such as skin (stratum corneum) multilayer membranes, which will be presented here for the first time.

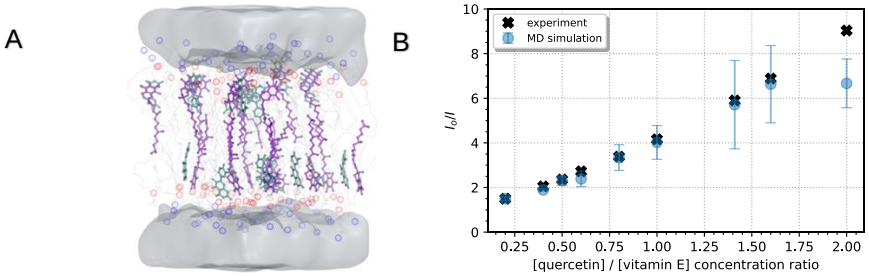

Figure 1: (A) Simulated noncovalent association (quercetin:vitaminE) in a lipid bilayer membrane, (B) Quenching Stern-Volmer plot

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OY.2.08 - INHIBITION OF NON-SMALL CELL LUNG CANCER (NSCLC) BY ROSEMARY EXTRACT AND ITS POLYPHENOL CARNOSIC ACID IS ASSOCIATED WITH ACTIVATION OF SESTRIN-2/LKB1/AMPK SIGNALLING

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Lung cancer is the leading cause of cancer-related deaths globally. Non-small cell lung cancer (NSCLC), accounting for approximately 80% of lung cancer cases, is aggressive, develops resistance to standard treatment strategies, and has low survival rates. Therefore, exploration of novel treatment strategies for NSCLC are urgently needed. Cancer is characterized by cells exhibiting enhanced proliferation and migration, and evasion of apoptosis due to aberrant cell signalling pathways. Historically, plant-derived chemicals such as paclitaxel and docetaxel have been established as anticancer agents and the search for novel chemicals with potent anticancer properties continues today. We have examined the effects of rosemary (*Rosmarinus officinalis*) extract (RE) and its constituent polyphenol carnosic acid (CA) in NSCLC cells *in vitro* (Figure 1). Treatment of A549 and H1299 NSCLC cells with RE resulted in a dose-dependent inhibition of proliferation and survival. In addition, treatment with RE induced apoptosis and these effects were associated with activation of AMP-activated protein kinase (AMPK) and inhibition of the mammalian target of rapamycin (mTOR). Furthermore, treatment of NSCLC cells with CA resulted in inhibition of proliferation and survival. These findings coincided with increased levels of sestrin-2, increased levels of phosphorylated (Ser^{428}) liver kinase B1 (LKB1), and increased activation of AMPK. Furthermore, CA induced autophagy as indicated by increased levels of autophagy marker light chain 3 (LC3), and induced apoptosis as indicated by increased levels of cleaved PARP and cleaved caspase-7. Overall, our data provide evidence that both RE and CA inhibit NSCLC *in vitro*. CA may be an active pharmaceutical ingredient (API) in RE with strong anticancer properties. Future studies involving siRNA and small molecule inhibitors are underway to better characterize the signalling mechanisms underlying the anticancer effects of CA as well as tumour xenograft models to identify whether our *in vitro* findings are reproducible using *in vivo* models.

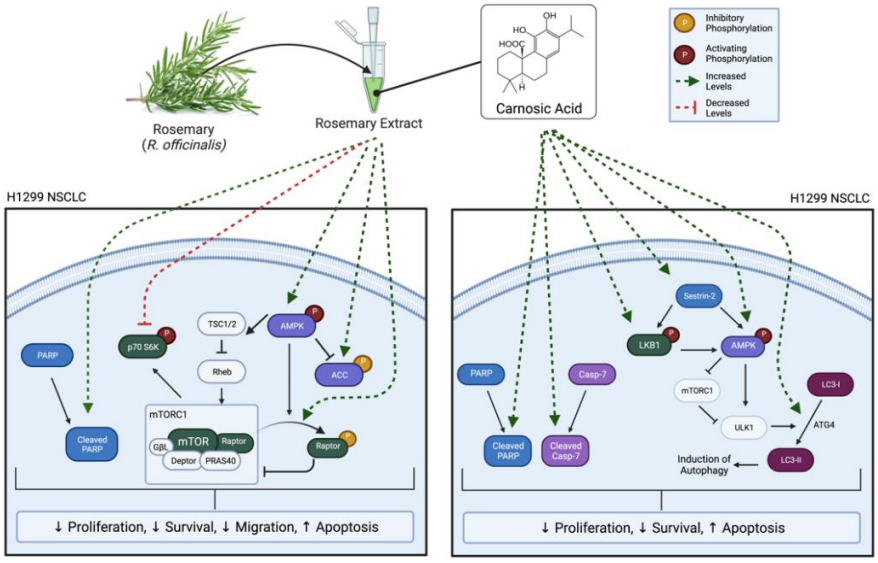

Figure 1: Effects of rosemary extract and carnosic acid in H1299 NSCLC cells

OY.2.09 - CAMU-CAMU (*MYRCIARIA DUBIA*) DECREASES HEPATIC STEATOSIS AND IMPROVES CIRCULATING MARKERS OF LIVER INJURY IN OVERWEIGHT INDIVIDUALS WITH *HYPERTRIGLYCERIDEMIA*.

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Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world, affecting 25% of the adult population. To date, no effective drug treatment against NAFLD is available. Camu-camu (CC) is a polyphenol-rich fruit and studies in mice have shown that CC administration prevents hepatic steatosis in association with changes in the gut microbiota composition. However, no clinical trials have yet investigated whether CC could improve those parameters in humans.
The objective was to determine the impact of 1.5 g/day of CC supplementation for 12 weeks on liver steatosis, gut microbiota composition, and metabolic profile changes in overweight subjects with hypertriglyceridemia.
Thirty adults overweight (body mass index >25 kg/cm^2) and hyper-triglyceridemic (>1.35 mmol/L) were recruited in the greater Québec city metropolitan area for a randomized, double-blind, placebo-controlled crossover trial. For two 12-week periods interspersed with a 4-week transition period, participants consumed CC capsules (1.5g) or placebo (1.5g) daily. Visceral, subcutaneous, and hepatic fat were assessed by magnetic resonance imaging at the beginning and end of each phase. Lipid profiles, fasting glucose and insulin levels, and gut microbiota composition were also analyzed.
CC supplementation for 12 weeks decreased the relative hepatic fat content by 14.6% (p=0.003) and plasma levels of aspartate (p=0.04) and alanine (p=0.0006) transaminases. These improvements were associated with changes in gut microbiota composition. CC supplementation induced the growth of *Enterococcus*, *Lactobacillus* and *Lactococcus*, whereas the placebo increased the relative abundance of *Adlercreutzia* and *Erysipelatoclostridium*.
Supplementation with 1.5 g of CC per day in overweight subjects with hypertriglyceridemia decreased hepatic steatosis and levels of aspartate and alanine transaminases, two markers of liver injury, as well as modifications in fecal microbiota, suggesting a prebiotic action of CC. These data support the hepatoprotective potential of CC against NAFLD.

OY.2.10 - PHENOLIC PROFILE OF *CHAENOMELES* LEAVES AND THEIR *IN VITRO* ANTICHOLINERGIC POTENTIAL

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We have been observing over the last two decades intensive development of the functional food segment and the growing demand for it. This is related to the search for new sources of phytochemicals to enrich various types of products. This is a challenge for food producers, and they are trying to transcend the limits of conventional food to develop new functional food products with targeted properties, such as anti-diabetic, anti-obesity and anti-aging [1].
There are many reports on the chemical composition and biological properties of leaves from seven commonly cultivated plants, for example lemons, mandarins, apples, pears or banana. But only a few studies have verified the composition and health-promoting properties of compounds contained in *Chaenomeles* leaves.
Chaenomeles is a frost-resistant shrub with thorny twigs, growing up to about 2 m in height. It is cultivated for the beautiful flowers that appear there from early spring, as well as for fruits that ripen in late summer and autumn, perfect for preserves [2, 3]. Several species of *Chaenomeles* are known, but mainly three are cultivated in Poland: flowering, also called Chinese quince (*Chaenomeles speciosa*); Japanese or Maule's quince (*Chaenomeles japonica*); and a hybrid, intermediate quince (*Chaenomeles* × *superba*). The leaves of the *Chaenomeles* genus are broad-ovate, small, 4-5 cm long, blunt at the top, wedge-shaped at the base. The leaf blade is dark green, shiny and coarsely serrated [3].
Therefore, this study was performed to investigate the content of phenolics (identification by UPLC/ESI-Q-TOF-MS, and quantification by UPLC-PDA-FL) and *in vitro* biological activities (acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition activity) of 12 cultivars of *Chaenomeles* × *superba*, *japonica* and *speciosa* leaves.
In the present work 39 phenolic compounds were tentatively identified, including polymeric procyanidins > flavan-3-ols > phenolic acids > flavonols > flavanones > flavones > ellagitannins. *Chaenomeles* leaves contained on average 16.8 g of phenolics per 100 g dry weight (dw), with *C*. × *superba* ˈNicolineˈ displaying the highest, and *C*. × *superba* ˈColour Trailˈ the lowest total phenolic content. The half-maximal inhibitory concentration (IC_{50}) of AChE and BuChE ranged from 20.2 to 39.8 and from 26.4 to 39.9 mg of dried fruit per mL of enzyme, with significant differences between samples. The calculated Pearson correlation coefficient revealed that phenolic compounds are more strongly correlated with the anti-AChE potential (r=0.756), than with the anti-BuChE activity (r=0.505). Interestingly, both of these activities show a very low positive correlation to each other (r=0.172).
In conclusion, *Chaenomeles* leaves might be used as new sources for the production of nutraceuticals, as well as for medical or/and cosmetic purposes.

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OY.2.11 - LIPID-LOWERING AND ANTIOXIDANT EFFECTS OF POLYPHENOL-ENRICHED EXTRACTS FROM THYMBRA SPICATA L. ON CELLULAR MODELS OF HEPATOSTEATOSIS AND ADIPOGENESIS

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Plants and plant-derived preparations are widely used in traditional medicine for the management of metabolism dysfunctions. Thymbra spicata, a member of the Lamiaceae family, is rich in phenolic compounds, particularly, phenolic acids as rosmarinic acid and phenolic monoterpenoids as carvacrol. These dietary polyphenols have large interest in preventing and/or treating the complex and multiple dysfunctions occurring in over-nutrition conditions leading to overweight and obesity. Obesity impacts across multiple organs, especially on the liver promoting steatosis and fatty liver diseases. In this study, we tested the lipid-lowering potential of two extracts prepared from T. spicata aerial parts: the ethanolic (TE) and the aqueous (TW) extracts. We investigated the possible beneficial effects of TE, TH and their main bioactive compounds (carvacrol and rosmarinic acid, respectively) on two in vitro models: a cellular model of hepatosteatosis (lipid-loaded FaO cells) and one of adipogenesis (differentiated 3T3-L1 cells). In the different conditions, we assessed the intracellular triglyceride content, lipid droplet accumulation, and lipid peroxidation level. The results revealed that both extracts and their major bioactive compounds ameliorated lipid accumulation and oxidative stress in both cellular models. Therefore, T. spicata could be of potential interest for developing natural therapeutic agents or dietary supplements to treat obesity and fatty liver diseases.

OY.2.12 - ROLE OF PLANT PHENOLIC EXTRACTS IN PREVENTING TYPE 2 DIABETES RELATED ENZYME INHIBITION

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The number of type 1 or 2 diabetics globally has exceeded 9.3% of the population. Without sufficient preventive action, the number of patients is projected to rise to a staggering 700 million (10.9%) by 2045, posing unavoidable public health and economic challenges [1]. Although oral synthetic antidiabetic drugs, carbohydrate digestive enzyme inhibitors, are commonly used to treat type 2 diabetes and hyperglycemia, plant-derived inhibitors are safer and, along with a balanced diet, are one way to control and regulate postprandial glycemia. Phenolic compounds are gaining popularity in this area however, the focus is mainly on medicinal plants, and recent literature data leaves many doubts within the bioavailability, extractability and impact of synergistic actions of the complex plant matrix [2].
Hence, the study aimed to determine the quantitative and qualitative profile of phenolic compounds and the antidiabetic activity of extracts of phenolic compounds obtained from commonly consumed plants (fruits, vegetables, herbs) and abundant in these compounds.
Analysis of the quantitative and qualitative profile of phenolic compounds, using liquid chromatography (UPLC-PDA/FL) coupled to a Q/TOF mass spectrometer equipped with electrospray ionization (ESI), ranked the plant phenolic extracts with respect to the predominant groups of phenolic compounds: anthocyanins in blackcurrant berries and red cabbage, flavonols in onion husks and sea buckthorn berries, flavan-3-ols and polymeric procyanidins in green tea leaves and Japanese quince fruits, as well phenolic acids in coffee beans and lemon balm leaves. Some of the flavonoids showed significantly more effective *in vitro* inhibition of intestinal *α*-glucosidase than pancreatic *α*-amylase ($p < 0.05$), key enzymes linked to postprandial hyperglycemia, also compared to the inhibitory activity of the drug acarbose (IC_{50}). The ability to inhibit a factor indirectly associated with postrandial hyperglycemia, i.e., inhibition of pancreatic lipase activity, was determined by the concentration of procyanidin polymers and phenolic acids including chlorogenic and caffeic acids. 3-*O*-Glucosides and 3-*O*-rutinosides of delphinidin and cyanidin enhanced antioxidant protection by reducing free radicals and scavenging reactive oxygen species.
Controlled reduction of enzyme activity using plant phenolic compounds is therefore a promising strategy for the prevention and treatment of type 2 diabetes in combination with reduced drug dosage. Natural sources of phenolic compounds should be considered as dietary supplements and useful functional food components for controlling both hyperglycemia and normal cellular redox status. Further research on improving the bioavailability and phenolic metabolite activity, as well clinical applications will optimize the beneficial antidiabetic effects of phenolic compounds.

This work was supported the National Science Centre (NCN) under the project Preludium 20, project no. 2021/41/N/NZ9/02790.

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OY.2.13 - SALIVA PROTEINS-TANNINS INTERACTIONS AND THE FATE OF COMPLEXES DURING DIGESTION

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Tannins may have detrimental effects on digestion, for example by inhibiting the digestive enzymes of the oro-gastro-intestinal tract [1]. Besides, some salivary proteins (such as PRPs or histatins) have a high affinity for tannins which is considered as a protective function of saliva against the deleterious impact of excessive tannins [2]. This research aims to describe the interactions between saliva proteins and tannins from apples and to follow the fate of the complexes during *in vitro* digestion.

In the experiments, we used on one hand a polyphenol mixture extracted from the apple cider variety Dous Moën containing 730 g/kg total polyphenols of which 44% were condensed tannins. On the other hand, clarified human saliva at a protein concentration of 0.46 mg/ml was used.

First, polyphenols and saliva were mixed at different ratios of tannins to saliva proteins (w/w) from 0.1 to 1.1. We measured the turbidity of the resulting samples by spectrophotometry at 400nm. Samples were then centrifuged at 10000 g, 4°C for 10 minutes in order to separate the larger aggregates in the pellet from soluble compounds and smaller aggregates in the supernatant. The concentration of tannins in the supernatants was measured by the Butanol-HCl method. Protein concentration in the pellets (resuspended in urea buffer) and in the supernatants was measured by the Bradford method, and protein profiles in both fractions were obtained by SDS-PAGE and compared to that of saliva alone.

When the ratio of tannins to saliva protein increased, turbidity in whole samples increased, suggesting the formation of increasingly larger saliva proteins-tannins aggregates. In accordance, the protein content in pellets increased while the protein content in supernatants decreased. Results of tannins in supernatants showed that 34 to 50 % of tannins remained soluble. Protein profiles revealed that some proteins were enriched in the pellets, particularly small proteins with molecular weights estimated at around 10-15kDa. This confirmed that tannins interact with and precipitate preferentially low molecular weight saliva proteins.

In a second step, polyphenol extracts-saliva mixtures were subjected to the INFOGEST static *in vitro* digestion model. Samples taken at the end of the gastric and intestinal phases of digestion were centrifuged and SDS-PAGE electrophoresis was performed on the pellets and supernatants. Apart from pepsin and trypsin, no proteins were detectable in gastric and intestinal supernatants respectively. In contrast, intestinal pellets showed a very clear band at around 10kDa on electrophoretic gels, suggesting the resistance of this small salivary protein to the digestive process. Additional experiments are currently being conducted to determine whether interaction with tannins plays a role in this persistence.

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OY.2.14 - EFFECT OF *HIBISCUS SABDARIFFA* AND POMEGRANATE EUTECTIC EXTRACTS ON VIABILITY AND PROLIFERATION OF BREAST CANCER CELLS

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The primary step to obtain phytochemicals from functional food like pomegranate (*Punica granatum* L.) and the roselle flower (*Hibiscus sabdariffa* L.) - and its agro-industrial residues - is solvent extraction. Their functional characteristics are attributed to the presence of polyphenolic compounds in their composition. In the aqueous and/or methanolic extracts of pomegranate husk, mainly hydrolysable tannins, phenolic acids and in lower concentrations some anthocyanins. It should be noted that punicalagin is the main phenolic compound in the husk. Thus, its influence on biological activity is relevant. Regarding *Hibiscus sabdariffa* L., the main constituents of the flower in aqueous and/or methanolic extracts are anthocyanins, as well as organic acids and phenolic acids.

However, the use of extracts obtained from traditional organic solvents could cause residual toxicity, when used as complementary therapeutic agents in the treatment of diseases, like breast cancer. As an alternative, the use of deep eutectic solvents (DES) has gained relevance [1]. Mostly, eutectic solvents are made up of mixtures of self-associated components with low or no toxicity.

Uncontrolled proliferation of malignant cells is one of the main characteristics of cancer. Additionally, breast cancer has gained epidemiological relevance due to its incidence in the population worldwide. In the case of so-called triple negative breast cancer (TNBC), patients do not benefit from targeted therapies. To deal with this problem, some studies have focused on the exploration of natural compounds. They could inhibit the growth of cancer cells more selectively, reducing or minimizing the toxic side effects [2,3].

Due to the above, the aims of this research were to evaluate: a) the extraction efficiency of polyphenols using eutectic mixtures; b) the characterization of the phenolic composition of the crude extracts and c) the *in vitro* effect of the phenolic extracts on the viability of TNBC cells.

Our results shows that both pomegranate and roselle eutectic solvent system made up of choline chloride: malic acid (DES 3) presents a similar extraction efficiency in comparison with the hydroalcoholic extracts. Moreover, the chromatographic profile of DES 1 (choline chloride: urea: glycerol) and DES 3 extracts from pomegranate husk showed punicalagin as the main compound. Also, DES 3 extracts from roselle presents characteristic peaks of anthocyanins (delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside) and some phenolic acids. In the *in vitro* evaluation of the biological activity of pomegranate extracts, the results showed a significant effect on the viability of Hs 578T TNBC cells, attributed to treatment with DES 1 and DES 3 extracts. The IC_{50} calculated for DES 3 extracts from pomegranate and hibiscus flower were 0.44 and 1.74 mg/mL, respectively. In the case of DES 1 extracts the value was estimated at 0.52 mg/mL for pomegranate and 1.52 mg/mL for hibiscus flower. The selectivity index (an index that shows the mortality of malignant cells in relationship with non-transformed cells) for the extracts indicates a high selectivity on the viability of TNBC cells in the DES 1, DES 3 and hydroalcoholic extracts of pomegranate, as well as in the DES 1 extract of hibiscus flower. Finally, TNBC cells treated with IC_{50} values showed morphological changes (shrinkage, giant cells, disorganized actin) compared with cells without treatment.

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OY.2.15 - POLYPHENOLS INDUCING DIFFERENT LEVELS AND PATTERN OF LIPID-DERIVED GUT MICROBIAL METABOLITES AFTER FERMENTATION OF FOODS

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Next to dietary fiber and proteins, undigested dietary lipids also enter the colon and can be used by human gut microbiota. Although the pathways through which lipids are metabolized by the gut microbiota is partly known, how the nature of the specific lipid-rich matrix modulates lipid metabolism is poorly explored. Here, the differences in the levels and patterns of lipid microbial metabolites produced from different food matrixes, sunflower seed, soybean, and walnut were investigated. Food samples were subjected to a simulated in vitro digestion, after which the undigested material was subjected to an in vitro colonic fermentation using faecal samples from three healthy donors for 48h. Several known linoleic acid (LA) metabolites were quantified by a targeted approach whereas several other FAs metabolites were identified or putatively annotated by the lipidomics untargeted approach using high-resolution liquid-chromatography mass spectrometry. Results showed that digested walnut produced the highest levels of FFAs and conjugated LAs (CLAs) after fermentation. To further explore the matrix effect, the same amount of defatted digested foods, as well as of fibre and polyphenol extracted from the digested materials were prepared and fermented with sunflower oil. The addition of defatted digested walnut to sunflower oil also produced the higher levels of FFAs and detected CLAs. This is ascribed to its fibre and polyphenols which addition produced the higher increase in CLAs than sunflower and soybean. Several LA metabolites, such as di- or tri-hydroxy-C18FAs, were putatively annotated by the untargeted lipidomics approach. Multivariate analysis of the profile of microbial lipid metabolites showed that the lipid profiles produced with sunflower seeds and walnuts were similar but distinct to soybean. In conclusion, foods with different compositions, such as polyphenol and fibre content can modulate the microbial production of lipid metabolites and the fatty acid composition after fermentation is more affected by the type of food matrices than oils.

OY.2.16 - IMMUNOMODULATING POLYPHENOLS FROM *SIDERITIS SCARDICA*

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Greek mountain tea, *Sideritis scardica*, has a long history in traditional medicine. Nowadays, *S. scardica* is investigated for its pharmacological activities in the central nervous system in which cognition-enhancing and neuroprotective properties have been described [1, 2]. Aerial parts of *S. scardica* were extracted with 80 % ethanol, suspended in distilled water and successively extracted with solvents of increasing polarity. The ethyl acetate and the butanol extracts were fractionated by different chromatographic techniques and isolated compounds identified by NMR spectroscopy. Isolated compounds and extracts were tested in antioxidant and anti-inflammatory *in vitro* systems, as well as in cholinesterase inhibition assays. The 80% ethanol extract contained flavones (glycosides of isoscutellarein and hypolaetin), phenylethanoids (mainly verbascoside) and chlorogenic acid as the main constituents. Observed antioxidant and anti-inflammatory effects are ascribed to the high content of polyphenols in the 80% ethanol extract. No inhibition of acetylcholine- or butyrylcholinesterases was observed. Antioxidant and anti-inflammatory effects are suggested to play a protective role in the pathogenesis of neurodegenerative diseases [3]. Our findings may be in accord with previous *in vivo* findings and should be followed up in future studies.

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OY.2.17 - POLYPHENOLS AND POLYPHENOL FUNCTIONALIZED NANOPARTICLES AS ANTIVIRALS AGAINST ENTEROVIRUSES

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To efficiently lower virus infectivity and combat virus epidemics or pandemics, it is important to discover broadly acting antivirals. Here, we investigated two naturally occurring polyphenols, Epigallocatechin gallate (EGCG) and Resveratrol (RES), and polyphenol-functionalized nanoparticles [1] for their antiviral efficacy. Concentrations in the low micromolar range permanently inhibited the infectivity of high doses of enteroviruses (10^7 PFU/mL). Sucrose gradient separation of radiolabelled viruses, dynamic light scattering, transmission electron microscopic imaging and an in-house developed real-time fluorescence assay revealed that polyphenols prevented infection mainly through clustering of the virions into very stable assemblies. Clustering and stabilisation were not compromised even in dilute virus solutions or after diluting the polyphenols-clustered virions by 50-fold. In addition, the polyphenols lowered virus binding on cells. In-silico docking experiments of these molecules against 2- and 3-fold symmetry axes of the capsid, using an algorithm developed for this study, discovered five binding sites for polyphenols (Figure 1), out of which three were novel binding sites. Our results altogether suggest that polyphenols exert their antiviral effect through binding to multiple sites on the virion surface, leading to aggregation of the virions and preventing RNA release and reducing cell surface binding.

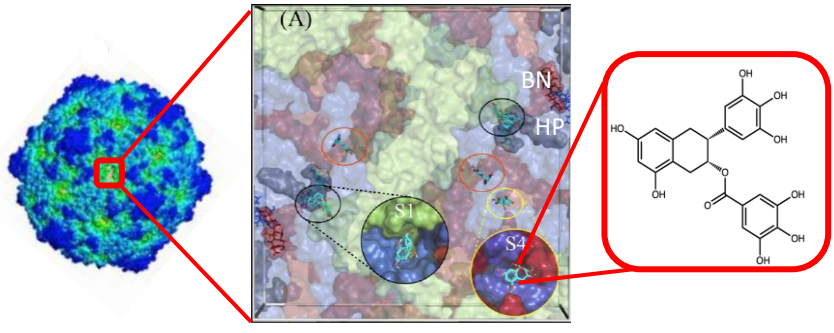

Figure 1: Docking sites identified for EGCG around the 2-fold axes of symmetry of Coxsackievirus A9 (docking performed by O. Sheik Amamuddy and Özlem Tastan Bishop (Rhodes University, SA)

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OY.2.18 - IN VITRO EVALUATION OF EFFICACY OF CORNELIAN CHERRY EXTRACT AGAINST NEURODEGENERATION

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Cornelian cherry *(Cornus mas* L.) is a plant used since ancient times in folk medicine, especially in Asia, because of its therapeutic potency. The nutritional value of the crop depends heavily on cultivation conditions, including soil properties and climate [1], but generally the fruit contains high concentrations of vitamins and minerals. At the same time, Cornelian cherry contains significantly higher amounts of polyphenols (phenolic acids, flavonoids, anthocyanins), compared to other fruit, to which the pro-health properties are attributed. The ingredients of Cornelian cherry, especially polyphenols and generally flavonoids, indicate potency of the fruit against various pathologies under oxidative stress. The central nervous system is one such system, especially vulnerable to oxidative stress [2] due to high concentration of poly-unsaturated fatty acids, low levels of antioxidant defence and relatively high rates of oxygen consumption.

In an effort to discover clinically effective nutrients as potential neuroprotective agents against cognitive disorders i.e., neurodegeneration, our research has focused on probing into a) neuroprotective properties of the extract against oxidative stress, and b) anti-inflammatory properties of Cornelian cherry extract obtained with aqueous solutions of cyclodextrins. The examined extract possesses the following characteristics: total polyphenolic content: 4613 ± 361 mg GAE/100 g d.w., DPPH radical scavenging activity: 21484 ±109 μmol TRE/100g d.w., total monomeric anthocyanines: 238 ± 6 mg cyanidin-3-o-glucoside /100 g d.w., loganic acid 1953 ± 38 mg /100g d.w. and total flavonoids: 136 ± 1 mg quercetin/100 g d.w.

Consequently, research was launched into the toxicity profile of *Cornus mas* L extract, as basic natural product isolated and characterized at the (bio)chemical level, using two different brain tissue cell lines, including pathological human neuroblastoma (SH-SY5Y) and physiological mouse neuronal (N2a) cell lines. The in vitro biological profile was formulated through investigation of the toxicity profile and further antioxidant and anti-inflammatory properties. In depth studies included a) viability, b) morphology, and c) chemotacticity, in a dose- and time-dependent fashion.

The results project a well-defined bioactivity profile of the enriched polyphenolic extract, attributed to atoxicity up to very high concentrations (5 mg/mL), thereby justifying further use as a neuroprotectant of nutritional value.

Acknowledgements: This research has been conducted in the frame of the Regional Operational Programme Central Macedonia 2014-2020 (“Development of natural product with neuroprotective action based on the plant Cornelian cherry”, action code: KMP6-0079229) that was co-financed by Greek national funds and the European Union (European Regional Development Fund).

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OY.2.19 - HOT WATER EXTRACTS OF WILLOW (*SALIX* SPP.) BARK SHOW ANTIVIRAL ACTIVITY AGAINST BOTH ENVELOPED CORONAVIRUSES AND NON-ENVELOPED ENTEROVIRUSES

Reshamwala D.*[a], Shroff S.[a], Tienaho J.[b], Liimatainen J.[b], Laajala M.[b], Kilpeläinen P.[b], Viherä-Aarnio A.[b], Jyske T.[b], Marjomäki V.[a]

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In the last few decades, the world has been witnessing several viral outbreaks. These epidemics and pandemics not only affect public health but also have a negative impact on the global economy. There is a great need to find broad acting, safe and sustainably produced antiviral agents to complement the vaccines and drugs in the fight against viruses. Willow is known to be a rich source of bioactive agents. The antiviral potential of hot water extracts of *Salix* spp. bark against the stable, non-enveloped enteroviruses was determined in our previous study [1]. Here, we expanded our research to study the antiviral property of these extracts against the enveloped human coronaviruses (Figure 1) and investigated their mechanism of action against both coronaviruses and enteroviruses. The native *Salix* bark samples were very effective in lowering the antiviral potential of the stable non-enveloped enteroviruses even at room temperature and only after 45 seconds of contact. Confocal microscopy verified the inhibition of infection by showing loss of staining, of the newly synthesized capsid or spike proteins. Time-of-addition studies demonstrated that *Salix* bark extract had a direct effect on the virus particles, but not through cellular targets. Negative stain electron microscopy and thermal assay showed that antiviral action on enteroviruses was based on the increased stability of the virions. In contrast, *Salix* bark extract caused disintegration of the coronaviruses, which was demonstrated by the negative stain electron microscopy and by an uncoating assay where the released RNA from the broken virions was measured with qPCR. The bark of *Salix* L. species is rich in phenolic compounds as secondary metabolites [1]. None of the well-known bioactive substances of *Salix* bark extract, such as salicin, salicylic acid, picein, and triandrin, had antiviral activity against viruses, suggesting that these pure compounds alone do not have sufficiently high antiviral activity [1]. The antiviral effect was based on the synergistic effect of different polyphenols (e.g., tannins) derived from the bark, rather than relatively unstable salicinoid structures.

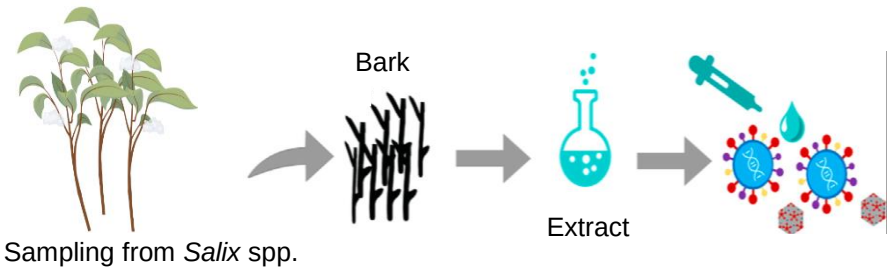

Figure 1: Schematic of the process of *Salix* bark extraction and exploring its antiviral potential

Keywords: Salix; antiviral; enteroviruses; coronaviruses

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P.2.01 - DERIVATIVE OF FLAVONOID METABOLITES, 3-METHOXYCATHECOL, INDUCES VASORELAXATION OF RAT AORTA *EX VIVO* AND THIS EFFECT INCLUDES ACTIVATION OF VOLTAGE-GATED POTASSIUM (K_V) CHANNELS

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Several catecholic compounds can be detected in human blood [1]. These compounds can arise from flavonoid metabolism by the gut microbiota. One of these metabolites, 4-methylcatechol (4-MC), exerts *ex vivo* vasorelaxant and *in vivo* blood pressure-decreasing effects in rats [2,3].
In this study, 22 closely-related derivatives of 4-MC were screened for *ex vivo* vasodilatory activity on the isolated rat aorta. Thirteen compounds were active and produced concentration-dependent vasodilation which was almost complete. 3-methoxycatechol (3-MOC) was the most potent one with an EC_{50} value of 9.6 µmol.L^{-1}. In mechanistic experiments, 3-MOC potentiated the vasodilatory effects of sodium nitroprusside (NO donor) or forskolin (adenylate cyclase activator). Subsequent testing showed that 3-MOC targeted directly the vascular smooth muscle probably via K_V7 channels as was observed in two different models using both non-selective (4-aminopyridine) and selective (linopirdine) K^+ channel blockers. On the other hand, direct effects on vascular endothelium, on muscular BK_{Ca}, K_{ATP}, K_{ir} or L-type Ca^{2+} channels, or on muscular sGC, PKG or SERCA can be excluded.
In short, 3-MOC-induced vasodilation *ex vivo* is based, at least in part, on activation of muscular K_V channels, direct or indirect, resulting in re/hyperpolarization of the vascular smooth muscle.

The study was supported by Czech Health Research Council (NU21-02-00135).

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P.2.02 - BIOACCESSIBILITY OF ANTHOCYANINS FROM EDIBLE FLOWERS USING GASTROTECHNIC APPROACHES

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The role of diet in human health remains one of the main concerns of modern society. New strategies to fulfill the necessity of healthy diets available to everyone, in a growing civilization, with increasing awareness of the consumers, and the need for more sustainable agriculture strategies to reduce climate and water footprints, led to an increase of research focused on such themes, and some forgotten traditions are being reinvented and adopted. In this perspective, edible flowers (EF), are gaining more and more popularity among consumers, chefs and confectioners, and although they still represent a niche market, EF constitute an emerging and sustainable dietary alternative. Amidst EF, there are some very good sources of such bioactive components, in particular, polyglycosylated ANT that have been reported as having better physical and chemical properties than monoglysocylated ANT [1]. Although a few health-benefits of ANT-rich EF have been reported, no knowledge about their bioavailability is at hand.

In this work, four species of Edible Flowers (*Viola tricolor L.*; *Centaurea cyanus*; *Cosmos bipinnatus*; *Clitoria ternatea*) – Figure 1 – were characterized in terms of anthocyanin content and for their stability towards different gastrotechnic approaches. Anthocyanin-rich extracts were submitted to different cooking temperatures and times and the degradation kinetics of the total anthocyanins was followed by UPLC-PDA. Also, the pH influence was assessed using the same methods. The influence of different food matrices elements including proteins, starch, and sugars, in the stability of anthocyanins to the cooking procedures was also assessed. And finally, the absorption of the resultant samples through an in vitro gastric cell model was evaluated using MKN-28 cells.

The results showed that the anthocyanins were mainly polyglycosylated. Temperature and pH had a significant effect on the degradation rate of anthocyanins. However, in some cases, a time-dependent rise in the amount of the total anthocyanin content in similar conditions of T and pH was observed. Different effects dependent of the food matrix element, were observed. Overall, all the anthocyanins regardless of the source were able to be detected on the basolateral side of the gastric cell model, indicating their ability to be absorbed and cross it.

This exploratory study showed the first insights on how cooking can influence the bioaccessibility and bioavailability of anthocyanins from edible flowers.

Figure 1: Edible Flowers utilized in the different experiments.

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P.2.03 - ANTIBACTERIAL ACTIVITY OF NATURAL EXTRACTS AND ITS INHIBITION OF *E. COLI* BACTERIA TOXINS

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Many natural plant polyphenols, including tannins, are intensively investigate due to their effective antibacterial properties [1].
The aim of this research was to determine antibacterial activity of six natural extracts: sweet chestnut extract, ellagitannin isolated from sweet chestnut extract (70% purity), ellagic acid, gallic acid, quebracho extract and mimosa extract. Studies were divided onto two parts:
(i) Analysis of MIC (minimum inhibition concentration) and MBC (minimum bactericidal concentration) of six compounds against *Escherichia coli* ATCC 35218, (ii) inhibition of bacteria hemolysin activity in the investigation where bacteria firstly grown in the presence of studied compounds (concentrations much lower than MIC) and then hemolysis was measured for sheep red blood cells exposed on the isolated bacteria growing medium.
From the all-tested compounds, the largest antimicrobial activity was observed for ellagic acid (Figure 1) with the MIC value sixteen times lower than for gallic acid (62.50 µg/ml and 1000 µg/ml respectively). Strong antimicrobial activity was also determined for sweet chestnut extract with the MIC four times lower (i.e., 250 µg/ml) than for the quebracho and mimosa (MIC was 1000 µg/ml for both). The strongest antihemolytic activity (protective effect of tested compounds against red blood cells lysis induced by *E. coli* hemolysin) was determined at ¼ MIC for ellagic acid (15.63 µg/ml corresponding to 8.18% of hemolysis inhibition) and chestnut extract (62.50 µg/ml corresponding to 7.66% of hemolysis inhibition).
According to obtained results it can be concluded that sweet chestnut extract, isolated from it ellagitannins and ellagic acid, as the main unit of ellagitannins and one of the active components in sweet chestnut extract, possess very high antimicrobial activity in comparison with gallic acid (from the group of gallotannins) as well as with quebracho and mimosa extracts (from the group of condensed tannins).

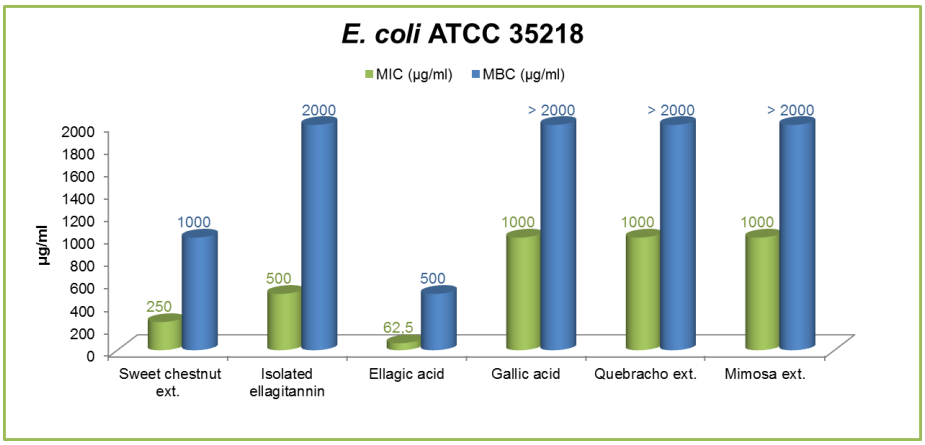

Figure 1: Antimicrobial activity of studied compounds demonstrated as MIC and MBC.

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P.2.04 - SHORT-TERM LIPID-INFUSION INDUCED GLUCOSE INTOLERANCE IN MICE IS ATTENUATED BY CARNOSIC ACID

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Obesity and type 2 diabetes (T2D) are associated with elevated plasma free fatty acids (FFA). Exposure to FFAs, such as palmitate, by lipid infusion in mice, results in insulin resistance as observed in obesity and T2D. Compounds found in herbs and plants have attracted attention, in recent years, for prevention and treatment of insulin resistance and T2D. In previous studies by our group, the polyphenol carnosic acid (CA), found in rosemary and other plants, increased glucose uptake and attenuated the FFA (palmitate)-induced insulin resistance in muscle cells and adipocytes. In addition, CA caused a robust activation of AMP-activated protein kinase (AMPK). Our present study examined the effects of CA in short-term FFA-induced glucose intolerance in mice.

To mimick elevated FFA levels seen in obesity, C57Bl/6J mice were cannulated in their jugular veins and infused for 48h with ethylpalmitate (EtP, nontoxic method of elevating circulating palmitate because of hydrolysis to palmitate and ethanol by plasma esterases). In addition, a group of mice were infused with EtP plus 50 mg/kg CA for 48h. Ethanol vehicle with or without CA were two additional control groups. At the end of the infusions, the mice were subjected to a 2hr hyperglycemic clamp (20 mM glucose) and the glucose infusion rate was measured to assess glucose tolerance.

Our data indicate that mice infused with EtP and CA had a higher glucose infusion rate (GIR; mg/kg*min) (66.5$\pm$10, mean$\pm$SEM) than mice infused with EtP alone (22.7$\pm$10). The GIR in the EtP and CA was comparable to the GIR of ethanol vehicle infused mice (73$\pm$9).

Analysis of collected plasma and tissue will be performed to assess insulin resistance and beta cell function in vivo and to elucidate the mechanisms of CA alleviation of FFA-induced insulin resistance.

Our data indicate that CA has the potential to counteract FFA-induced glucose intolerance in mice.

P.2.05 - APIGENIN-TANKYRASE 1 INTERACTION REVEALED AS A NOVEL THERAPEUTIC APPROACH IN COLORECTAL CANCER

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Anticancer properties of apigenin (a naturally occurring polyphenol) have been characterised recently in different cancers, including colorectal cancer (CRC). However, it becomes indistinct if one looks deeper inside the cell regarding this polyphenol's action. Wnt/β catenin signalling pathway is the focal point in developing targeted therapy in CRC. Tankyrase 1 (*TNKS*) is not only known for its Poly-ADP-ribosyltransferase action on Axin proteins, which leads to Axin degradation and activation of oncogenic Wnt/β catenin signalling but is also linked to the maintenance of telomere length (critical for cancer cell survival). Tankyrase 1 achieves telomere elongation by counteracting the Telomere repeat factor 1 (TRF1) action [1] (antagonises telomere elongation). Thus, tankyrase 1 (*TNKS*) comes out as an exciting target in CRC due to its multifaceted role, and nevertheless, no tankyrase 1 inhibitor is clinically available yet. Thus, we hypothesised apigenin-tankyrase 1 interaction as a possible anticancer strategy in colon cancer (Figure 1). The prognostic effect of *TNKS* in CRC was explored by investigating genetic alterations, expression data, tankyrase 1 expression and tumour immune infiltration by analysing TCGA data. Genetic alterations data and expression of Telomere repeat factor 1 (TRF1) were also checked, the principal interacting partner of tankyrase to regulate telomere length. 12% of CRC patients harbour alterations in *TNKS* and *TERF1* (TRF1) in combination (CBioportal). Increased expression of *TNKS* was further linked to poor overall survival in CRC. The top interacting partners of *TNKS* were revealed by developing the PPI network via STRING. Functional enrichment analyses (ShinyGO) revealed *TNKS* and its interacting partners are linked to telomere maintenance, cell cycle, and Wnt signalling. Ankyrin-repeat clusters (ARCs) and the PARP catalytic domain of tankyrase 1 were checked for possible interactions with apigenin. Molecular docking (Autodock Vina) with apigenin and its metabolite luteolin showed moderate to good binding affinities with both ARC (-7.1 and -6.6 Kcal/mol) and PARP catalytic domain (-9.6 and -9.8 Kcal/mol) of tankyrase 1. Deeper insights through MD simulation analyses (RMSD, RMSF, H-bond) further characterised protein-ligand interactions. Finally, in CRC cells (COLO-205, HCT-116), apigenin and luteolin exerted anti-tumour effects by suppressing β-catenin, indicating apigenin as a probable anti-tankyrase 1 inhibitor.

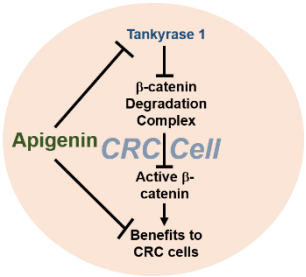

Figure 1: Schematic representation of possible action of apigenin on tankyrase 1 in CRC.

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P.2.06 - STUDIES ON POTENTIAL PROTECTIVE EFFECTS OF DIFFERENT FRUIT JUICE EXTRACTS ON THE INTESTINAL BARRIER.

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The antioxidant effects of fruits and fruit juices are already well described in the scientific literature. These positive effects are mainly attributed to their polyphenols [1]. Many studies have already confirmed positive influences of e.g., polyphenols from apples on the intestine and the intestinal epithelium. For example, they are associated with preventive effects in chronic intestinal inflammation [2]. Oxidative stress and the consumption of sugary drinks are associated with increased intestinal permeability [3]. The possible antioxidant effect of polyphenols in fruit juices could mitigate or even prevent the negative effects of sugars on intestinal permeability when consumed.
The aim of the study was to investigate the influence of sugars individually and in combination with the prepared fruit juice extracts on the permeability of the intestinal barrier and their antioxidant properties. To enrich the polyphenols, a sugar-free XAD7 extract was generated from different fruit juices, such as chokeberry, grape, pomegranate, cranberry and elderberry. The polyphenols contained were characterized and quantified by HPLC-DAD. In addition, the antioxidant activity of the extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the total phenolic content was determined according to Folin-Ciocalteau. For the *in vitro* human intestinal barrier model, a coculture of Caco2 and HT29-cells was differentiated to form the intestinal epithelium. To investigate the effects on intestinal barrier permeability, the measurement of intestinal membrane transepithelial electrical resistance (TEER) was used as an indicator. As a second method, the transport of the fluorescent marker molecule across the membrane was measured using the Lucifer Yellow assay, with a high transport rate equating to high permeability.
The polyphenol profile typical of each fruit species as well as the antioxidant properties were determined in XAD7 extracts. In the *in vitro* transwell system with Caco2 and HT29-cells, significant permeability-altering effects were observed when treated with sugars and sugar alcohols. Glucose, fructose, sucrose, and sorbitol, which are present in the fruit juices, led to a higher permeability. However, regeneration of the intestinal barrier is observed after 24 hours. Based on these data, the influence of oxidative stress in connection with the pre-treatment of the cells with different fruit juice extracts will also be analyzed in more detail. In order to induce stress response in cells, they will be treated with hydrogen peroxide. This will be followed by a treatment with the fruit extracts to observe a possible faster regeneration of the intestinal barrier. The preventive effects of fruit juice extracts are also investigated by treating the cells with the fruit juice extracts prior to the stress response to possibly mitigate damage to the intestinal membrane by the subsequent stress response. Furthermore, the mechanistic effect and influence of the individual polyphenols of the fruit juice extracts on the intestinal barrier, including oxidative stress on the cells, will be investigated on a transcriptional level by analyzing the expression of the glucose transporters such as GLUT 4 and GLUT 5, as well as SGLT1 and occludin.

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P.2.07 - ANTI-INFLAMMATORY POTENTIAL OF PIGMENTED POTATOES ENRICHED IN CHLOROGENIC ACID, CAROTENOIDS OR ANTHOCYANINS

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Since chronic inflammation is involved in the pathogenesis and/or in the degeneration of many diseases, such as obesity, cancer, cardiovascular and neurodegenerative disorders, the study of the anti-inflammatory activity of bioactive compounds is gaining great interest. In this scenario, pigmented potatoes could represent a source of different phytonutrients known for their potential health-promoting activity, including phenolic acids, carotenoids and anthocyanins (ACNs). Among these, ACNs are known to exert beneficial properties against inflammation-related diseases, such as neuroinflammation and obesity [1, 2], while carotenoid consumption inversely correlates with the incidence of many chronic diseases [3]. Thus, we aimed to study the anti-inflammatory properties of three different upland potato varieties enriched in chlorogenic acid (CGA) (yellow-skinned and white-fleshed Kennebec), carotenoids (red-skinned and yellow-fleshed Desiree) or ACNs (purple skinned and fleshed Bleuet) in THP-1 derived macrophages.
The phytonutrient composition of tubers extracts has been characterized by HPLC-DAD and spectrophotometric analysis allowing us to consider Kennebec, Desiree and Bleuet extracts as CGA-rich, carotenoid-rich and ACN-rich respectively. Their anti-inflammatory effect was tested on THP-1 macrophages pre-treated with the three extracts and then challenged with LPS. The dose-dependent effects on gene expression and/or protein levels of pro-inflammatory factors, such as cytokines and other mediators, were evaluated.
While at higher doses the three extracts exhibit nearly comparable anti-inflammatory properties, our results show that, when provided at plasmatic concentrations, only the carotenoid- and the ACN-rich varieties are able to counteract LPS-induced inflammation. Our findings suggest that the consumption of the pigmented potatoes enriched in carotenoids or ACNs, such as Desiree and Bleuet, may represent a strategy to prevent chronic inflammation.

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P.2.08 - CARNOSIC ACID INHIBITS PROLIFERATION, INDUCES APOPTOSIS AND ACTIVATES AMPK AND ASK1 IN PROSTATE CANCER CELLS

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Prostate cancer is the second most prevalent cancer in men worldwide and accounted for 375,304 deaths in 2020. While many treatments exist for prostatic carcinoma, novel therapeutic agents with increased efficiency are needed to target more aggressive and hormone-resistant forms of prostate cancer, while sparing healthy cells. Traditionally, plant-derived chemicals have been established to treat cancers including prostate cancer. Such examples of plant-derived chemotherapy drugs are docetaxel and paclitaxel. Carnosic acid (CA), a polyphenol found in the herb rosemary (*Rosmarinus officinalis*) has been shown to have biological effects including anticancer properties but its effects in prostate cancer and its mechanisms of action have not been examined. In our preliminary studies, CA has been shown to cause a dose dependent inhibition of cell proliferation (IC_{50}: 66 µM) and cell survival in PC-3 prostate cancer cells. Furthermore, CA induced apoptosis and decreased phosphorylation/activation of Akt. A notable increase in phosphorylation/activation of AMP-activated kinase (AMPK), acetyl-CoA carboxylase (ACC) and Liver kinase B1 (LKB1) was seen with CA treatment (Figure 1). In addition, treatment with CA increased the phosphorylation/activation of apoptosis signal-regulating kinase 1 (ASK1) (Figure 1). Our data indicate that CA activates LKB1-AMPK signaling leading to phosphorylation/activation of ASK1 and induction of apoptosis. The use of inhibitors and small RNA interference (siRNA) approaches will be employed, in future studies, to elucidate the mechanisms involved in carnosic acid's inhibitory effects of prostate cancer.

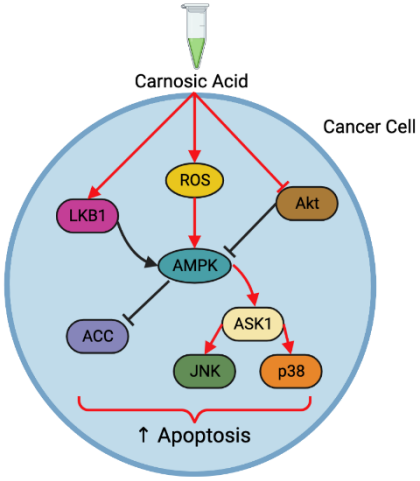

Figure 1: Hypothesized Effects of Carnosic acid on prostate cancer cells. Black arrows = established, red = hypothesized

P.2.09 - NOVEL PHENOLIC-ENRICHED FRUIT EXTRACTS WITH PHOTOPERIOD-DEPENDENT EFFECTS FOR THE PREVENTION OF METABOLIC DISORDERS

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Fruits are rich in bioactive compounds such as (poly)phenols, and the obtaining of extracts enriched in these compounds can reduce fruit loss. (Poly)phenols have shown beneficial effects in preventing metabolic diseases. Moreover, there is growing evidence that these compounds act as signals in the regulation of biological rhythms. Their effects depend on the time of day and the season in which they are consumed. This study aimed to investigate the effects of seasonal-fruit extracts on lipid metabolism and inflammation under short- and long-photoperiods. Male Fischer 344 rats were housed in two photoperiods: short (L6, 6h light) and long (L18, 18h light), fed a standard diet, and orally supplemented with 100 mg/kg body weight of each extract or water (control) for 2 weeks. Different metabolic challenge tests were designed to temporarily induce a degree of disturbance in homeostasis and evaluate the benefits of each extract depending on photoperiod consumption. An oral lipid tolerance test (OLTT) and lipopolysaccharide (LPS)-induced inflammatory challenge were performed to assess the modulation of inflammation. The results from the OLTT showed differences in the area under the curve, depending on the fruit phenolic extract and photoperiod of administration. Inflammatory markers (interleukin 6, IL-6 and tumour necrosis factor alpha, TNFα) were significantly higher in L6, and winter fruit extracts were the most efficient in lowering pro-inflammatory cytokines. These results suggest that specific extracts from seasonal fruits may be more effective in preventing or treating metabolic disorders by restoring disturbed homeostasis depending on the season of consumption.

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P.2.10 - INVESTIGATING THE INTERACTIONS BETWEEN HYDROLYZABLE TANNINS AND ANTHELMINTICS BY ISOTHERMAL TITRATION CALORIMETRY

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Plant tannins are known for their anthelmintic activity and have been extensively studied to battle the ever-growing problem of anthelmintic resistance. While tannins have been shown to possess anthelmintic activity on their own, one approach would be to use them as complementary nutrients alongside commercial anthelmintics. So far, research on the interactions between tannins and anthelmintics is limited, and few studies have reported both synergistic and antagonistic effects depending on the type of the tannin and method used [1,2]. These interactions could either strengthen or weaken the anthelmintic activity of the commercial anthelmintic, especially if tannin-rich diets are combined with oral commercial anthelmintics. This warrants for more research to determine whether tannins can interact directly with commercial anthelmintics or if these observed effects are the results of an indirect mode of action.

To study these interactions, a series of hydrolyzable tannins (HTs) was chosen, and direct interactions between thiabendazole (TBZ) and HTs were evaluated by isothermal titration calorimetry (ITC). ITC is a powerful technique to study the thermodynamics of different interaction and allows the detection of roles and significances of different structural features of tannins in these interactions. The results indicate that HTs interact with the chosen commercial anthelmintic, as shown for pentagalloylglucose in Figure 1, and the strength of the interaction is dependent on the structural characteristics of HTs.

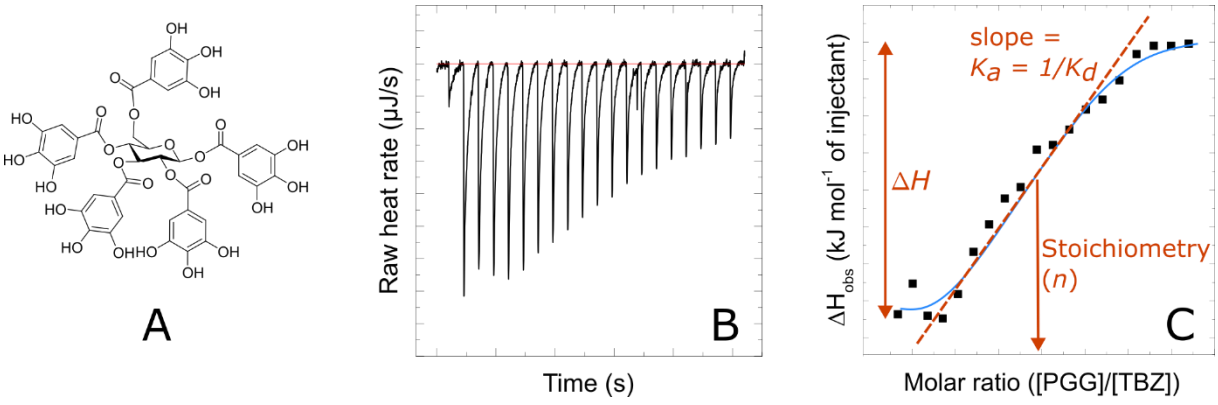

Figure 1: An example of data obtained from titrating pentagalloylglucose (PGG, A) into thiabendazole (TBZ). The isotherm (B) is first integrated, and observed heat rates of injection as a function of the molar ratio (C) is obtained. From this plot, we can determine the thermodynamic parameters of the interaction such as enthalpy (*ΔH*), binding affinity (K_a), and binding stoichiometry (*n*).

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P.2.11 - SYNTHESIS AND *IN VITRO* BIOLOGICAL ACTIVITIES OF HYDROXYTYROSYL OLEATE ON SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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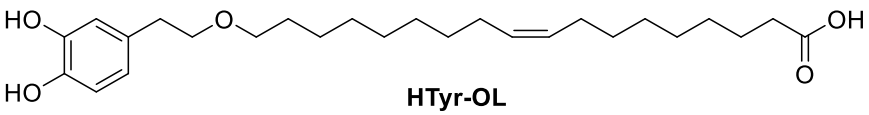
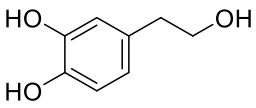
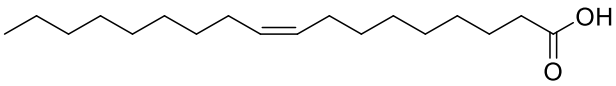
The biological activities of natural substances have been exploited since ancient times and could be employed to treat various human diseases. Today, the anticancer properties of some plant molecules, including synthetic and semi-synthetic compounds are well-studied and used against different types of cancer.
Polyphenols derived from extra-virgin olive oil as hydroxytyrosol (HTyr) have been studied extensively. However, the use of this compound as a therapeutic agent for clinical applications is restricted by its low bioavailability and rapid excretion in humans [1]. To overcome this limitation, several synthetic strategies have been optimized to prepare lipophilic HTyr derivatives. One very promising compound is hydroxytyrosyl oleate (HTyr-OL, Figure 1), synthesized in our laboratories with satisfactory yield by a simple procedure [2].
This communication describes the *in vitro* cytotoxic, antiproliferative, and apoptotic induction activities of HTyr-OL against SH-SY5Y human neuroblastoma cells, compared with those of HTyr and OA (Figure 1). The cell viability assays were followed up by Western blot, flow cytometry, and electron microscopy techniques. HTyr biological activity was occurring in HTyr-OL treatments at lower dosages. Increased expression of caspase-3 and annexin-V and propidium iodide staining confirmed the apoptosis-induced mechanism after HTyr-OL treatment, with a high apoptotic rate in the cytofluorimetric analysis. Furthermore, ultrastructural changes such as alterations in the cell surface, reduction of protrusions, and increase of membrane blebbing were observed by scanning electron microscope [3]. Overall, HTyr-OL is found to be an attractive molecule for pharmaceutical applications.



HTyr-OL



HTyr



OA

Figure 1: Tested compounds

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P.2.12 - PH-DEPENDENT STRUCTURAL MODIFICATIONS OF THE EPITOPES OFB-LACTOGLOBULIN WITH PHENOLIC COMPOUNDS FOR TARGETING ALLERGENIC EFFECTS

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Allergies are immunological overreactions where antibodies of the immune system interact with specific protein regions, so called epitopes. For this interaction, protein sequence and folding (secondary, tertiary and quaternary structural elements) are crucial. Many food allergies are caused by milk proteins. One new approach to reduce the allergenic effect of milk proteins is the structural modification of their epitopes using phenolic compounds (PC), which can interact non-covalently and covalently with the proteins. Due to a variety of chemical properties (quinone formation) of PC and the influence of the reaction conditions (pH), a wide range of possible interaction or even reaction products is possible.
Therefore, the aim of this work is the systematical investigation of structural modifications (secondary, tertiary, and quaternary) of milk proteins by PC. As a model protein, whey protein β-lactoglobulin (β-LG) was used and the PC p-coumaric acid (COU), caffeic acid (CAF), chlorogenic acid (CA) and phlorizin (PHL) were applied at pH 6 and 9. To investigate the changes in secondary structure such as α-helix, intramolecular β-sheet, and/or random coil as well as quaternary structure like dimer formation Fourier-transform infrared spectroscopy (FTIR), fluorescence spectroscopy (surface hydrophobicity), and a modified Ellman assay (free thiol groups) were used for analysis. The allergic effect was measured using modified enzyme-linked immunosorbent assays (ELISA).
The FTIR showed that β-LG is more folded at pH 6 compared to pH 9. At pH 6, the reaction with PC caused a significant increase in α-helix, intermolecular β-sheet, and a decrease in random coil, whereas at pH 9 PCs induced a significant increase in α-helix and intermolecular β-sheet, indicating a stronger folding of β-LG at pH 9 and dimer formation. Fluorescence spectroscopy showed β-LG being more folded at pH 6 than at pH 9, thus, supporting the FTIR data. The addition CAF, CA, and PHL significantly increased the surface hydrophobicity at pH 9. PC forced hydrophobic amino acids like tryptophan to be exposed to the protein surface, causing an unfolding of the protein. Ellman assay showed the increase of free thiol groups by PHL at pH 9 and that confirmed the unfolding. The first results of ELISA showed a significant decrease in antigenic binding capacity at pH 6 for CA, indicating a modification of β-LG's epitopes and therefore, a decrease in the allergenic effect of β-LG. CAF, and PHL at all PC at pH 9 increased antigenic binding capacity, suggesting a better access of the epitopes induced by the PC. It is assumed that an increase in α-helix caused by PC correlates with an increase in allergenicity and may underline the results of the ELISA.
These results contribute to the understanding of structural modifications of β-LG by PC. In particular, FTIR has the potential to show new data of structural modifications of epitopes to reduce allergic effect.

P.2.13 - FLAVONOIDS' DUAL BENEFITS IN DIABETES AND CANCER: A POTENTIAL TREATMENT ON THE HORIZON?

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Flavonoids are natural products ubiquitously found in fruits and vegetables. They are classified based on their chemical structure and their degree of oxidation. Flavonoids possess potential therapeutic properties that target diabetes and cancer. Observing the impact of flavonoids on the impaired pathways in both conditions is essential for properly understanding their influence. The challenge with such a topic is the lack of systemic studies that address and compare the potential therapeutic role of flavonoids in both conditions.

Additionally, limited studies report the gut microbiome's influence on flavonoid metabolism. Here we show the effect of flavonoid consumption on four metabolic pathways that are impaired in diabetes and cancer: apoptosis, AMPK, NF-KB, and enzymatic modification pathways. We also highlight the impact of the gut microbiome's enzymatic modification on flavonoid metabolism.

Throughout our research with more than 11 publications, we found that consuming flavonoids improved the metabolic outcome of the impaired pathways in diabetes and cancer [1]. Also, the same flavonoid can influence the impaired pathway in a different direction based on the metabolic disorder, which suggests a possible dual effect of flavonoids. Additionally, we highlighted the importance of the enzymatic bioconversion by the human gut microbiome on flavonoids and their observed biological properties in diabetes and cancer, as 90% of the ingested flavonoids reach the colon for further modifications. Unfortunately, the literature still lacks data that shows the effect of combination therapy between flavonoids and currently used treatments, as the aim is not to replace the available therapeutic tools but to complement them. Our results demonstrate how the consumption of flavonoids in specific concentrations may influence the complexity of the metabolic pathways, partly improving diabetes and metabolic cancer outcomes (Fig. 1) [2]. We anticipate our research to be a starting point for more systemic studies that highlight the main challenges with this topic, such as the bioavailability of flavonoids, their appropriate administered concentration, and their side effects. Additionally, we suggest conducting more clinical trials to assess the validity of flavonoid's observed biological effects.

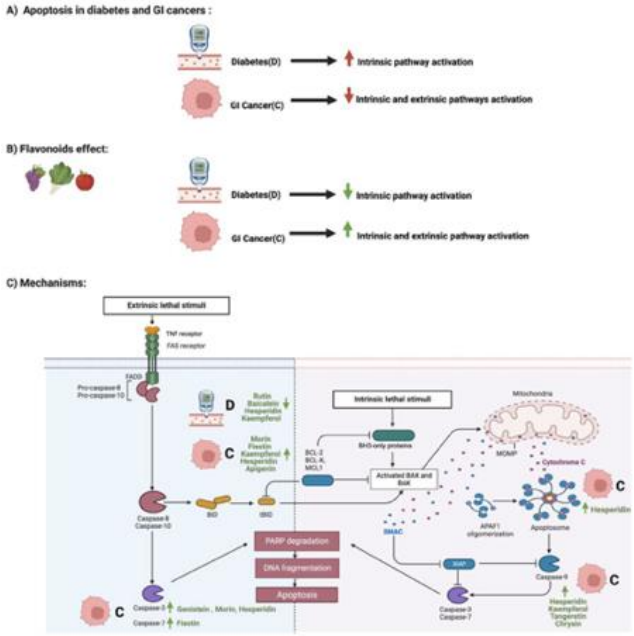

Fig. 1: *Illustrations of the influence of specific flavonoids on apoptotic pathways in diabetes and GI cancers.* Created with BioRender.com.

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P.2.14 - CHOCOLATE POLYPHENOLS: BIOACCESSIBILITY AND BIOAVAILABILITY

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Chocolate is one of the most important food sources of polyphenolic compounds. The basic ingredients necessary for the manufacture of chocolate are cocoa nibs/cocoa liquor, sugar, sweeteners, cocoa butter/butter fat, milk powder, and emulsifiers. Cocoa is the polyphenol-rich ingredient of chocolate. Flavan-3-ols, especially catechin, epicatechin, and polymerization products of flavan-3-ols (procyanidins) are the most abundant phenolics in chocolate. Phenolic compounds are antioxidants and are attributed to many health effects such as reducing blood pressure and cardiovascular disease risk, cancer-protective effects, anti-atherosclerotic effects, and anti-tumoral effects.

The bioaccessibility and bioavailability of phenolic compounds are very important in exerting health effects. The bioaccessibility and bioavailability of phenolics depend on many factors such as the initial concentration of phenolic compound, food matrix, and gastrointestinal conditions. Flavan-3-ols are the most found compound in dark chocolates but have low bioaccessibilities. Hydroxycinnamic acid has higher bioaccessibility values than flavan-3-ols in dark chocolates. Absorbed monomeric flavan-3-ols metabolize into several metabolites such as O-glucuronidated, sulfated, and O-methylated conjugates bearing an intact flavan-3-ol ring. Unabsorbed flavan-3-ols arrive colon, where they are transformed by gut microflora into numerous low molecular weight compounds. Intake of dark chocolate with milk or intake of milk chocolate may affect the absorption of polyphenols. Consumption of chocolate with/without milk did not affect or slightly affect the absorption of polyphenols in several pieces of research, but in another study consumption of chocolate with milk protein decreased the absorption of epicatechin. Carbohydrates such as sucrose may enhance the bioavailability of phenolics from the chocolate matrix. Chocolate spreads with hazelnut was higher phenolic content, but lower bioaccessibility values than chocolate spread without hazelnut. The lower bioaccessibility of phenolics in chocolate spread with hazelnut is probably dependent on high protein content, due to the protein-phenolic interactions of these products. This review aims to discuss the bioaccessibility and bioavailability of phenolics in chocolate and chocolate products.

P.2.15 - INTERACTION OF STILBENES FROM VITIS VINIFERA FOR THEIR ANTI-RADICAL, ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES

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Polyphenols are naturally occurring in plants and are derived from their secondary metabolism. Resveratrol, a polyphenol that belongs to the stilbene family, is the object of numerous studies. Many in vitro studies highlight the anti-inflammatory and antioxidant activities of resveratrol and its derivatives or of plants extracts containing a mixture of these polyphenols. In vivo, these compounds also exhibit beneficial effects in various pathologies that involve inflammatory or oxidant processes in their physiopathology, despite their very low bioavailability. In a plant extract those compounds are present as a complex mixture and the effect of the whole extract probably results from their interactions. In our laboratory, we have shown that several monomers and polymers of resveratrol possess antioxidant and anti-inflammatory activities on their own but the effects that result from their interactions are unknown.

The main objective of the present work is to study the interactions between two stilbenes, resveratrol and ε-viniferin (a dimer of resveratrol) for their anti-radical, antioxidant and anti-inflammatory activities in an acellular model (reduction or scavenging of the DPPH or NO radical, respectively) and in a cell model of rat macrophages (RAW 264.7) stimulated by LPS (production of NO and reactive oxygen species, or ROS). Also, insofar as these compounds possess biological activities in animals despite their very low bioavailability, we were interested in the biological effects of their metabolites.

In a cell-free model, resveratrol and ε-viniferin alone have the ability to dose-dependently reduce and scavenge the DPPH and the NO radicals, respectively. When they are used in an equimolar combination these capacities become synergistic. In a mouse macrophages model, exposure to LPS induces the production of NO as well as the production of ROS in a dose-dependent manner. Resveratrol and ε-viniferin alone possess the ability to inhibit the production of NO as well as that of ROS in a dose-dependent manner. When these two stilbenes are used in an equimolar combination, they exhibit a synergistic effect in inhibiting NO production and an additive effect in inhibiting ROS production. In vivo, we have previously demonstrated the appearance of 4 sulfates and 4 glucuronides metabolites of ε-viniferin in rats. Insofar as we are able to produce the 4 glucuronidated forms of ε-viniferin, we are actually conducting studies in order to identify to which part is contributing each form, native and glucuronidated forms, to the whole biological activity.

The use of a mixture of polyphenols or of a plant extract could therefore be beneficial to health insofar as, when mixed, they can present a synergistic activity. Moreover, their metabolites could be involved in their in vivo effect. Overall, our results underline the interest of using these natural compounds for the prevention of diseases whose pathophysiology involves oxidative stress or inflammation.

P.2.16 - ROLE OF SGLT-2 INHIBITION IN THE RECOVERY OF ENDOTHELIAL FUNCTION OF ISOLATED RAT THORACIC AORTA EXPOSED TO HIGH GLUCOSE

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It is well known that apple is a great source of polyphenols. Among them, phloridzin, a member of the dihydrochalcone class, is well known to have inhibitory effects on sodium-glucose cotransporters (SGLTs) [1]. A decrease in SGLTs activity could allow a better cardiovascular health. They are functionally expressed on the surface of endothelial cells and are responsible for the intracellular uptake of circulating glucose. However, an increase of circulating glucose, which is a risk factor for the development of metabolic syndrome, may lead to endothelial dysfunction. We investigated the effects of short-term exposure (2 hours) to High Glucose (HG) and SGLT2 inhibition on thoracic aortic rings from Sprague-Dawley rats (12 weeks old). The rings were incubated in isolated organ baths (Emka Technologies, Paris, France) and pre-contracted with phenylephrine (Phe ; 3.10^{-6} M). After a 2-hours incubation in presence of control glucose (11.1 mM) or HG (44.4 mM), we performed Cumulative Concentration-Relaxation Curves (CCRCs) to acetylcholine (ACh) (Figure 1). The results are analyzed by a Non-Linear Mixed Effects (NLME) model with the R software. ACh-induced vasorelaxation was impaired in the presence of HG compared with the control group (respectively E_{max} = 93.37 ± 1.25 *VS* 98.94 ± 0.57; p<0.001 and pD_2 = 6.625 ± 0.061 *VS* 7.129 ± 0.045; p<0.001). The use of tempol, an antioxidant agent, and L-Arginine+BH4, respectively a substrate and a cofactor of eNOS, have both improved Ach-mediated vasorelaxation in HG group. Pretreatment with a SGLT-2 inhibitor, Dapaglilfozin (10 nM), significantly improved vasorelaxation in presence of HG (E_{max} = 100.26 ± 1.04; pD_2 = 7.229 ± 0.039) indicating that an inhibition of SGLT-2 could have an endothelio-protective effect. Furthermore, it has been reported that the attenuation of endothelial dysfunction by gliflozins may involve a role of sirtuin proteins, particularly SIRT1. Resveratrol is a polyphenol extracted from black grapes, found in red wine, it has the ability to activate SIRT1 [2]. We also found that Sirtinol (8 µM), an inhibitor of sirtuin-1, completely inhibited the improvement of Dapagliflozin-modulated Ach vasorelaxation (E_{max} = 90.81 ± 3.49; pD_2= 6.673 ± 0.110), suggesting that the mechanism of action of that gliflozin mainly involves the SIRT1 pathway.

Furthering biochemical analyses evaluating the expression level of eNOS, phosphorylated eNOS, SGLT-2 and SIRT1 are in progress. The next step will also be to evaluate the ability of polyphenols (extracted from apple and black grape (rich in resveratrol)) to modulate the activity of SGLT-2 and SIRT1 in rat thoracic aorta exposed to high glucose.

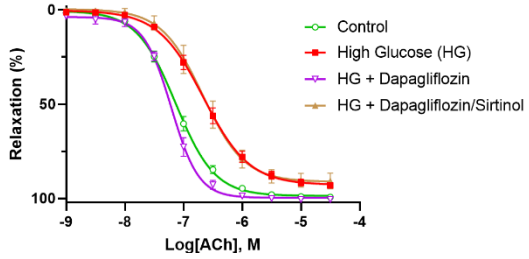

Figure 1: Cumulative concentration-relaxation curves to acetylcholine

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P.2.17 - COFFEE SILVERSKIN EXTRACT AS A POTENTIAL COLORECTAL CANCER PREVENTIVE AGENT: A COMPARISON WITH ROASTED COFFEE

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Colorectal cancer is currently the third type of cancer most incident worldwide, and the second most lethal. However, it is closely related to lifestyle and diet, and its prevalence and malignancy can be preventable [1]. Some studies have indicated that coffee consumption may be associated with a lower risk of developing this tumor due to the presence of certain bioactive compounds (*e.g.,* chlorogenic acids) relevant to the physiology of the colon [2]. Coffee silverskin (CS), the major by-product of coffee roasting, has a composition in bioactive compounds similar to that of coffee [3]. Therefore, the present work aimed to investigate its potential effects in the prevention of colorectal cancer (in comparison with roasted coffee (RC)). For that, RC and CS were used to prepare extracts (RCE and CSE, respectively) by aqueous ultrasound-assisted extraction and their effects on cell viability (LDH assay), proliferation (^{3}H-thymidine incorporation), culture growth (SRB assay), angiogenesis (VEGF-A commercial kit), and oxidative stress (malondyaldehyde (MDA) commercial kit) levels were tested in a human colorectal adenocarcinoma cell line (HT-29). The results showed that CSE was cytotoxic to this cancer cells, while RCE had no effect on cell viability. Furthermore, none of the extracts had any effect on culture growth, but both similarly and significantly reduce cell proliferation rates. Regarding the effects on angiogenesis, RCE significantly reduced VEGF-A levels, while no effects were found with CSE. Finally, CSE (but not RCE) significantly reduced the oxidative stress levels. Overall, these results showed that CSE and RCE possess anticancer potential, but by different mechanisms.

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P.2.18 - INFUSIONS OF CHAMOMILE PREPARED WITH THERMAL WATERS: IS THERE ROOM FOR BIOACTIVE IMPROVEMENT?

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German chamomile (GC, *Matricaria recutita* L.) is an herbaceous flowering plant traditionally prepared as an infusion with water to make an herbal infusion used for medicinal purposes. The health-promoting effects of GC infusions have been associated to phenolic compounds (PC) [1]. Thermal waters (TW) are natural mineral waters with therapeutic applications, which health beneficial effects have been related to their chemical and mineral composition. Given that both matrices show interesting biological properties, in this study it was hypothesized that preparing GC infusions with TW rather than with drinking-water (DW) could improve the bioactive benefits. Thus, this study aimed to evaluate the PC composition, the antioxidant, the hepatoxicity, antimicrobial, anti-inflammatory and anti-tumour activities of GC infusions prepared with TW or DW. Infusions were prepared according to Guimarães et al. (2013), PC were determined using LC-DAD-ESI-MS^n, antioxidant activity was analysed through a chemical method analysing the radical scavenging capacity (DPPH) and a biochemical method that measures the lipid peroxidation inhibition (TBARS), while the remaining bioactivities were evaluated using cell-based assays. Fifteen phenolic compounds were tentatively identified in the samples, 8 flavonoids and 7 phenolic acids, being diosmetin-rutinoside, apigenin 6,8-di *C*-hexoside, chlorogenic acid and ferulic acid hexoside, the major compounds detected. The type of water used for infusion preparation (TW or DW) showed no effect on the PC profile, but it seemed to have an effect on the observed concentration. However, antioxidant, hepatoxicity, antimicrobial, anti-inflammatory and anti-tumour activities are still under discussion and will be presented at the conference.

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P.2.19 - NEW INSIGHTS ON BIOACTIVE EFFECTS OF CHLOROGENIC ACIDS: ACTION ON PROLIFERATION AND DIFFERENTIATION OF C2C12 MYOBLAST CELLS

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Currently, the search for new molecules with pro-regenerative activity has raised clinical and scientific interest, since poor regeneration and/or remodeling following injuries to skeletal muscle tissue can lead to loss of tissue function, affecting the quality of life. A recent therapeutic approach has been to investigate the potential effect of bioactive compounds from plants and vegetables, such as phenolic compounds. Chlorogenic acids are among the most widely distributed phenolic compounds in nature, with well-characterized anti-inflammatory and antioxidant activities. In this study, it was hypothesized that chlorogenic acid (CA) and 3,5-dicaffeoylquinic acid (3,5-DQA) may exert pharmacological actions in the context of tissue repair and regeneration. Thus, this study aimed to investigate the effect of CA and 3,5-DQA on the proliferation and differentiation of myoblasts (C2C12), which mimetizes skeletal muscle tissue. Viability assays indicated that 25 µM (CA) and 15 µM (3,5-DQA) were the appropriate concentrations to be applied in cells. For proliferation studies, C2C12 cells were grown in DMEM supplemented with 10% fetal serum bovine for 48 hours (37°C, 5% CO_2), followed by treatments with the compounds for 24 hours. For differentiation assays, after achieving confluence (>80%), cells were treated with each compound and left for 72 hours in DMEM supplemented with 2% of horse serum. The expression of transcription factors was evaluated, namely Pax7, MyoD (proliferation phase); MyoD and myogenin (differentiation phase). Compared with the control samples (no compounds added), in the proliferation phase, there was an increase in the expression of MyoD, but not Pax7, for both CA and 3,5-DQA. In the differentiation phase, none of the compunds influenced the expression of MyoD, while 3,5DQA promoted an increase in myogenin expression. These results sugest that CA and 3,5DQA may induce the begining of differentitation by increasing MyoD expression during proliferation. Moreover, by increasing myogenin in the differentiation phase, the results sugest that 3,5-DQA may favor the differentiation of myoblasts into myotubes, and generation of new fibers. This is the first report on the pro-regenerative effect of these compounds, and the promising results obtained should be further validated in future studies.

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P.2.20 - EFFECT OF SUPPLEMENTATION WITH WINE POMACE PRODUCT ON THE EXPRESSION OF CYTOKINES IN C57BL/6 MICE INFECTED WITH *CAMPYLOBACTER JEJUNI*

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Foodborne illnesses are one of the most serious public health problems and one of the leading causes of illness and death. Among them are the disease called campylobacteriosis, which is the world's first diarrheal disease caused by a bacterial agent, *Campylobacter jejuni.* The clinical presentation of *C. jejuni* infection is intrinsically linked to an induced inflammatory response within the intestinal epithelium. *C. jejuni* virulence is multifactorial, requiring both intrinsic and exported bacterial components, some of which facilitate host cell interactions. A hallmark of *Campylobacter* enteritis is an acute inflammatory response localized to the epithelium of the distal colon.

Treatment of mild campylobacteriosis cases in otherwise healthy adults typically involves the administration of antibiotics, rehydration, and clinical monitoring, however antimicrobial resistance of antibiotics has been increasing over the past two decades. The selection of a natural antimicrobial product from winemaking residues rich in phenolic compounds [1,2] can be an alternative to the use of antibiotics in the fight against *Campylobacter*.

The aim of this work is to study the supplementation of a red wine pomace product, rich in phenolic compounds, on the expression of cytokines of C57BL/mice infected with *Campylobacter jejuni*.

An animal model of infection was developed using C57BL/6 infant mice with a depleted microbiome. Mice were infected with 10^{10} CFU of *C. jejuni* on two consecutive days and wine pomace product was administered for 6 days. After sacrifice gene expression in colon of IL-6, TNF-α and MCP-1 cytokines was examined by quantitative reverse transcription PCR (RT-qPCR).

The infection model shows high *Campylobacter* levels in feces after the two infective doses and a significant increase in cytokines expression in colon. The administration during 6 days of the wine pomace product allows to reduce between 40 and 60% the levels of cytokines. In conclusion, in this work it has been observed the activation of the innate immune system during *C. jejuni* infection induced proinflammatory and anti-inflammatory cytokines and a reducing effect of this expression with supplementation, contributing to understand the possible antimicrobial mechanism exerted by the phenolic compounds of the wine pomace product.

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P.2.21 - WINE POMACE PRODUCT INHIBITS HYPOXIA-INDUCED OXIDATIVE STRESS IN SH-SY5Y CELLS. COMPARATIVE STUDY IN 2D AND 3D CELL CULTURES

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Hypoxia or ischemia-mediated brain injury is a major cause of neurodegenerative disorders. Oxidative stress and inflammatory response are involved in the progression of secondary hypoxic-ischemic brain injury modulated by NF-KB and Nrf2. In previous studies we showed that wine pomace product (WPP)-mediated anti-inflammatory and antioxidant effects due to modulation of Nrf2/ARE and IKK/NF-κB pathways cross-talk, indicating that it may have therapeutic potential as a neuroprotective agent [1].

The aim of the present study was to investigate the neuroprotective effect of WPP against hypoxia injury in SH-SY5Y cells by treatment with $CoCl_2$. A comparative study was carried out on two-dimensional (2D) and spheroids cells (3D) to establish a hypoxic model of neuronal cells. WPPs showed a high content of phenolic compounds mainly anthocyanins, flavonols such as myricetin and kaempferol, flavanols like epigallocatechin, and phenolic acids such as gentisic, homoprotocatechuic and caffeic acids. The fraction bioaccesible of WPPs was obtained by in vitro digestion following a dialysis step to model the mechanical aspects of the epithelial barrier. The SH-SY5Y cells were differentiated with sequential cell treatments with retinoic acid (RA) and 12-O-tetradecancyl-phorbol-13-acetate (TPA). NeuN and Beta III tubulin was used as neuronal markers. Cell viability was tested using an MTT assay and the ROS levels were analyzed based on microscopy images obtained from immunofluorescence and cytometry.

Spheroids (3D) cells develop different characteristics in comparison to 2D cells. However, the bioaccesible fraction of WPPs showed a significant protective effect on cell viability in both models. Furthermore, the WPPs treatment result in a decrease of the intracellular ROS levels induced by $CoCl_2$ and modulated by the Nrf2/NfkB transcription factors. The results showed that the 3D model was more sensitive to damage by $CoCl_2$ and the response of WPPs bioaccessible fraction than the 2D model. In conclusion, this study established that the sensitivities and tolerances to bioactive compounds could be better understood with a 3D cell culture system. Furthermore, the results suggest the potential neuroprotector benefits of wine pomace product in the treatment of neurodegeneration exposure to hypoxia.

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P.2.22 - ANTI-INFLAMMATORY EFFECTS OF WINE POMACE PRODUCT IN AN IN VITRO EPITHELIAL-ENDOTHELIAL CO-CULTURE MODEL

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Bioactive phenolic compounds of wine pomace (WPP) exert protection against chronic diseases such as cardiovascular and cancer pathologies in which the endothelial barrier plays an important role [1]. The aim of this study was to identify the anti-inflammatory effect of a bioaccesible fraction (WPGI) obtained by in vitro digestion of WPP on activated human umbilical vein endothelial cells (EA.hy929) after their transport across an epithelial intestinal cell (Caco-2). The study was carried out in a transwell epithelial-endothelial co-culture system with Caco-2 cells as intestinal layer, and EA.hy929 as endothelial cells stimulated with TNF-α. Transport of phenolic compounds were determined by HPLC/DAD and the effect on inflammation events such as changes in the expression of adhesion molecules, cytokines and transcription factors (Nrf2 and NF-κb) after crossing the intestinal layer was evaluated.

Our results provide evidence that the bioavailable polyphenolic constituents of the WPGI cross intestinal barrier to observed the phenolic compounds flavanols epicatechin and catechin, and phenolic acids such as vanillic, cumaric, gallic, protocatechuic and p-hidroxy benzoic acids. The bioavailable fraction attenuated changes in cell permeability-TNF-α induced, increased the expression of adhesion and tight-junction molecules (E-cadherin, claudin and occludin) and reduced mRNA levels of transcription factors such as NF-κB. In conclusion, the gastrointestinal fraction of WPP crossed the intestinal cell layer and ameliorated the inflammatory response in endothelial cells.

Acknowledgment: The authors thank the financial support of Ministry of Science, Innovation and Universities, Spanish State Research Agency and European Regional Development Fund (Project PGC2018-097113-B-I00).

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P.2.23 - NOVEL ARYL SULFOTRANSFERASES FOR (POLY)PHENOLS SULFATION

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Natural (poly)phenolic compounds are largely metabolized via the II phase (conjugation) of biotransformation, especially by sulfation and glucuronidation [1]. Due to their complex structure, a large number of structurally different metabolites, such as mono-, di- or trisulfates, can be formed. Their isolation from biological material is impractical; however, they can be synthesized *in vitro*. Recombinant bacterial aryl sulfotransferases (ASTs) that use available *p*-nitrophenyl sulfate (*p*-NPS) as a sulfate donor can be readily produced in *E. coli*. There are only a few aryl sulfotransferases that have been characterized and used for the sulfation of (poly)phenols. The best studied is the recombinant AST from *Desulfitobacterium hafniense* [2]. Enzymatic sulfation of polyphenols with bacterial ASTs is an effective method for producing metabolites identical to metabolic intermediates occurring in the human body.
In the present study, five potential recombinant aryl sulfotrasferases were produced in *E. coli*, purified to homogeneity, and characterized. These enzymes were then tested in sulfation reactions with *p*-NPS as donor and various phenolic compounds as acceptors: Flavonoids, flavones, phenolic acids and even phenolic glycosides. The reactions were performed both on analytical and preparative scale. The tested enzymes showed different substrate preferences, which helped us to find the optimal conditions for the preparation of sulfate derivatives of phenolic compounds. The products were detected by HPLC and some of them were isolated for NMR analysis. Obtaining sufficient amounts of sulfated compounds should allow us to characterize human metabolites in terms of their stereochemistry and sulfation sites.

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P.2.24 - PROTECTIVE EFFECT OF EPICATECHIN GALLATE-4B-GLUTATHIONE METHYL ESTER ON THE ETHANOL-INDUCED INJURY OF BRL-3A CELLS VIA ACTIVATION OF KEAP1-NRF2-HO-1/NQO1 PATHWAY

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(-)-epicatechin gallate-4β-glutathione methyl ester (ECG-G) is a small molecule active substance obtained after the degradation of persimmon leaf polymeric proanthocyanidins with glutathione as a nucleophile. Both proanthocyanidin epicatechin gallate (ECG) and nucleophile glutathione have liver protective effects due to their strong antioxidant activity. However, the liver protective effect and mechanism of ECG-G remain unclear.

In this study, an in vitro model of alcoholic liver injury was established by a modified ethanol bath method using BRL-3A cells. The cell viability and the LDH leakage rate were analyzed by MTT and LDH kit. The mitochondrial ROS and mitochondrial membrane potential (MMT) were detected by fluorescence microscope. The cell ultrastructure was detected by transmission electron microscope. To further investigate the mechanism of ECG-G, the Keap1-Nrf2-HO-1/NQO1 pathway was assessed by western blot and immunofluorescence analysis. The results showed that 350 mM ethanol incubation for 24 h was the best condition for modeling, which could be used in subsequent experiments. After 4h pre-protection, ECG-G with different concentrations without affecting BRL-3A cell survival rate could significantly improve the decrease of survival rate of BRL-3A cells induced by ethanol in a dose-dependent matter, ECG-G also dose-dependently reduced the LDH leakage rate. And its activity was better than that of its parent compound ECG. Further study revealed that 50 μM ECG-G reduced significantly the increase of mitochondrial ROS level, improved significantly the damage of cell ultrastructure, and increased the mitochondrial membrane potential in BRL-3A cell, ECG-G activated the Nrf2 nuclear transposition, down-regulated the expressions of Keap1, and up-regulated the expressions of HO-1 and NQO1. In conclusion, ECG-G has a protective effect on ethanol-induced BRL-3A cell injury. The mechanism may be related to inhibiting mitochondrial oxidative stress, then activating the Keap1-Nrf2-HO-1/NQO1 antioxidant pathway in BRL-3A cells.

P.2.25 - THE CONTRIBUTION OF OLEACEIN AND ITS METABOLITES FOR THE ANTI-INFLAMMATORY ACTIVITY OF OLIVE OIL POLYPHENOLS

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Epidemiological data show that adherence to the Mediterranean diet reduces the incidence of chronic human diseases caused by inflammation and cellular oxidation, such as cardiovascular and neurodegenerative diseases. These health effects have been partially associated with the high concentration of phenols in extra virgin olive oil (EVOO), the most important fat in this diet. Oleacein is usually the main antioxidant polyphenolic compound found in EVOO and is believed to be responsible in part for the anti-inflammatory activity of olive oil consumption. The bioactivity of dietary phenolic compounds, however, should be evaluated taking into account their bioavailability once ingested [1].
The anti-inflammatory potential of oleacein and its main metabolites were characterized by their effects on RAW 264.7 macrophages challenged with lipopolysaccharide (LPS), and by their ability to inhibit enzymes of the arachidonic acid metabolism with a key role in the synthesis of pro-inflammatory lipid mediators.[2] Oleacein at 12.5 µM significantly decreased the amount of L-citrulline and •NO generated by LPS-stimulated macrophages. Hydroxytyrosol, hydroxytyrosol acetate and hydroxytyrosol acetate sulfate were also able to reduce the cellular amount of •NO, although to a lesser extent. In contrast, hydroxy-tyrosol glucuronide and sulfate did not show detectable effects. Oleacein was also able to inhibit the coupled PLA2 + 5-LOX enzyme system (IC50 = 16.11 µM), as well as the 5-LOX enzyme (IC50 = 45.02 µM). Although with lower activity, both hydroxytyrosol and hydroxytyrosol acetate were also capable of inhibiting these enzymes at a concentration of 100 µM. None of the other tested metabolites showed a capacity to inhibit these enzymes. In contrast, all compounds, including glucuronides and sulfate metabolites, showed a remarkable capacity to inhibit both cyclooxy-genase isoforms, COX-1 and COX-2, with IC50 values lower than 3 µM. Therefore, oleacein and its metabolites have the ability to modulate •NO- and arachidonic acid-dependent inflammatory cascades, contributing to the anti-inflammatory activity associated with olive oil polyphenols [2].

[1] Costa, M., Costa, V., Lopes, M. & Paiva-Martins, F., *Critical Reviews in Food Science and Nutrition*, https://doi.org/10.1080/10408398.2022.2116558, 2022.
[2] Costa. V., Costa M., Videira, R., Andrade, P.B. & Paiva-Martins, F., *Biomedicines*,10(11), 2990, https://doi.org/10.3390/biomedicines10112990, 2022.

This work was funded by Project cLabel+ POCI-01-0247-FEDER-046080. We acknowledge FCT/MCTES (UID/QUI/50006/2020) and PTDC/OCE-ETA/32492/2017 – POCI-01-0145-FEDER-032492 – PHENOLIVA.

P.2.26 - METABOLITE PRODUCTION THROUGH *IN VITRO* CAMELLIA CULTURE

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Plants are known to produce a diverse array of secondary metabolites, which are chemical compounds that are not essential for their growth, development, or reproduction but are crucial for their interaction with the environment. These secondary metabolites are synthesized by specialized pathways and are responsible for various functions, including defence against herbivores and pathogens, attraction of pollinators and seed dispersers, and adaptation to environmental stress. The importance of secondary metabolites extends beyond their biological roles, as they have significant economic value in various industries such as food, pharmaceuticals, chemicals, and cosmetics.

The cosmetics industry alone has a global annual turnover of 412 billion Euros and a market value of 2.8-4.6 billion Euros in the European Union, making it a highly competitive and desirable field. This increasing demand for secondary metabolites has led to a potential discrepancy between their demand and availability. Over the last decade, there has been a growing interest in plant cell culture extracts, which have been found to contain a wide range of primary and secondary metabolites. Plant biotechnology techniques, and in particular plant *in vitro* systems, offer a reliable and sustainable platform for bioproduction of plant-derived metabolites. The potential of plant cells and the ability to produce desired metabolites in high yield and consistency make plant *in vitro* systems a promising alternative to traditional plant-based production methods. The process is also carried out under controlled aseptic conditions, which guarantees the safety of the final product without contamination of the environment and allows strict quality and production control.

In this work, the production of catechins from *in vitro Camellias japonica L.* was analysed (Figure 1). *C. japonica* is a widespread species found in Galicia (NW Spain), where it has been largely exploited with ornamental purposes. Recent findings on its phytochemical characterization showed thousands of bioactive ingredients, mostly represented by phenolic compounds. The results shown an interesting potential for increasing metabolite production by exploring biosynthetic pathways and optimising cultivation conditions. *In vitro* plant systems can be used as a model to unravel biosynthetic pathways and investigate the factors that modulate these pathways, increasing the possibility of establishing commercial processes for the bioproduction of high-value secondary metabolites.

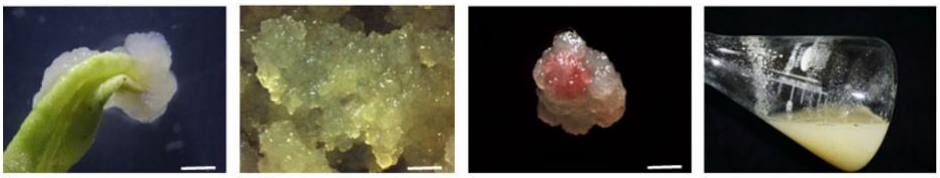

Figure 1: Callus induction from the *Camellia* explant.

P.2.27 - BERRY ANTHOCYANINS EXERT ANTI-CANCER PROPERTIES AGAINST LUNG CARCINOGENESIS

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Haskap (*Lonicera caerulea*) berry has been used as a traditional medicine in Russia, Japan, and Northeastern China for centuries. We have found that the antioxidant capacity of haskap was significantly greater compared to that of other commonly consumed fruits. We have demonstrated for the first time that anthocyanin-rich haskap berry extracts could reduce carcinogen-induced DNA damage in cultured lung epithelial cells as well as in carcinogen-induced lung tumorigenesis in A/Jcr mice. Pre-treatment of cultured human lung epithelial BEAS-2B cells with the anthocyanin-rich haskap berry extracts significantly reduced carcinogen-induced DNA damage, DNA fragmentation, and intracellular reactive oxygen species and upregulated the ATM-dependent DNA damage repair cascade compared to non-treated BEAS-2B cells. Dietary supplementation of anthocyanin-rich haskap berry powder (262 mg C3G/kg body weight/day) for 22 weeks significantly reduced the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, (NNK)-induced lung tumor multiplicity and tumor area. Immunohistochemical analysis showed reduced expression of proliferative cell nuclear antigen (PCNA) and Ki67 in lung tissues. The integrative mechanisms of action of haskap anthocyanin include carcinogen detoxification, anti-inflammatory and immune-modulatory properties. Scientific evidence suggests that this ancient berry of Asia can be established as a cancer-preventive superfood.

P.2.28 - SILYBIN AND ITS CONGENERS: FROM TRADITIONAL MEDICINE TO MOLECULAR EFFECTS

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Silymarin, an extract from the fruits of milk thistle (*Silybum marianum*), is used in various medicinal applications since ancient times. A major component of silymarin is the flavonolignan silybin [1] (Figure 1) and its relatives isosilybin, silychristin, silydianin, 2,3-dehydrosilybin, and several others. With the exception of silydianin, they occur naturally as two stereomers.
This contribution focuses on recent developments in chemistry, biosynthesis, modern advanced analytical methods, and transformations of flavonolignans specifically reflecting their chirality. Recently described chemotypes of *S. marianum*, as well as recent findings on the pharmacokinetics, hepatoprotective, antiviral, neuroprotective, and cardioprotective activity, modulation of endocrine functions, modulation of multidrug resistance, and safety of flavonolignans are discussed.
A growing number of studies show that the respective stereomers of flavonolignans have markedly different activities in anisotropic biological systems. Moreover, it is now clear that flavonolignans do not act as antioxidants *in vivo*, but as specific ligands of biological targets and therefore their chirality is crucial. Controversy frequently arises, mainly due to the nonstandard composition of silymarin-containing phytopreparations, the use of various undefined mixtures, the misclassification of silymarin and silybin, and the failure to consider the chemistry of the respective components of silymarin.

Supported by Czech Science Foundation 21-00551S and LT-CZ bilateral project LAS-21-04.

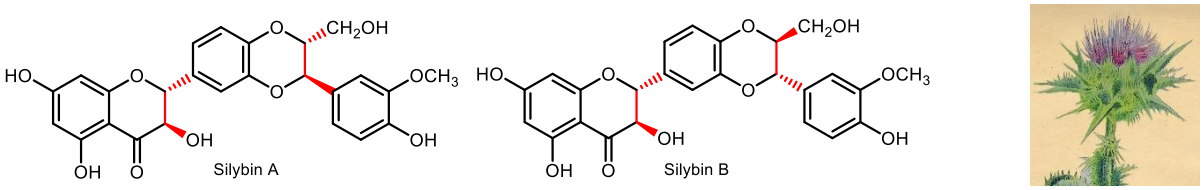

Figure 1 : Silybin A and silybin B; milk thistle (*Silybum marianum*)

[1] Křen V. & Valentová K. *Nat. Prod. Rep.*, 39, 1264-1281, 2022.

P.2.29 - THE IMPACT OF GROWTH STAGE AND GEOGRAPHICAL ORIGIN ON IN-VITRO ENZYME INHIBITORY EFFECT AND PHENOLIC PROFILE OF *AGRIMONIA EUPATORIA* EXTRACTS

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Agrimonia eupatoria (Rosaceae), commonly known as agrimony, is a plant widely spread in the northern hemisphere that has been used in traditional medicine due to its antioxidant, anti-inflammatory, analgesic and hypotensive properties, amongst others. There is both in vitro and in vivo evidence [1] that the aforementioned health beneficial properties are related to agrimony high abundance in phenolic compounds. Although published studies have provided some insight on agrimony bioactivity and its phenolic profile, still there is not much information on how these parameters may be impacted by the plant growth stage as well as its geographical origin. To this end, in this study, we applied a multidisciplinary approach to analyse aqueous and ethanolic extracts originating by agrimony plants in four different growth stages (vegetative stage, beginning of flowering, full bloom and senescence) and cultivated in two different geographical locations in the Czech Republic (Hlohovec and Milovice). In total 30 samples were tested and their inhibitory effect against enzymes with important biochemical functions was monitored, namely acetylcholinesterase (AChE, EC 3.1.1.7), pancreatic lipase (PNLIP, EC 3.1.1.5) and tyrosinase (TYR, EC 1.14.18.1). Worthy to mention is that all enzyme assays were developed in-house providing high-throughput and cost-efficiency [2]. Indicatively, the TYR assay optimisation will be presented to demonstrate the analytical challenges faced in order to deliver robust and accurate results. Based on the preliminary results, the highest AChE inhibitory effect was monitored in the senescence (98% ± 2.5%, extract concentration 100 mg mL^{-1}) whilst in the case of PNLIP at the beginning of flowering (74% ± 3.2%, extract concentration 100 mg mL^{-1}). This indicates that maybe phenolic compounds expressed in different growth stages inhibited the enzyme active site in each case. Following the performed enzyme assays, a suspect screening metabolomic workflow was performed using an ultra-high-performance liquid chromatography hybrid quadrupole time-of-flight mass spectrometry (UHPLC-q-TOF-MS) method. The attained polyphenol profile varied depending on the growth stage, with the most significant differences to be noticed in the senescence stage. Interestingly, differences were noticed for specific compounds for plants originating from a different location. All in all, the presented approach combines in vitro bioactivity measurements to high-end metabolomics delivering a dynamic profile of the phenolic content and its enzyme inhibitory potency.

[1] Malheiros J., Simões D.M., Figueirinha, A., Cotrim M.D. & Fonseca D.A., *Journal of Ethnopharmacology* 296, 115498, https://doi.org/10.1016/j.jep.2022.115498, 2022.
[2] Tsagkaris A.S., Loučková A., Jaegerova T., Tokárová V. & Hajslova J., *International Journal of Molecular Sciences*, 23, 11204, https://doi.org/10.3390/ijms231911204, 2022.

P.2.30 - SYNTHESIS AND CHARACTERIZATION OF BIOACTIVE POLYTHYMOL

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Thymol is an aromatic monoterpene occurring in essential oils from plants belonging to different plant families including Lamiaceae, especially from *Thymus* and *Ocimum* genera [1]. In the present work, a new biobased material derived from thymol, obtained from the essential oil of *Thymus vulgaris* (Lamiaceae), was prepared using this natural product and formaldehyde (Figure 1). Polythymol (PTF) was synthesized through several reactions, aiming to optimize the reaction conditions to obtain a macromolecule with high purity, and yield. The macromolecule was prepared and characterized by different techniques such as nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy (UV-Vis), fluorescence, gel permeation chromatography (GPC), thermogravimetry analysis (TGA) and differential scanning calorimetry (DSC). The obtained results confirmed the proposed structure for the macromolecule of PTF and its optical and thermal properties. In order to evaluate the bioactivity of the formed macromolecule against different microorganisms such as bacteria and yeast, a bioassay of a minimum inhibitory concentration of the synthesized fractions of PTF and thymol was carried out. The obtained results show that the bioactivity of the PTF (MIC = 31,2 µg/mL and 500,0 µg/mL for *Staphylococcus aureus* and *Candida albicans* respectively) is interesting once is higher than the pure monomer (MIC = 500,0 µg/mL for *S. aureus* and no activity for *C. albicans)*. Therefore, the preparation of a bioactive macromolecule from thymol has been proven, allowing its application in this important area of science.

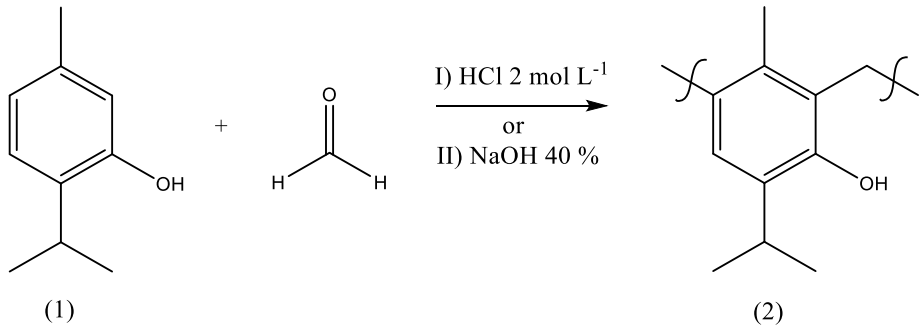

Figure 1: Synthetic route scheme to prepare polythymol (PTF).

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P.2.31 - TANNIN-LIPID INTERACTIONS: WHAT, WHERE AND HOW?

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Plant tannins have many potentials applications and bioactivities, such as antioxidant, antimicrobial and antiviral activities, which are highly structure-dependent and linked to their bioavailability and interactions with different biomolecules, such as proteins, lipids and fibres. The tannin-protein interactions are well-known but the interactions of tannins with the other macromolecules have not been studied as comprehensively. Here we focus on tannin-lipid interactions and shortly introduce the chemical methods used for their analysis, the molecular aspects related to these interactions and the effects of tannins on the membrane structure [1]. Tannin-lipid interactions can be studied by *in vitro* membrane models, cellular biological studies or chemical techniques. The chemical methods include partition coefficient measurements by octanol-water or by membrane models, calorimetric methods, such as isothermal titration calorimetry (ITC) and differential scanning calorimetry, spectroscopic techniques, such as Fourier transform infrared spectroscopy or NMR spectroscopy, and molecular dynamics simulation. For example, ITC (Figure 1) has been used to study the interactions between hydrolysable tannins and lipid vesicles from the phospholipid extract of *Escherichia coli* [2] and high-resolution magic angle spinning NMR for the investigation of the location and orientation of hydrolysable tannins within *E. coli* lipid membranes [3].

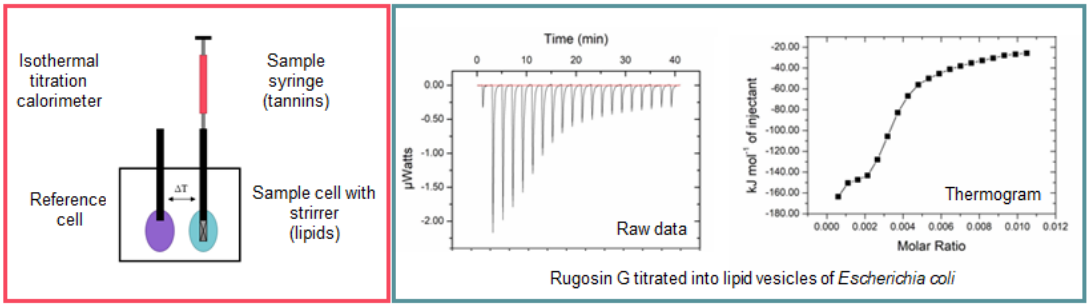

Figure 1: Ellagitannin rugosin G titrated into lipid vesicles by isothermal titration calorimetry

Different tannins can interact with lipid bilayers with varying efficiency depending on the structures and concentration of tannins, the composition of lipids and the conditions used. When tannins penetrate the lipid bilayers, they can cause changes in the structure and biophysical properties of lipids, for example, by increasing or decreasing acyl chain order, fluidity or rigidity [1]. In general, polyphenols are located close to the polar headgroups of the lipids, but due to the different structural features, the location may vary. Similarly, the changes caused to the lipid order vary depending on the polyphenol and lipid studied. For high affinity to lipids, two factors are important: the hydrophobicity of tannins as more hydrophobic ones can penetrate deeper into lipid bilayers, and the forces stabilizing the location and orientation of tannins in the lipid bilayer. The main structural features of tannins affecting their interactions with lipid bilayers seem to be the degree of polymerization, galloylation and interflavanoid linkages for proanthocyanidins and the galloyl moieties, molecular size and structural flexibility for hydrolysable tannins [1].

[1] Karonen M. *Plants*, 11, 1809, https://doi.org/10.3390/plants11141809, 2022.
[2] Virtanen V., Green R.J. & Karonen M. *Molecules*, 27, 3204, https://doi.org/10.3390/molecules27103204, 2022.
[3] Virtanen V., Räikkönen S., Puljula E. & Karonen M. *Molecules*, 26, 373, https://doi.org/10.3390/molecules26020373, 2021.

P.2.32 - FUNGISTATIC ACTIVITY OF CONIFER TANNINS AGAINST FIVE SAPTOTROPHS

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Bioactive phytochemicals in wood affect the decomposition and utilization of these ecologically and economically important materials. Saprotrophic fungi specialized on wood decomposition can be divided into species causing white or brown rot, the latter being mainly restricted to conifers and lacking ligninolytic enzymes. Coniferous condensed tannins (proanthocyanidins) are often cited as antifungal compounds but earlier reports show inconsistent results regarding their antifungal capacity [1,2].

To investigate how source material, polymer structure or fungal species affects the growth-reducing activity of condensed tannins purified from the needles and bark of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) saplings, we conducted *in vitro* bioassays against three brown rot and two white rot causing fungi (Fig. 1). Condensed tannins were extracted with 80% acetone, and Sephadex LH-20 fractionation [3] was used to purify tannin polymers mixed into malt agar at 0.07% (w/w) for bioassays.

Most conifer tannins and the commercially available quebracho (Colatan GT10) tannins inhibited mycelial growth on average by 12–24% (Fig. 1). The sensitivity to condensed tannins varied strongly amongst the brown rot fungi, *Rhodonia placenta* and *Gloeophyllum trabeum* being sensitive and *Coniophora puteana* resilient to conifer tannins. On average, bark tannins were more effective than needle tannins, and pine tannins more effective than spruce tannins, but antifungal activity of conifer tannins did not depend on their mean degree of polymerization or the proportion of procyanidin-type subunits.

Taken together, our results indicate moderate antifungal activity for condensed tannins from conifers. We found no support for the hypothesis that conifer tannins would be particularly effective against brown rot causing fungi specialized in conifers; instead, differences in fungal sensitivity or source-specific tannin efficiency fit to the evolutionary framework of adaptations and counter-adaptations between plants and their antagonists.

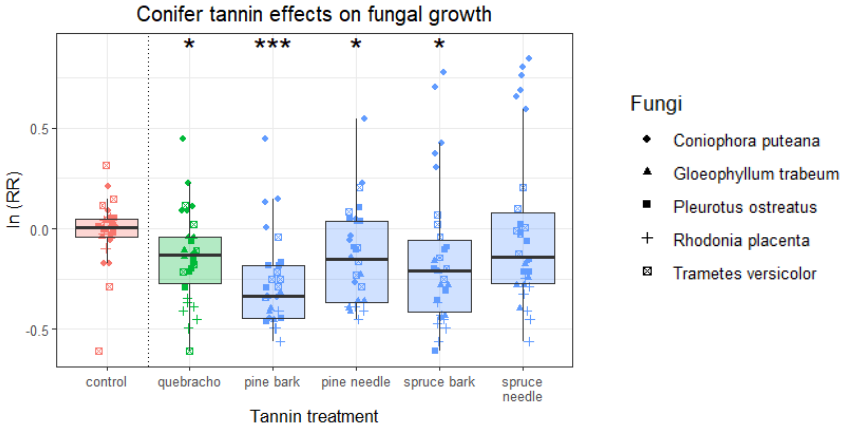

Figure 1: Bioactivity of studied tannin fractions against five fungal saprotrophs, shown as log-response ratio (ln(RR)) of growth relative to the control treatment (* 0.01<p<0.05, *** p<0.001)

[1] Anttila A.-K., Pirttilä A.M., Häggman H., Harju A., et al., *Holzforschung*, 67, 825-832, 2013.
[2] Tascioglu C., Yalcin M., Sen S. & Akcay C., *International Biodeterioration & Biodegradation*, 85, 23-28, 2013.
[3] Salminen J.-P. & Karonen M. *Functional Ecology*, 25, 325-338, 2011.

P.2.33 - CHLOROGENIC ACID AND ITS MICROBIAL METABOLITES POORLY INHIBIT TRIMETHYLAMINE OXIDATION BY HEPATIC FLAVIN MONOOXYGENASE 3

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Trimethylamine *N*-oxide (TMAO) is an endogenous metabolite positively correlated with atherosclerosis. TMAO is produced through a microbiota-host axis. Choline and other dietary quaternary amines are converted to trimethylamine (TMA) by gut bacteria. TMA is metabolized to TMAO by hepatic flavin-containing monooxygenase 3 (FMO3). Circulating TMAO contributes to atherosclerosis development and cardiovascular mortality risk. Controlling TMAO levels could significantly benefit public health. Phenolics have cardioprotective effects, but the mechanisms are incompletely understood. Recently, use of phenolics to reduce TMAO levels has been considered. We previously focused on bacterial metabolism of choline and identified chlorogenic acid as inhibiting TMA production. However, phenolics and their microbial metabolites are absorbed and reach the liver. Inhibiting TMA conversion by FMO3 may be a complementary mechanism for reducing TMAO. We evaluated if chlorogenic acid and its bioavailable metabolites [quinic, caffeic, ferulic, isoferulic, dihydrocaffeic (DHC), dihydroferulic (DHF), dihydroisoferulic (DHIF), *p*-coumaric and *m*-coumaric acids] inhibit hepatic TMAO production *in vitro* at nutritionally relevant (1 µM) and pharmacological (50 µM) doses. Methimazole (MTM), an FMO3 inhibitor, was a positive control. In pilot experiments, human (HepG2) and mouse (Hepa-1) hepatocytes did not possess FOM3 catalytic activity, and thus rat hepatic microsomes were employed. Conversion of TMA to TMAO depended on the presence of microsomes and NADPH. MTM inhibited TMA transformation at ≥50 µM. Overall, few phenolics inhibited TMAO production (Figure 1). At 1 µM dose, isoferulic and *p*-coumaric acids yielded greater TMA area-under-the-curve (AUC, i.e. less TMA utilization) vs. control (Figure 1C), and DHC was the only phenolic with reduced TMAO AUC vs. control (Figure 1D). Even at a 50 µM supraphysiological dose only achievable via injection, inhibition was minimal. Our results show that bioavailable metabolites of chlorogenic acid do not strongly inhibit FMO3. *In vivo* experiments are needed to elucidate if chlorogenic acid or its metabolites inhibit TMAO by other mechanisms involving FMO3 (downregulation of gene expression, etc.).

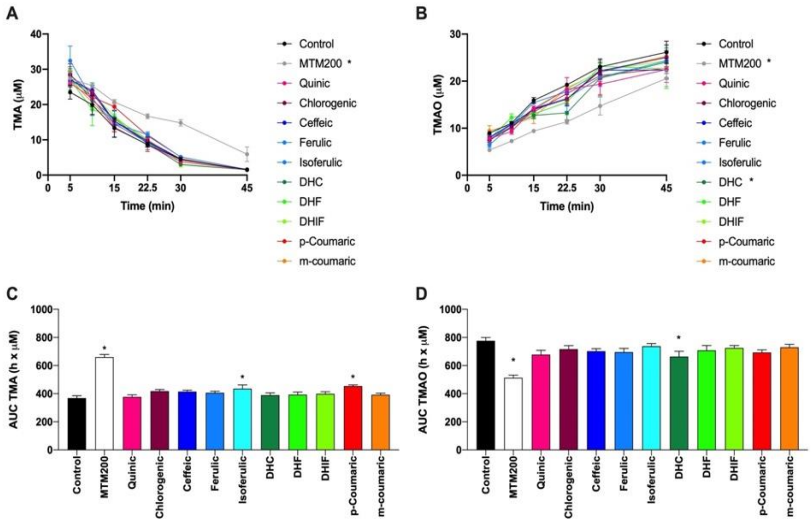

Figure 1: Effect of chlorogenic acid-related compounds on kinetics of TMA (A) and TMAO (B), and areas under the curve of TMA (C) and TMAO (D). *different vs. control by Two-Way (A&B, factors: time, treatment) and by One-Way (C&D) ANOVA (Dunnett's *post hoc* test, $p<0.05$).

P.2.34 - DRY GRAPE EXTRACT SUPPLEMENTATION IN SOWS IMPROVES COLOSTRUM QUALITY AND PIGLETS' PERFORMANCES

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Pigs are one of the most important species raised on the planet for human food consumption. Amongst the different challenges during the various production stages, reproduction, especially birth and the lactation phase that follows are critical for the future generation of pigs. Lactation is indeed a critical aspect of swine production for both the sow, which mobilises different body reserves to produce milk, and for the piglets, which depend on efficient transmission of essential nutrients and biological substances through the colostrum and milk. The aim of this experiment was to study effects of supplementation with a commercial standardised grape extract (SGE, Nor-Grape®, Nor-Feed, France) on colostrum and sow and piglet performances.

A total of 305 sows were randomly divided into two groups when entering the nursery: a control group (CTL, 146 sows) and a supplemented group (NG, 159 sows, CTL feed + 50 g/T of SGE). The parameters studied were the Brix value of colostrum (indicator of the colostrum richness in immunoglobulin G) and its antioxidant composition (Vitamin A, C and E, Glutathione peroxidase); piglets' individual birth weights and weaning weights; the total number of piglets born, live born, stillborn and mummified per litter, average daily gain of piglets and litters, pre-weaning mortality, loss of sow backfat and piglets IgG and antioxidant levels at weaning. Individual daily feed intake was also recorded for the sows during lactation.

At farrowing, no significant difference was observed between groups in the number (total, live born, stillborn or mummified) or weight of piglets. However, the Brix values of colostrum from supplemented sows were significantly higher than those of CTL sows (27.8 vs 26.4, respectively, $P < 0.05$) as well as the colostrum GPx level ($p<0.05$). Whilst no difference was observed between groups of sows in terms of lactation feed consumption or backfat loss, NG piglets were significantly heavier than CTL ones at weaning (5.62 vs 5.33 kg, respectively, $P < 0.001$), resulting in a significantly heavier weaned litter weight (66.5 vs 63.6 kg, respectively, $P < 0.05$). Blood sample analyses of piglets at weaning also revealed that the sows' supplementation during the lactation phase led to an increase in blood antioxidants and IgG level for the progeny.

Supplementing sows with a commercial SGE during lactation thus improve colostrum quality (in terms of immunity and antioxidant transfer) and piglets' performances. Furthermore, the absence of difference in feed intake for the sows or back fat loss indicate that these improvement in piglets' performances may be linked to a better transformation of the diet in milk by the supplemented sows, resulting in a better conversion of the lactation feed into piglet gain.

P.2.35 - DIETARY CONSUMPTION OF HOP (*HUMULUS LUPULUS* L.): AN ASSESSMENT OF GLUCOSE CONTROL EFFECTIVENESS ON AN ANIMAL MODEL OF OBESITY

Alves M.R.[a]; Nascimento R.P.*[a], Machado A.P.F.[a], Santos P.[b] Aledo E.C.A.[b], Vuolo M.[b], Maróstica Junior M.R.[a]

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An unbalanced energetic diet is the main cause of obesity. Subsequently, body fat deposition can generate a low-grade inflammatory state that might predispose the tissue to insulin resistance and type 2 diabetes. Several species of plants have powerful bioactive compounds that can minimize the effects of hyperglycemia and inflammation. The hop plant (*Humulus lupulus* L.) has high contents of alpha and beta acids, besides polyphenols, which have been used to treat and/or alleviate chronic diseases and metabolic-correlated disorders. Therefore, the current project aimed to investigate the composition and antioxidant capacity of an extract from hop, and its protective role on mice with obesity induced by a high-fat diet. The drying process and the extraction of hop were performed by supercritical carbon dioxide under controlled temperature and pressure conditions. The extract was chemically analyzed regarding its composition in macro and micronutrients, alpha and beta acids, and total phenolic compounds (TPC) by Folin-Ciocalteu. The antioxidant capacity was measured by the oxygen radical absorbance capacity (ORAC) method. A high-fat diet (35% calories from lard) supplemented with two doses of hop's bitter alpha acids (0.05 or 0.5% mg/kg) was given to C57BL/6 male mice for 12 weeks. Upon reaching the 9th and 10th weeks of experiment, glucose and insulin tolerance tests (GTT and ITT), respectively, were performed. At euthanasia, the weight of the adipose tissue was measured. Dry hop extract showed to be mostly a source (dry weight) of total carbohydrates (64.71%), lipids (17.68%), alpha acids (15.24%), and beta acids (4.11%), with a few contents in protein (0.13%), ashes (0.07%), calcium (0.01%), and sodium (0.04%). Especially, the extract showed to be a relevant source of TPC, around 24.46 ± 1.52 mg of gallic acid equivalent/g, and had an ORAC value of 22.68 ± 5.80 µmol of Trolox equivalent/g (dry weight). Regarding the animal assay, the hyperlipidemic control group showed higher resistance to normalizing the GTT rates in comparison to hop's higher dose (time 120', $p<0.05$). The high-fat groups treated with higher or lower doses also showed reduced glucose levels in the ITT test (times 15' and 45', respectively, $p<0.05$). The body weight and epididymal fat weight of animals were decreased due to hop's intervention (low and high doses) ($p<0.05$). However, the bitter taste of the diet with the highest dose of hop promoted a reduction of animals' ingestion over the weeks. Therefore, the results of this experimental group in particular should be taken with caution. In conclusion, *Humulus lupulus* L. showed to have a promising effect as a glycemic control agent, which could be related to its bioactive composition.

Acknowledgments
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P.2.36 - JABOTICABA (MYRCIARIA JABOTICABA) PEEL WATER EXTRACT REDUCES THE SURVIVAL OF COLON CANCER CELLS IN VITRO

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Colorectal cancer (CRC) is the third most common and the second most deadly type of cancer worldwide. Observational studies indicate that most cases of CRC are associated with environmental factors, such as physical activity and diet. While red meat consumption can increase the risk for CRC, fruit and vegetable intake have the opposite effect. Recently, a meta-analysis indicated that the consumption of anthocyanins, which are mostly found in berry fruits, is inversely associated with the risk of CRC in men and the colon site. An improved biological understanding of these bioactive dietary components may determine whether anthocyanins can be exploited to improve current CRC management. To address this, this study aimed to investigate the survival of Caco-2 colon adenocarcinoma cells after treatment with a water extract from the peel of anthocyanin-rich jaboticaba-sabará (*Myrciaria jaboticaba* (Vell.) O. Berg), a promising berry native to Brazil. Two samples of freeze-dried jaboticaba peel powder were used in the experiments: one was collected in May and the other in the peak season of August-October. The samples were extracted according to the literature by immersing the powders in boiling water at a 1:20 sample-to-solvent ratio, and subsequently freeze-dried again. Samples were analyzed regarding their content in monomeric anthocyanins according to the differential pH methodology. For the *in vitro* assay, Caco-2 cells were maintained under standard cell culture conditions and seeded at 10000 cells per well for the viability and proliferation assays or 500 cells per well for the colony formation assay (CFA). After 24h, for the viability assay, cells were treated at 5, 25, 50, 100, 500, 1000, and 2000 µg/mL dosages for 48h. For the proliferation assay, cells were treated for 24, 48, and 72h, and for the colony formation assay, for nine days, using the most efficient extract and dosages. The crystal violet assay was applied in the analyses. Extract 2 (peak season jaboticaba) showed significantly ($p<0.05$) more levels of monomeric anthocyanins (8.13 ± 1.32 mg/g) than extract 1 (3.60 ± 0.15 mg/g). When applied *in vitro*, our preliminary results showed that both extracts were able to statistically ($p<0.05$) reduce the viability of Caco-2 cells starting at dosage 500 µg/mL. Dosages 5 to 100 µg/mL showed no ability to significantly induce cell toxicity. The survival of Caco-2 cells reduced gradually to 63-68%, 34-40%, and 11-18% (average) after treatment with 500, 1000, and 2000 µg/mL of the extracts, respectively. No statistical differences were noticed between the toxicity of the two extracts, although extract 2 showed a lower numeric IC50 and therefore, a more promising bioactivity. In the proliferation assay, cells growth progressively from 24h to 72h in all groups. However, when treated with extract 2, in all moments (24, 48, 72h), survival decreased to 70% down to 10%, accordingly to the dosages applied (500 to 2000 µg/mL). In the CFA, between 40 to 50 colonies were detected in the control cells, while no colonies and only single cells were seen in wells treated with extract 2 at the highest dosages. In conclusion, anthocyanin-rich jaboticaba extract appears to be a promising crop against CRC, being necessary further molecular investigations to strengthen the existing scenario.

Acknowledgments
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P.2.37 - PHENOLIC PROFILE AND IN VITRO BIOACTIVITIES OF SAFFRON FLOWER BY-PRODUCTS EXTRACTS

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Bioactive compounds from wastes and by-products of plant origin have recently increased due to their potential applications in food, cosmetics, and pharmaceuticals. *Crocus sativus* L. petals, a waste obtained from saffron flowers, are of particular interest to develop a circular economy approach, which attempts to turn them into resources with high antioxidant capacity and polyphenol contents. The project aimed to quantitative determinations in total phenolic and flavonoid contents (TPC and TFC) and evaluation of antioxidant capacity and cytotoxic activities of *C. sativus* L. hydromethanolic petal extracts (CsHMPE). The TPC and the TFC were determined using Folin–Ciocalteu test and aluminum chloride colorimetric assay respectively. Two methods DPPH and FRAP were carried out to investigate the antioxidant activity. Finally, the cytotoxic effect was assessed on human colon carcinoma (HT-29), human breast cancer (MCF-7) and normal (Vero) cell lines, using the MTT test (3- (4,5-dimethylthiazol-2) bromide -2,5 -diphenyltetrazolium).
CsHMPE showed an important phenolic and flavonoid content of 85,97±6.85 µg EAG/mg extract and 14.24±0.25 µg CA/mg extract respectively, with a free-radical-scavenging ability recording an IC_{50} of 425,25 ± 11.20 µg/mL and a ferric reducing antioxidant power (0,675±0.019). The cytotoxic activity showed that the extract decreased the cell viability of the three cell lines of HT-29, MCF-7 and Vero in a dose dependent manner, causing morphological alterations.
Taken together, these results suggest a potential use of these Saffron flower waste materials as important source of bioactive compounds. Additional investigations are needed to evaluate CsHMPE's most essential bioactive phenolic constituents responsible for antioxidant and cytotoxic proprieties. Other additional biological studies could lead to a better understanding of the mode of action of these molecules.

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P.2.38 - *ACTINIDIA ARGUTA* LEAVES: AN OVERVIEW OF ANTIOXIDANT ACTIVITY, INTESTINAL PERMEATION AND HEALTH EFFECTS

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Actinidia arguta crops are expanding in different world regions, from Europe to Asia and North America, mainly due to the nutritional and healthy benefits associated with the fruit's consumption [1]. This study intends to evaluate the *in vitro* antioxidant/antiradical capacity and the intestinal permeation as well as the *in vivo* antioxidant effects of an *A. arguta* leaves extract in animals (rats), aiming to validate it as new nutraceutical ingredient.

Briefly, samples from a local farm were extracted with ultrasound-assisted extraction (UAE) according to Silva *et al.* [2]. After extraction, samples were filtrated, lyophilized, and characterized regarding antioxidant/antiradical capacity (through total phenolic content (TPC), ABTS, DPPH and ferric reducing antioxidant power (FRAP) assays). A 3D co-culture intestinal model, composed by Caco-2 and HT29-MTX, were prepared for the permeation assays during 240 min, coupled to LC/DAD-ESI-MS analysis. For the *in vivo* assays, Wistar rats (n = 6/group) were orally treated during 7 days with water (Group I), *A. arguta* leaves extract (50 and 75 mg/kg bw/d, respectively, Group II and III) or vitamin C (Group IV). After this period, the animals were sacrificed, the organs (liver and kidneys) were removed, and the blood collected. The antioxidant enzyme activities, namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were determined in the organs and serum.

The results demonstrated that the extract has a high TPC (97.50 mg of gallic acid equivalents (GAE)/g dw) and antioxidant/antiradical activities (IC_{50} = 249.46 µg/mL for ABTS assay; IC_{50} = 547.34 µg/mL for DPPH assay; 1440.13 µmol of ferrous sulfate equivalents (FSE)/g dw for FRAP). The LC/DAD-ESI-MS analysis revealed the presence of coumaroyl quinic acid in all time-points, achieving an intestinal permeability of 34.23% after 240 min. Regarding the biochemical and antioxidant parameters, the highest SOD activity in kidneys and livers was observed for Group III, respectively, 183.36 and 175.26 units/g protein. Similarly, groups II and III achieved the best CAT results for livers (7840180 and 7526357 nmol/min/g protein, respectively), while Group II significantly increased the GSH-Px activity (kidneys = 205.35 units/g protein, livers = 133.60 units/g protein and serum = 64.57 units/mL protein). Regarding the MDA levels, groups II and III exhibited the lowest levels (54994 and 44968 nmol/g protein for livers, and 67598 and 54566 nmol/g protein for kidneys, respectively). These results highlight the efficacy and safety of *A. arguta* leaves extract as nutraceutical ingredient. Further studies should be performed to identify the metabolites responsible for these activities.

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P.2.39 - INFLUENCE OF ACETYLSALICYLIC ACID AND FLAVONOID METABOLITE, 4-METHYLCATECHOL, ON PLATELET AGGREGATION IN PATIENTS SUFFERING FROM METABOLIC DISEASES

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Introduction: Dyslipidemia and diabetes mellitus are two of the main risk factors for developing atherosclerosis, leading to coronary artery disease. Increased plasmatic levels of low-density lipoprotein cholesterol and glucose are tightly associated with platelet hyperaggregability. Acetylsalicylic acid (ASA) is the most frequently used drug in clinical practice to reduce this risk, but the profit of this therapy is limited due to resistance development by patients. It is known that flavonoid metabolites might have health benefits in humans. One of these metabolites, 4-methylcatechol (4-MC) has shown to be a strong antiplatelet compound in healthy donors [1]. The aim of this study was to compare its activity with ASA in patients suffering from metabolic diseases.
Material and methods: Blood samples from 15 familial hypercholesterolemic patients and 50 diabetic type I patients were tested for comparison of the effect of 4-MC and ASA on platelet aggregation in whole blood by impedance method.
Results: Effect of 4-MC as a strong antiplatelet inhibitor in healthy donors, was confirmed in patients suffering from metabolic diseases as well. Its inhibitory activity was even stronger than the activity of ASA at least in arachidonic acid and collagen-triggered platelet aggregation.
Conclusion:4-MC was shown to have beneficial effects decreasing platelet aggregability also in patients suffering from familial hypercholesterolemia and type 1 diabetes.

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P.2.40 - THE NUTRACEUTICALS MIXTURE BERBERINE, CITRUS AND APPLE EXTRACTS MODULATES THE METABOLIC PARAMETERS AND MICROBIOTA IN WISTAR RATS FED TO HIGH FAT AND HIGH FRUCTOSE DIET

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Nutraceuticals help to prevent various metabolic disorders such as those related to metabolic syndrome (MetS). The latter is generally caused by a sedentary lifestyle and the consumption of ultra-processed products [1,2]. We evaluated the beneficial effects of a nutritional mixture based on Berberine, Citrus (Citrus pericarp-35% bioflavonoids-aqueous extraction) and Apple (phloridzin-5%-hydroethanolic extraction) (BCA) extracts in Wistar rats subjected to a high fat and high Fructose diet (HFHF).

Thirty two 8-week-old Wistar rats were randomly divided into 4 groups: untreated control (U-C;n=8), BCA-treated control (BCA-C;n=8), untreated rats fed with HFHF (U-HFHF;n=8) and BCA-treated rats fed with HFHF diet (BCA-HFHF;n=8). All rats received daily water or BCA mixture (200mg/kg *per os*) for 14 weeks. Morphological data, metabolic parameters were measured before and at the end of the treatment. Stool samples were collected before and at the end of the treatment. DNA extracted from each stool sample was used as a template for amplification of the V3-V4 region of 16S rDNA genes.

Compared with the control group, rats fed with HFHF diet showed an increase in body weight, abdominal circumference, and adiposity index calculated from perirenal and peri-epididymal f adipose tissue ($p<0.001$). The HFHF diet induced atrophy of the caecum ($p<0.001$). The BCA treatment significantly decreased visceral fat weight and reduced ceacal atrophy ($p<0.01$) compared to the HFHF untreated group. The HFHF diet decreased oral glucose tolerance, but the BCA mixture reduced this alteration. The HFHF diet altered the microbiota profile in comparison to the control group, particularly an increase in Phylum Firmicutes and a reduction in Bacteroidetes. The BCA mixture modulated the microbiota in a different way in the BCA-C and BCA-HFHF groups indicating a different response in the pathophysiological state. In addition, short-chain fatty acid species producers such as *Akkermansia muciniphila* were also increased by the BCA mixture.

We found that the BCA nutritional blend improved oral glucose tolerance, probably through mechanisms involving the microbiota, particularly *Akkermansia muciniphila* [3], and further research is in progress.

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P.2.41 - ANTI-AGING EFFECTS OF CONSTITUENTS IN MERLOT WINE AND ITS COMPRESSION RESIDUE

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The anti-aging properties (AGEs (advanced glycation end-products) inhibitory activity) of Merlot wine and its compression residue were studied in this study. Oleanolic acid (**1**), 5-carboxypelargonidin-3-glucopyranoside (**2**), vitisin A (**3**), 3-*O*-glucopyranosyl-5,7,4'-tetrahydroxy-3',5'-dimethoxy flavone (**4**), 3',3'-dimethoxy procyanidin B2 (**5**), procyanidin B2 (**6**), 3'-methoxy procyanidin B2 (**7**) and 3-*O*-glucopyranosyl-3'-methoxy ethyl procyanidin B2 (**8**) were isolated from Merlot wine and its compression residue, and their structures were established on the basis of MS and NMR spectroscopy. In this study, three compounds, **5**, **7** and **8** were isolated from Merlot wine and its compression residue as new compounds. Even at concentrations lower than the IC50 of the positive control (aminoguanidine), several isolated compounds and extracts prevented AGEs generation. TIG-110 fibroblasts have been used to study the prevention of AGE generation by glycation (glyoxal addition) [1]. Thus, each isolated compounds and extracts significantly boosted cellular viability, showing that they inhibited AGE development by a mechanism other than their own. Catechin dimers with a methoxy group, such compounds **5** and **7**, were particularly active. Due to the extraordinary AGE-inhibiting effect of Merlot wine compression residue, it might be employed in food or cosmetics as it has a remarkable ability to halt the aging process.

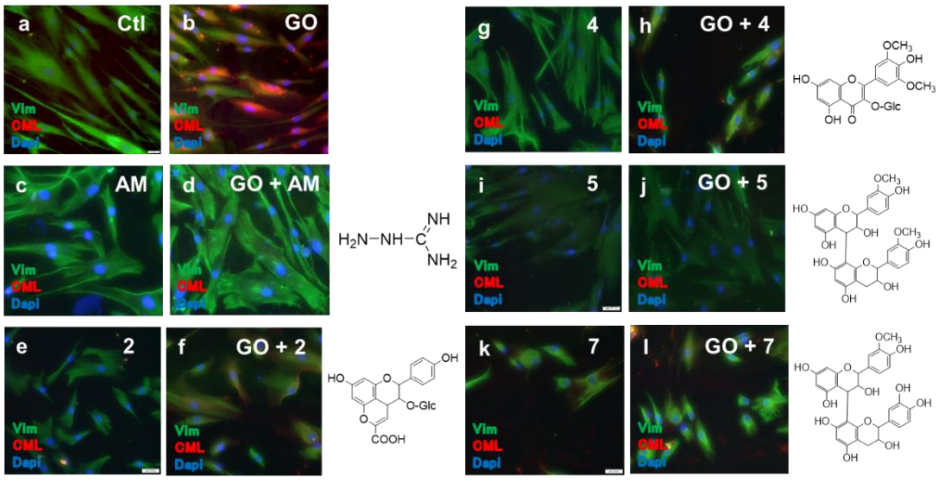

Figure 1: Images of immunofluorescent-stained glycation end products (CML) with the isolated compounds. CML (red), Vimentin (green), DAPI (blue).

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P.2.42 - PURPLE CORN ANTHOCYANINS AS A NUTRACEUTICAL APPROACH AGAINST THE PROGRESSION OF MULTIPLE SCLEROSIS AND ASSOCIATED TRIGEMINAL PAIN: EFFECT ON NEUROINFLAMMATION AND AUTOPHAGY

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Anthocyanins (ACNs) are important health-promoting components in the human diet with protective effects against many chronic diseases, including cardiovascular and neurodegenerative disorders and obesity [1]. Using isogenic ACN-rich and ACN-free corn, we have previously demonstrated that the administration of an ACN-rich purple corn extract has a protective effect on the development of orofacial allodynia in an *in vivo* model of inflammatory trigeminal (TG) pain [2]. Here, we investigated whether purple corn can exert beneficial effects on TG pain associated to multiple sclerosis (MS) and on the onset and progression of the disease.
Experimental autoimmune encephalomyelitis (EAE) was induced in Dark Agouti rats as previously described [3]. Eleven days before EAE induction rats were assigned to drink water, yellow or purple corn extracts. The development of EAE was evaluated by a scale of ascending paralysis and spontaneous TG pain was measured by von Frey test. Faecal samples were collected at significant time points for the analysis of microbiota composition and ACN metabolites. Results show that, thanks to gut ACN metabolism, purple corn positively influences the progression of EAE motor symptoms and protects from associated TG pain by modulating glia activation, pro-/anti-inflammatory mediators and autophagy. Our findings suggest a possible application of a purple corn-based dietary supplement as co-adjuvant to pharmacological treatments against MS and associated symptoms.

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P.2.43 - THE ADMINISTRATION OF GRAPE SEED FLAVANOLS TO OBESE RATS SUBJECTED TO A SUDDEN CHANGE IN PHOTOPERIOD IMPROVES THEIR DISRUPTED ACTIVITY AND EXHIBITS AN ANTIHYPERTENSIVE EFFECT

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Light/dark cycles trigger changes in behavior and other physiological parameters, and their disruption can increase the risk of metabolic diseases. Grape seed flavanols have shown beneficial effects on hypertension and other components of metabolic syndrome. Moreover, modulatory effects of these (poly)phenols on circadian rhythms have been described. The aim of this study was to evaluate the effects of a grape seed proanthocyanidin extract (GSPE) on activity, blood pressure, and body temperature in obese rats that were abruptly switched from a standard photoperiod (12 h light, L12) to a long (18 h light, L18) or short (6 h light, L6) photoperiod. Telemetry was used to study 24 Fischer rats fed a cafeteria diet (CAF) for 6 weeks under L12 conditions. Subsequently, the animals were switched to L18 or L6 conditions and administered either vehicle (VH) or GSPE (25mg/Kg) for 1 week. The results showed that photoperiod change induced alterations in activity and diurnal blood pressure oscillation in these animals, while GSPE administration improved the disrupted activity rhythmicity and exhibited an antihypertensive effect, also attenuating the non-dipper pattern, i.e., the absence of blood pressure drop during the rest period, under L18 conditions. Although further research is needed, these findings suggest that GSPE may prevent circadian disruptions in activity and mitigate blood pressure changes caused by photoperiod disruption.

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P.2.44 - BIOAVAILABILITY OF HYDROLYZABLE TANNINS AFTER ORAL ADMINISTRATION OF TRAPA BISPINOSA EXTRACT IN RATS

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Polyphenols are found widely in nature, such as in foods and medicinal plants, and have been shown to have various biological activities including antioxidant and anti-inflammatory effects. Bioavailability of hydrolyzable tannins including ellagitannins and gallotannins has been studied in recent years, and various functionalities as antiglycation and anti-aging effects of metabolites such as urolithins have also been reported. However, there are many unclear points about bioavailability such as absorption, distribution, metabolism, and excretion after hydrolyzable tannins ingestion. An appropriate intake of functional foods can be suggested to establish by understanding *in vivo* behavior, but there are few reports on bioavailability after ingesting polyphenol-rich sources. In this study, we investigated the *in vivo* behavior of hydrolyzable tannins and the related metabolites after administration of polyphenol-rich *Trapa bispinosa* pericarp extract (TBPE) to rats.

After oral administration of 100 mg/kg of TBPE to SD male rats, blood was collected from the inferior vena cava at 1, 3, 6, 12, 24, 48, and 72 h. In addition, urine was collected up to 72 h after the administration. The collected plasma and urine samples were deconjugated and then extracted with ethyl acetate to prepare samples for quantitative analysis. 18 metabolites, including 12 urolithins and ellagitannin related metabolites, and 6 gallotannin related metabolites were quantified by HPLC-ESI-MS/MS.

Urolithins and gallotannin related metabolites were detected in the urine of rats administered TBPE, but they showed different excretion patterns. Urolithins were excreted in urine with a sigmoidal curve up to 72 h, indicating that the excretion was delayed, and most gallic acid related metabolites were rapidly excreted earlier after the administration. On the other hand, urolithins showed to reach the maximum blood concentration around 24 h after the ingestion, while gallic acid related metabolites tended to reach around 1h. Although ellagitannin metabolites take time to be absorbed and excreted, gallotannin metabolites are shown to be rapidly absorbed and excreted in the body, indicating to clarify that each type of metabolite exhibits different *in vivo* behavior (Figure 1).

These results provide basic data on the pharmacokinetics of hydrolyzable tannins contained in TBPE, which is expected to lead to the demonstration of the functionality of *Trapa* ingredient.

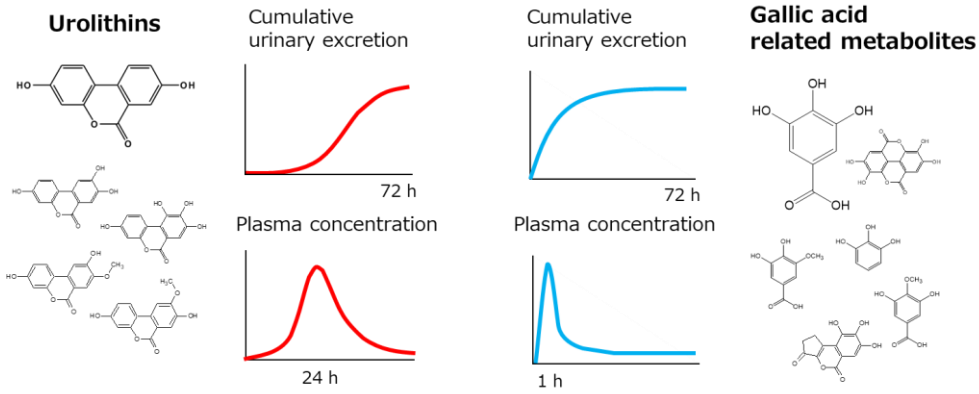

Figure 1: Patterns of urinary excretion and blood kinetics of urolithins and gallic acid related metabolites after oral administration of TBPE to rats

P.2.45 - FRUIT TREE LEAVES EXTRACTS FROM *PRUNUS ARMENIACA* L.: VALUABLE SOURCE OF POLYPHENOLS COMPOUNDS DETERMINED BY LIQUID CHROMATOGRAPHY-QUADRUPOLE/TIME OF FLIGHT-MASS SPECTROMETRY (LC-MS/QTOF)

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Fruits and berries are excellent sources of phytochemicals such as various polyphenols, tri- and tetraterpene, and other nutritional substances such as pectins, fibers, minerals, and vitamins [1,2]. However, the permanent quest for early disease prevention and health promotion inspires a continual search for new natural plant sources with high pro-healthy properties. Owing to the high contents of these compounds, leaves have received additionally higher attention in the last few years as a potential component of a healthy diet. As Ferlemi and Lamari [3] mentioned, leaves are one of the alternative sources of bioactive compounds alongside widely consumed fruits and berries. In view of the limited information available in the literature concerning leaves as by-products of Prunus armeniaca L. cultivation, the aim of this work was to identify and characterize their principal polyphenolic constituents by LC-ESI-QTOF-MS/MS and screening in vitro biological potency as antioxidant capacity (online ABTS), antidiabetic (α-amylase, α-glucosidase), anti-obesity (pancreatic lipase) inhibitory activity.
The leaves of *Prunus armeniaca* L. (FTLe) were collected after tree blooming and polyphenols fractions was isolated [4]. Polyphenols from pFTLe was carried out using an Acquity system as described previously by Wojdyło et al. [4,5]. pFTLe antidiabetic potential as α-amylase, α-glucosidase and pancreatic lipase enzyme activity were determined using the method proposed previously by Wojdyło et al. [4,5].
Comparison of different polyphenolic extracts of *P. armeniaca* cultivar leaves ectracts according to their quantitative composition revealed them to be exceptional sources of hydroxycinnamic acids, and to a lesser extent as sources of flavonols.
Polyphenol-rich apricot leaf extract showed the most effective anti-obesity action through inhibition of pancreatic lipase, and antioxidant capacity, especially the oxygen radical absorbance capacity, which was particularly correlated with polyphenolic compounds. Online ABTS radical UPLC-PDA-PDA analysis clearly demonstrated that the three predominant compounds of pFTLe are quercetin-3-*O*-rutinoside > 5-*O*- and 3-*O*-caffeoylquinic acid, which basically contribute to antioxidant potential. These results assist in the evaluation of plant sources of potential new raw materials for application in different commercial sectors, especially for food, cosmetics and pharmaceuticals production.
The results of this work suggest that extracts from the fruits tree leaves in future can be applied in different commercial sectors such as food, cosmetics, and pharmaceuticals. Finally, the polyphenols leaf extracts could be an important source of interesting molecules for the prevention and treatment of other diseases of the 21st century such as cancer.

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This work was supported by the NCN by project UMO-2018/29/B/NZ9/02872. The work is the result of activity of the research group *Plants4Food*.

P.2.46 - PROFILING OF POLYPHENOLS BY LC-QTOF/ESI-MS-, CHARACTERISTICS OF NUTRITIONAL COMPOUNDS AND IN VITRO BIOLOGICALLY EFFECT OF *PRUNUS AVIUM* AND *P. CERASUS* LEAVES

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In recent decades numerous studies have demonstrated that fruits, berries, vegetables, and herbs and their roots and bark have been known as a good source of various phytonutrients, dominantly vitamins, polyphenols, minerals, amino acids, fiber, etc. [1]. It is well known that these phytonutrients are characterized by biological activities that exert a protective effect against many different of human diseases [2]. Leaves of fruits and berry are recognized, in the word as “superfoods” due to the high content of bioactive natural products and the health benefits deriving from their consumption. Some berry leaves have been used in folk and traditional medicine [3]. Sweet cherries (*Prunus avium* L.) and sour cherries (*Prunus cerasus* L.) are among the most important fruits, widely growing crops, and consumed fresh (mainly sweet cherries) or processed (mainly sour cherries) in many countries worldwide.

With regard to this topic, the main objective present work was to comprehensively study polyphenolics by LC-MS-ESI-QTOF, amino acids and triterpenic detailed analysis of sweet and sour cherry leaves and to evaluate antioxidant capacity, and in vitro biological activities against enzymes related to obesity (pancreatic lipase), diabetes (α-amylase, α-glucosidase), cholinesterase (AChE, BuChE), and anti-inflammatory (COX-1, COX-2) effects.

Sweet (Rivan cv.) and sour (Łutówka cv.) cherry leaves (500±10 g) cultivars cultivated in the Research Station for Cultivar Testing in Zybiszów near Wroclaw were collected, freeze-dried and finally pulverized to powder with a mill. Analysis of polyphenols, triterpenic and amino acids was carried out on an LC-MS/PDA-QTOF and Acquity UPLC-PDA-FL system (Waters Corp., Milford, MA, USA) [4,5]. Antioxidant capacity, inhibition of pancreatic lipase, α-glucosidase, α-amylase, acetylcholinesterase (AChE) and butylcholinesterase (BChE), cyclooxygenase (COX-1, COX-2) were analysis [4,5]. A total of 27 phenolic compounds were identified by LC-MS-ESI-QTOF, and they included flavanols > phenolic acids > flavonols and flavanols for leaves. Pearson’s correlation and PCA showed that phenolic compounds, minerals and amino acids were the main contributors to the *in vitro* enzymatic activity. Components of leaves were the most active in ORAC capacity, and present high potential for inhibition of some enzymes including AChE, BuChE, α-amylase, COX-1 and COX-2.

Therefore, the sour cherry leaves may be used as a potential source of valuable ingredients in herbal teas, additive for functional foods, medicinal products, dietary supplements, and other products, which could open opportunities in novel applications. The results also increase the interest and potential use of leaves of sweet and sour cherries as a consolidated source of pharmaceutical agents.

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TOPIC 3 - METABOLOMICS, TARGETED ANALYSIS & BIG DATA

PL.3.01 - CHILDREN OF NATURE – TARGETED AND UNTARGETED ANALYTICAL APPROACHES TO DECIPHER POLYPHENOL REACTIVITY IN FOOD PROCESSING AND METABOLISM

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Following 25 years of polyphenol research in our laboratory, the astonishing chemical and metabolic reactivity resulting in considerable chemical diversity, has emerged as the most remarkable attribute of this class of secondary natural products. To illustrate this concept, we will present selected data from black tea and coffee chemistry.

In black tea chemistry enzymatic fermentation converts six catechin derivatives into an estimated 30 000 different polyphenolic compounds via a process we have termed the oxidative cascade process [1]. In coffee roasting around 45 chlorogenic acids are converted into an estimated 250 novel derivatives following a series of diverse chemical transformations [2].

Following ingestion by humans these dietary polyphenols, whether genuine secondary metabolites or food processing products, encounter the microorganisms of the gut microbiota, converting them into a myriad of novel structures [3]. In the case of coffee only two out of 250 chlorogenic acids are absorbed intact, with most others being subject to gut microbial metabolism.

Modern mass spectrometry (MS) has been key in unravelling the true complexity of polyphenols subjected to food processing and metabolism. We will accompany the lecture with analytical strategies developed, including ultra-high-resolution MS, tandem MS, multivariate statistics and molecular networking that allow an insight into the fascinating chemical processes surrounding dietary polyphenols. Finally, experimental results studying biological activity of polyphenols will be presented, highlighting a general promiscuity of this class of compounds associated with non-selective protein binding leading to loss of enzymatic function.

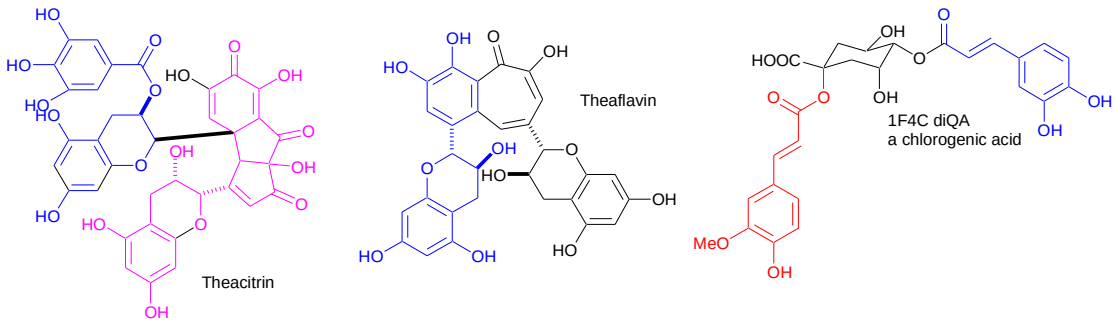

Figure 1: Selected polyphenol structures from black tea and coffee

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PL.3.02 - UTILIZING NEW TANNIN ANALYTICS TO ESTIMATE THEIR STRUCTURE-ACTIVITY FUNCTIONS

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The polyphenol family contains some high-molecular weight compounds that are more challenging than others to detect and quantify accurately as individual compounds or even as groups of compounds. This fact has created and continues to create subsequent challenges for understanding their structure-activity functions. Two such groups of complex polyphenols are hydrolysable tannins and proanthocyanidins. Especially their oligomeric and polymeric forms may be difficult to separate by chromatographic tools thus making their individual detection by for instance mass spectrometry a daunting task. The presence of hundreds of hydrolysable tannins or proanthocyanidins, and even their complex mixtures in one plant species, makes it practically impossible to separate or isolate them all for structure-activity studies. This issue is further highlighted in efforts trying to widely screen the presence of various types of hydrolysable tannins and proanthocyanidins in the thousands of species present in the plant kingdom. For these large-scale efforts to be worth while, we need efficient liquid chromatography mass spectrometry tools that can accurately detect the relevant compounds, their functional units and other properties that are central in causing their bioactivities such as e.g. oxidative, protein precipitation and anthelmintic activities. In my talk, I will summarize the current knowledge of tannins' structure-activity linkages and will show how rapid group-specific UHPLC-MS/MS analytics can be used to reveal the causes of plant bioactivity even directly from the crude plant extract. I use tens of plant species as examples and focus especially on different types of subgroups of both hydrolysable tannins and proanthocyanidins. At the same time different types of flavonols and quinic acid derivatives detected with their group-specific MS/MS tools will be used to explain a part of the observed bioactivities as well.

O.3.01 - HOW DEVELOPMENTAL AND DROUGHT FACTORS AFFECT POLYPHENOLS OF GOJI (*LYCIUM BARBARUM*) LEAVES AND BERRIES?

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Goji berries (*Lycium barbarum*, *L. chinense* and *L. ruthenicum*, perennial species from the Solanaceae family) are traditionally eaten in Asia for their high nutritional value [1]. Although goji consumption is increasingly growing in Europe, there is no significant field production or research program on this plant. Thus, the ecophysiological and genetic determinisms of the organoleptic and nutritional quality of goji berries remain unexplored. Moreover, it is unclear how yield components and berry quality traits may be affected by agricultural practices or abiotic stresses, yet *Lycium* is reputed to be drought-tolerant.

To gain further knowledge on the mechanisms underlying goji nutritional value and drought tolerance, both untargeted and targeted metabolite analyses were performed by UPLC-ESI-QTOF or UPLC-DAD-ESI-TQ on leaves and berries of *Lycium barbarum* accessions growing under well-watered (soil water potential of -0.1 MPa) or water stress conditions (soil water potential of - 0.5 MPa).

The dataset included 46 phenolic compounds, half of which were identified with a level 1 confidence [2] by comparing their retention time, absorbance spectra and MS/MS data with authentic standards. The water deficit applied during the reproductive period affected plant growth and carbon allocation. A significant reduction of leaf fresh and dry biomass was reported for all accessions. In addition, the plants under water deficit decreased fruit production. Interestingly, the soil water deficit modified the fruit dry matter's composition. The fruits from ‘FPW07’ concentrated more glucose with the declining of the soil water potential. In addition, the soil water deficit decreased quercetin and phenolic acids in *Lycium* accessions.

We have collected a reference dataset for key physiological traits that were not previously documented in *Lycium*, and characterized their response to drought. These data help to gain knowledge on *Lycium* physiology and development.

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O.3.02 - TEA PHENOLICS: SUSTAINABLE SOURCING, AUTO-OXIDATION, AND RAPID ANALYSIS BY ION MOBILITY MS

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Tea is one of the most consumed beverages world-wide and is rich in phenolic compounds. Tea phenolics are generally considered to possess health-promoting properties, in part due to their prebiotic potential [1]. Therefore, it is of scientific and industrial interest to explore the sustainable sourcing and the stability of tea phenolics.

In our work, we investigated the possibility to valorise phenolics from old tea leaves, which are an agricultural waste stream. Our research shows that old tea leaves possess a significantly different phenolic profile than the young leaves that are typically used for tea production. Despite this, we concluded that old tea leaves still contain a relatively high quantity of tea phenolics [2]. We also explored whether pulsed electric field (PEF) can be used as a selective and energy-efficient pre-treatment for the extraction of these valuable compounds.

A challenge for application of tea phenolics is their instability, which may result in undesirable changes in their properties. For example, catechins (flavan-3-ols) in ready-to-drink green teas can undergo poorly understood non-enzymatic browning reactions during storage. Thus, we studied catechin auto-oxidation under accelerated shelf-life-like conditions and found that major reactions are epimerization, degalloylation, and oxidative coupling. Our results indicate that the main brown products are dehydrodicatechins, which are also formed upon enzymatic browning of tea phenolics. We also show that oxidative coupling in the absence of enzymes proceeds via *o*-quinone mediated reactions, similar to the reactions observed during enzymatic oxidation [3]. In our most recent work, we developed an analytical method for more in-depth investigation of the reactions of catechins using state-of-the-art cyclic ion mobility mass spectrometry (cIMS-MS). Using this technique, catechin epimers (e.g. catechin and epicatechin) in complex samples, like green tea, can be rapidly separated and (semi-)quantified without the need for relatively time-consuming chromatographic separation. Thereby, cIMS-MS enables real-time monitoring of epimerization and facilitates determination of the reactivity of individual catechin epimers. We expect that coupling of (cyclic) ion mobility MS to LC will enable even more powerful multi-dimensional separation that can be used for future studies on highly complex mixtures of catechin oxidation products.

To conclude, our work on tea phenolics has revealed opportunities for sustainable sourcing of these compounds, sheds light on their auto-oxidative browning, and demonstrates the potential of ion mobility MS for their analysis.

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O.3.03 - A PROTEOMIC APPROACH FOR UNREVEALING THE ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF 5-(3',4'-DIHYDROXYPHENYL)-Γ-VALEROLACTONE, THE MAIN METABOLITE OF A STANDARDIZED EXTRACT OF GRAPE SEED PACS

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Proanthocyanidins (PACs) are a class of secondary metabolites present in many foods and beverages as oligomers and polymers PACs of flavan-3-ols. The health benefits of PACs on cardiovascular diseases, cancer, and diabetes are supported by a huge number of *in vitro* and *in vivo* studies. Recent evidences support that their health promoting activities is also due to their effects on the GI tract. Moreover, only monomers and dimers are absorbed, while oligomeric and polymeric PACs are not bioavailable. A large portion of orally ingested PACs reach the colon where they are catabolized by colonic microflora into phenyl-γ-valerolactones and phenyl-4-hydroxyvaleric acids, which showed anti-inflammatory activity [1]. Aim of the present work is to study by LC-ESI-MS/MS the metabolic conversion of high polymeric PAC_S contained in Ecovitis® [2], a new grape seed extract characterized by a high concentration of oligo-polymeric PACs and prepared using selected seeds obtained from Northeast Italian wineries, to bioactive valerolactones in healthy volunteers in a placebo-controlled intervention study. Among the different valerolactone metabolites, 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (γ-V) was identified as the main plasma (~ 35 nM) and urinary (as sulfate and glucuronide phase II metabolites) metabolite (~ 40 µmoles/24 hours). We then synthesized γ-V [3] to evaluate its anti-inflammatory and antioxidant effect by using two phenotypic cell models: HEK293 with the cell reporter for Nrf2 activation and R3/1 with cell reporter for NF-κB. A dose-dependent activity was observed in both experiments, over the ranges 50-200 µM for Nrf2 and 10-150 µM for NF-κB. In addition, the ability of γ-V to modulate the NF-κB and its phosphorylated form p(Ser276)-NF-κB levels was confirmed on human colon Caco-2 cells. Quantitative proteomics studies were then carried out on Caco-2 cells to go deeper inside the mechanism of action of γ-V (75 µM), in both physiological and inflammatory conditions. In physiological condition, γ-V improved the mitochondrial activity, by boosting the oxidative phosphorylation. When a pro-inflammatory stimulus is used (TNFα), γ-V acts by reverting the expression levels of certain key genes belonging to the integrin signalling pathway, usually triggered by an inflammatory state. Moreover, the activation of the Nrf2 cascade is indirectly observed through the modulation of the downstream gene products, including COX2 found upregulated in the inflammatory state and downregulated in the presence of γ-V. In conclusion, γ-V is the main circulating metabolite after the oral intake of Ecovitis® and it is one of the putative responsible for the anti-inflammatory and antioxidant effect usually related to PACs assumption.

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O.3.04 - LIGNIN-RICH BIRCH GLUCURONOXYLAN AS A DIETARY FIBER: THE METABOLIC FATE OF LIGNIN IN A RAT MODEL

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Dietary fiber has been traditionally defined as the plant cell wall remnants remaining by human digestive enzymes, including polysaccharides and lignin, and indigestible protein, lipids, and inorganic constituents [1]. Although recent studies suggest metabolic reactions of lignin in the gastrointestinal tract, the research on metabolic fate of lignin using modern analytical tools is lacking.

We have recently demonstrated the prebiotic potential of birch glucuronoxylan (GX) as a dietary fiber supplement in a rat model [2]. The positive effects on colonial health were accompanied with the formation of short-chain fatty acids (SCFAs) and higher abundance of Bifidobacteria [2]. The same experimental setup was used in this research to explore the metabolic fate of lignin, which is isolated together with hemicelluloses (GX) during hot-water extraction. The GX can be purified further using physical or chemical methods, such as centrifugation and ethanol precipitation [3].

The rats were divided into three groups, 14 animals per group, which were fed with lignin-rich GX (GXpoly), purified GX (pureGX), or cellulose (reference). Urine and fecal samples of both GX groups were analyzed using state-of-the-art analytical tools. The lignin content in fecal samples was evaluated using a semi-quantitative pyrolysis – gas chromatography / mass spectrometry (pyr-GC/MS) method. Furthermore, the lignin was isolated from the fecal samples and the chemical structure was analyzed using a nuclear magnetic resonance (NMR) spectroscopy. The smaller phenolic metabolites were analyzed and quantified from the urine samples using ultra high-pressure liquid chromatography (UHPLC) in combination with mass spectrometry (LC-MS). Finally, the microbiota was extracted and sequenced, and the abundance of different bacteria was statistically analyzed for associations with the lignin-content.

All the complementary results showed that lignin was partially metabolized. The NMR spectrum of the fecal lignin from GXpoly group showed significant fragmentation. The pyr-GC/MS data showed that the guaiacyl (G) units of lignin were preferred in the metabolic reactions over syringyl (S) units, observed by the increasing S/G-ratio. The urine samples contained six different small phenolic metabolites, comprising of ferulic and sinapic acids, and their derivatives. Some bacterial taxa were associated with the amount of lignin in the samples.

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O.3.05 - INTEGRATED MULTI-OMICS ANALYSES OF LIGNIN-REDUCED *ARABIDOPSIS* LINES ON INTERNATIONAL SPACE STATION

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Polyphenol metabolism in terrestrial plants can largely be attributed to withstanding gravitational and radiation (e.g., UV) effects, as well as providing polyphenolic biopolymers and bioactives that help withstand and/or limit the damage by opportunistic herbivores and pathogens. That is their functions for terrestrial plant life on Earth. What about their requirements though outside of Earth's gravitational field, e.g., for long duration space missions or extraterrestrial colonization? Here we report insights gained on the integrated multi-omics (big data) analyses of various lignin-reduced lines of the model plant species, *Arabidopsis thaliana*, from two 6-week grow-outs in the Advanced Plant Habitat (APH) on International Space Station (ISS); corresponding ground controls were conducted at Kennedy Space Center (KSC). On Earth, lignin biopolymers enable vascular plants to stand upright against forces of gravity by reinforcing their cell walls in the vasculature, to afford conduits to enable water/nutrient transport, and as a protective barrier to opportunistic pathogens. Additionally, lignin in different plant tissues and organs significantly reduces overall plant digestibility (by mammals) and nutritional benefit of vascular plants. *Arabidopsis* lignin-reduced mutants (*adt5*, *adt4/5*, *adt3/4/5*, and *adt3/4/5/6*) were obtained by generating homozygous knockouts of genes encoding *arogenate dehydratase* (*ADT*) as well as those with a carbon concentrating mechanism, *CCM* (e.g., *adt3/4/5/6/CCM*). Plants were daily monitored telemetrically, where unusual stem growth orientation and anatomy was noted particularly with lignin-reduced mutant lines. Aerial tissues (stems and leaves) were harvested on ISS at approximately 6 weeks, then immediately freezer stored and returned later to Earth. Stem and leaf tissues of all lines were individually subjected to integrated multi-omics (metabolomics, transcriptomics, lipidomics, proteomics and phospho-proteomics) analyses, with multi-omics integration results compared to their corresponding wild type (WT) lines. Here we discuss our consortium's integrated multi-omics analyses and the new insights gained, as well as the current rationale for the unusual *Arabidopsis* stem growth and anatomy behavior on ISS, together with the potential for lignin reduced plant lines in extra-terrestrial environments, while maintaining needed levels of other important bioactive polyphenols. Supported by a grant from the National Aeronautics and Space Administration: NNX15AG56G.

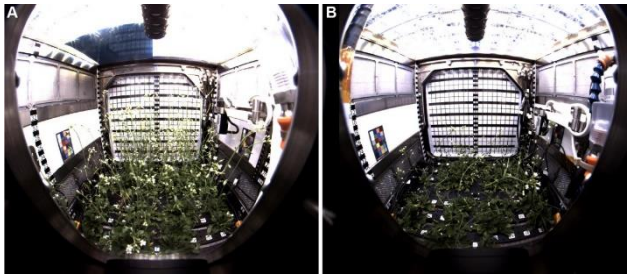

Figure 1. Flowering stems of *Arabidopsis* plants in KSC ground controls (A) grew mostly upright (vertical), whereas ISS flowering stems (B) deviated away from upright (vertical) growth from Grow-out #1 on day 36.

OY.3.01 - DECIPHERING THE RED FLESH TRAIT IN APPLE BY IMAGE ANALYSIS AND METABOLITE PROFILING

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Red-fleshed apple varieties are becoming increasingly popular among fruit consumers. This trait is associated with anthocyanin accumulation, which is known for its beneficial effects on human health [1]. The genetic basis of colour development has been widely characterised [2], however, current models do not explain the observed variations in red pigmentation intensity and distribution.

We developed a multifactorial approach to gain knowledge on the control of the red flesh trait in apple. This methodology was applied to investigate the phenotypic diversity in five hybrid families segregating for this trait (450 genotypes) by combining image-based phenotyping with the determination of several biochemical traits that influence colour expression: phenolic compound contents, dry matter contents, and pH values. We used image-based phenotyping to evaluate intensity and distribution of red colour in progenies using colour parameters. We then defined morphometric descriptors to assess the significant variation of colour patterns in the dataset and to study the inheritance of the colour distribution. Important variations in phenolic profiles between hybrid families were identified. Ten of the most discriminating phenolic compounds involved in the expression and stability of red flesh were selected. Targeted analyses were performed to quantify these compounds by UPLC-DAD over two years of fruit harvest (2021 and 2022). Correlation analyses were performed to assess the robustness of our image analysis pipeline. As a result, we propose a model including dry matter, pH, phenolic compound contents to describe red flesh colour development.

[1] Yuste, S., Ludwig, I.A., Romero, M.-P., Motilva, M.-J. & Rubió, L., *Food Funct.*, 13, 4861–4874, 2022.
[2] Wang, N. & Chen X., Genetics and Genomics of Fruit Color Development in Apple, In *The Apple Genome* (Korban S.S., Ed.), Springer, 271–295, https://doi.org/10.1007/978-3-030-74682-7_13, 2021.

OY.3.02 - UHPLC-QQQ-MS TARGETED QUANTIFICATION OF SYRAH RED WINE PHENOLIC AGEING MARKERS DURING BOTTLE AGEING

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During red wine ageing, many reactions take place which will modify and improve the organoleptic parameters of the wine over time [1]. Concerning phenolic composition, the content of the "native" polyphenols decreases during ageing. They may react through oxidative and non-oxidative reactions with other wine constituents, leading to the formation of more stable compounds [2]. Oxygen transfer through the cork stopper is a major parameter in the evolution of red wines and thus on the formation of these new compounds.

The objective of this study was to examine the evolution during ageing of different polyphenols in experimental Syrah red wines. These wines were bottled using different micro-agglomerated cork stoppers with different oxygen transfer rates (OTR) and stored during 2 years. Specific polyphenols ageing markers were synthesized (pyranoanthocyanin, ethyl linked flavan-3-ol and flavan-3-ol sulfonate) and a new and sensitive UHPLC-QqQ-MS method of quantification of these compounds was developed and validated. In parallel, the quantification of monomeric anthocyanins in red wine samples was also performed by UHPLC-DAD-MS.

The results showed that "native" polyphenols content in general decreased with wine ageing, especially monomeric anthocyanins (glucosylated, acetylated, coumaroylated) following a first order kinetic. Moreover, significant differences between corks stoppers were observed which could be due to the reactions of anthocyanins with other constituents in relation with the stopper OTR. In addition to that, the levels of anthocyanin-derived pigments changed during bottle ageing. This lead to an evolution in the red wine hue measured by spectrophotometric analysis depending on both Syrah red wine et stopper studied.

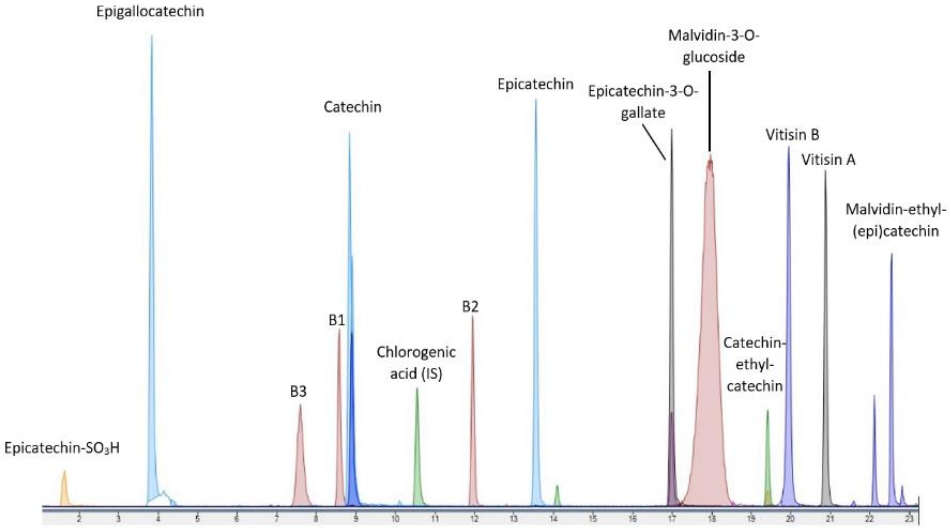

Figure 1: Extracted MRM chromatogram of studied compounds

[1] Tao Y., García J.F. & Sun D.-W., *Crit. Rev. Food Sci. Nutr.*, 54, 817–835, 2014.
[2] Echave J., Barral M., Fraga-Corral M. & Prieto M.A., *Molecules*, 26, 713, https://doi.org/10.3390/molecules26030713, 2021.

OY.3.03 - THE APPLICATION OF UNTARGETED METABOLOMIC APPROACHES FOR THE SEARCH OF COMMON BIOAVAILABLE METABOLITES COMING FROM DIFFERENT BIOACTIVE EXTRACTS

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Clinical evidence shows that numerous extracts rich in bioactive compounds exhibit similar potential benefits in human chronic diseases. Although most of these extracts are rich in the same families of chemical compounds, such as phenolic compounds, the specific compounds characterised in them are often very different between species and matrices [1]. In fact, more than 8.000 phenolic compounds have been reported to date. Considering that these compounds can be metabolized following phase I and phase II reactions in the organism, we hypothesized that there may be common bioavailable metabolized compounds contributing to the reported bioactive effects, which may come from different bioactive compounds present in the vegetal matrices. In this context, the objective of this study was to detect bioavailable common metabolites from 5 bioactive extracts (*Hibiscus sabdariffa*, *Olea Europaea*, *Silybum Marianum*, *Theobroma Cacao*, and *Lippia Citriodora*) rich in phenolic compounds but with different chemical compositions. To achieve this goal, we proposed an acute double-blind dietary intervention in humans. 48 volunteers divided into 6 groups (5 from the extracts, and placebo), were recruited from whom plasma and urine samples were collected at different times. These biological samples were analysed using an untargeted metabolomic methodology based on HPLC-ESI-QTOF-MS. The acquired data was processed using mzMine 2.53 and R packages (notame, batchCorr). A methodology based on QA/QC approaches was followed to control the quality of the data, using QC-pool, blanks, sequence randomization, etc. The variables of interest were selected based on the Fold-Change parameter calculated by the ratio of the area of the signals at the different times with respect to time 0 (i.e. biological samples collected just before the consumption of the bioactive supplements) of each volunteer. Those variables that presented a significant FC in any of the groups that took the bioactive supplements and non-significant in the placebo group were proposed for the metabolite annotation step. Those signals were annotated based on the comparison of the MS/MS spectrum, with those reported in metabolomic databases (HMDB, KEGG, etc.), massbanks, and with annotation computational tools, such as Sirius or GNPS. The preliminary results allowed the detection of more than 70 significant signals. Among this set of significant signals, some have been shown to be common in at least two of the supplements, highlighting a subset of common signals that come from the extracts of *Lippia Citriodora* and *Olea Europaea*. In addition, it is also worth noting that several of these common metabolites have their maximum absorption in plasma at different times depending on the matrix of origin, demonstrating different degradation/absorption mechanisms. We are currently working on testing the bioactive properties of these annotated bioavailable metabolites, which will allow us to obtain a better understanding of the bioactive mechanisms of phenolic compounds that could have an important impact for the development of future applications based on them as functional foods or pharmaceuticals.

[1] Villegas-Aguilar M.C, Fernández-Ochoa Á, de la Luz Cádiz-Gurrea M., Pimentel-Moral S., Lozano-Sánchez J., Arráez-Román D. & Segura-Carretero A., *Molecules*, 25, 596, https://doi.org/10.3390/molecules25030596, 2020.

OY.3.04 - SELECTIVE IDENTIFICATION OF CONJUGATED POLYPHENOL COMPOUNDS IN PLANT MATRICES BY LC-MS/MS: A MULTI EXPERIMENT APPROACH

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Phenolic compounds (PCs) have biological role in plants, mainly related to antioxidant capacity [1], and it is closely linked to their chemical structure since they have many nucleophilic sites available for different reactions, such as: glycosylation, esterification, methylation, oxidation etc [2]. This leads to the formation of a large number of conjugated forms and hence to the challenge to develop analytical methods capable of providing a complete characterization of the samples, and to selectively identify a set of PCs belonging to a specific phenolic class.
In this work, the potential of the hybrid linear ion trap – triple quadrupole mass spectrometry coupled with high-performance liquid chromatography (HPLC-LIT-QqQ) was exploited, in order to obtain more structural information for the putatively identification of PCs by the development of different approaches, capable of selective identification of the common moiety of interest. A selective determination by means of precursor ion scan (PI), neutral loss (NL), enhanced product ion (EPI) and MS^3 (MS/MS/MS) acquisition modes was achieved, based on the study of the PCs structures and their relative conjugated forms, such as phenolic acids esters, glycosidic derivatives of flavonoids, and more complex molecules, such as proanthocyanidins.
In our opinion, this approach represents a complete and useful tool for the determination of PCs in different matrices, as it can provide exhaustive information on the forms conjugated with specific molecules, allowing a rapid and effective putative identification for wide range of PCs derivates, such as mono-, di- and tri-glycosylated or other conjugates, without the need to employ a large number of standards. In figure 1, an example of the fragmentation pattern for a conjugate of Kaempferol is displayed.

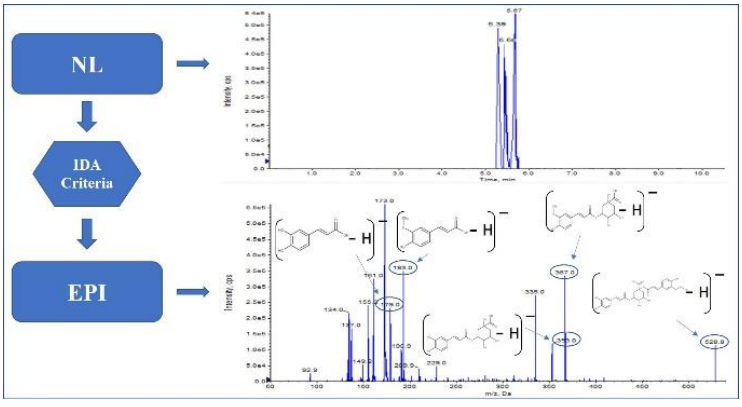

Figure 1: Kaempferol-glucosyl-(1->2)-(6''-acetylgalactoside)-hexoside fragmentation pattern.

[1] Kurek-Górecka A., Rzepecka-Stojko A., Górecki M., Stojko J., Sosada M. & Świerczek-Zięba G., *Molecules*, 19, 78-101, 2014.
[2] Manach C ., Scalbert A., Morand C., Rémésy C. & Jiménez L., *The American journal of clinical nutrition*, 5, 727-747, 2004.

OY.3.05 - AN ORIGINAL NON-TARGETED LC-MS APPROACH FOR POLYPHENOL OXIDATION PRODUCTS ELUCIDATION IN APPLE JUICE.

Bourseau C.*[a], Guilois-Dubois S.[a], Le Quéré J-M.[a], Chereau S.[b], Sylvain Guyot S.[a]

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Apples and derivate products are rich in polyphenols, the consumption of those specialized metabolites has potential health benefits. Through apple juice processing (crushing pressing), the oxidation naturally occurs caused by the contact of the plastidial polyphenol-oxidase (PPO) and its vacuolar phenolic substrates. Coupled to oxygen presence, the PPO catalyzes the oxidation of apple native polyphenols to numerous oxidation products (OPs) with specific structures and functional properties. Organoleptic (color, bitterness, astringency, turbidity) and nutritional apple juice qualities might therefore be modified by oxidation processes. To better understand its mechanism and consequences on juices and ciders, it is necessary to know the molecules formed by oxidation.

In contrast to previous works dealing with targeted analysis of OPs on the basis of oxidative reactivity of polyphenols [1,2], the present study aims to set up a standardized non-targeted approach for detecting Ops in juices of different apple varieties depending on the amount of oxygen consumed .

Juices from five cider apple varieties were prepared under anoxic conditions. They then underwent a controlled oxidation by cumulative additions of oxygen reached thanks to an experimental prototype. Thus, five oxidation levels (null to maximum) were analyzed for each experiment by HPLC-UV-MS. Samples were injected before and after depolymerization by phloroglucinolysis to characterize both native and oxidized procyanidins. Raw mass spectrometry data were processed using Galaxy Workflow 4 Metabolomics (W4M) to obtain the full ions list.

An original workflow was developed using a four steps data filtering to retain OPs related ions. A linear regression model (polynomial 2nd order) was applied to each ion, variety by variety to fit the intensity of each ion to the cumulative injected oxygen. The model enabled (i) the collection of all ions that responded to oxidation, (ii) the removal of weak oxygen dependent ions, (iii) the selection of ions that increase with the supply of oxygen (OPs). Then the last step (iv) used a hierarchical agglomerative clustering to annotate ions (retention time, molecular ion, isotopes, fragments, adducts, stacking) into “molecular signals”. This approach enabled the identification of 32 OPs previously identified by targeted approaches and 22 potential OPs that had not been detected before.

The presented non-targeted approach associated to quantitative sorting is strengthened through the detection of already known OPs and even provided supplemental information about OPs. Ultimately, the approach will contribute to the deep understanding of oxidations processes and their effects on the juice quality and composition.

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[2] Mouls L. & Fulcrand H., *Journal of Mass Spectrometry*, 47, 1450-1457, https://doi.org/10.1002/jms.3098, 2012.

P.3.01 - LC-UV-MS QUANTIFICATION OF NATIVE AND OXIDIZED PHENOLIC COMPOUNDS IN EXPERIMENTAL AND COMMERCIAL APPLE JUICES REVEALED HIGHLY CONTRASTED COMPOSITIONS.

Guilois-Dubois S.*[a,c], Castillo-Fraire C.M.[a,c], Poupard P.[b,c], Le Quéré J.M.[a,c], Salas E.[d], Guyot S.[a,c]

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In the first step of apple juice production (crushing and pressing), phenolic compounds undergo enzymatic oxidation, forming newly formed molecules. More information is needed concerning the concentrations of those oxidized phenolic compounds (OP) in apple juices to better understand their real contribution to organoleptic and nutritional properties. In this study, native phenolic compounds and oxidized phenolic compounds were quantified in 54 commercial and experimental apple juices. HPLC-MS method was specially developed and validated to quantify two families of OP (5-*O*-caffeoylquinic acid dehydrodimers and epicatechin-5-O-caffeoylquinic acid heterodimers). A statistical clustering analysis (Euclidian Ward's method) was performed on all phenolic compounds data sets. Interestingly, these two series of oxidation products could represent more than 14% of the total phenolic compounds quantified (TPQ). To the best of our knowledge, it is the first time that both families of OP (CQA dehydrodimers and EC-CQA heterodimers) were successfully quantified in apple juices. Noticeably, these compounds exhibit very original polyphenolic structures that are not encountered in fresh apples and may contribute to specific nutritional and organoleptic properties in the juices. In addition, we noted that most of the experimental juices (made from "cider apple varieties") and some juices from local cider producers (mostly made from "cider apple varieties"), were found to be on average eight times more concentrated in polyphenols than juices mainly found in supermarkets (mostly made from "dessert apples"). These results of experimental apple juices underlined the importance of apple varieties on the polyphenol content of the juices. In addition, the part of the phenolic compounds represented by the oxidized forms should not be neglected.

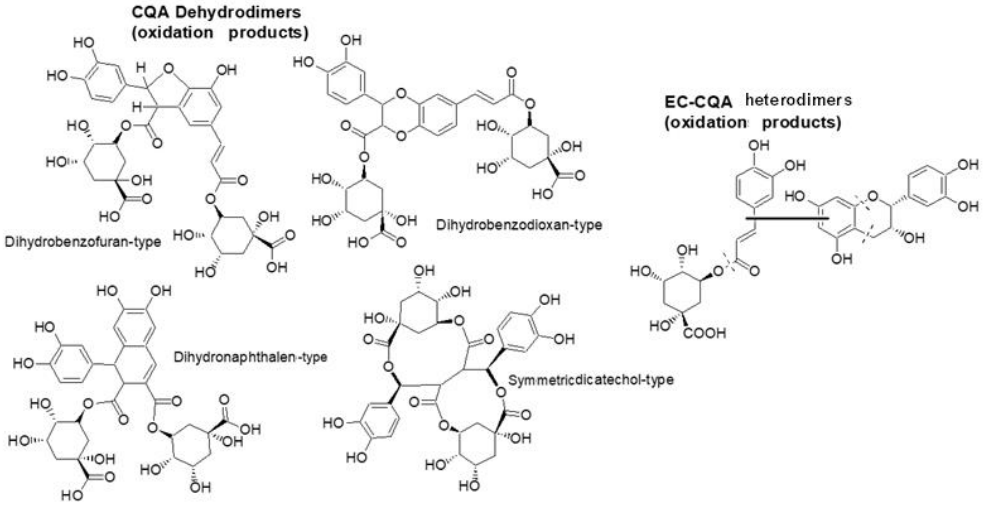

Figure 1: Families of oxidized phenolic compounds (OP) studied.

P.3.02 - A COMPERATIVE POLYPHENOLIC ANALYSIS OF DOMESTIC CITRUS GERMPLASM FROM GREECE

Sarrou E.*[a], Michailidis M.[b], Martens S.[c], Ziogas V.[d], Ganopoulos I.[a], Molassiotis A.[b]

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Citrus crops are a key pillar of the agricultural production of Greece as it occupies the first place in the list of exportable fruits. The country is considered as the third major producer of citrus fruits across Europe following Spain and Italy [1]. In Greece there are several indigenous citrus varieties/clones adapted to the climate conditions of the country also with remarkable taste characteristics and quality. Such germplasm is underlined as a new agricultural production model focused on products of high nutritional value obtained from small-medium size orchards.

In order to evaluate aspects of the nutritional value of the Greek citrus-germplasm, a comparative phytochemical analysis including polyphenols, carotenoids and primary metabolites content was applied in fourty-nine domestic genetic accessions belonging to different species of the Rutaceae family including some commercial citrus cultivars. A targeted Liquid Chromatographic (MS/MS, MRM) analysis [2] revealed hesperidin, narirutin, eriocitrin and quercetin glycosides as the major polyphenolic compounds identified in orange, lemon, and mandarin flesh tissue. Hesperidin content varied between 6500-13200 mg/100g dry weight among twenty-six orange cultivars, 2500-9500 mg/100g dry weight among thirteen lemon cultivars, and 4000-12000 mg/100g dry weight among ten mandarin cultivars. The content of narirutin followed the following tendency: orange > mandarin > lemon (identified in considerable lower amounts). In contrary, eriocitrin was a predominant metabolite (2800-4100 mg/100g dry weight) of lemons flesh tissue, while identified only in minor amounts in orange and mandarin flesh.

The analysis of the”nutr’tional characteristics of citrus flesh together with genomic characterization of the Greek germplasm via a Whole Genome Sequencing approach applied to Greek germplasm, can lead to commercial cultivars, laying the basis for the diversification of Greek-oriented citrus germplasm. This research was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T2EΔK-01318, MIS 5072531) entitled “Rescue, preservation and exploitation of native citrus genetic material via modern bio-analytic approaches” (Acronym: GoCitrus).

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[2] Multari S., Licciardello C., Caruso M. & Martens, S., *Food Research International*, *134*, 109228, https://doi.org/10.1016/j.foodres.2020.109228, 2020.

P.3.03 - METABOLOMICS SCREENING OF *VITIS* SP. INTERSPECIFIC HYBRIDS TO SELECT NATURAL INGREDIENTS WITH COSMETIC PURPOSES.

Ferrier M.[a,*], Abdallah C.[a], Garros L.[b], Malinowska M.A.[c], Gémin M-P.[a], Giglioli-Guivarc'h N.[a], Hano C.[b], Lanoue A.[a,*]

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Introducing natural ingredients using green chemistry practices is a major challenge in cosmetic industry to follow the market trend. Among the plants of cosmetic interest, vine products show a remarkable diversity of natural substances with high potential for the cosmetic and dermatological sectors. To date, research focuses on well-known compounds like *E*-resveratrol and *E*-ε-viniferin, however grapevine contains many bioactive polyphenols for which biological activities remains unknown. Furthermore, complex polyphenol-rich grape canes extracts display activities against oxidative stress [1] and skin aging through tyrosinase inhibition as well as in the delay of skin senescence by sirtuin activation [2].

The domesticated grapevine (*Vitis vinifera* L.) presents a huge varietal diversity with over 10,000 varieties worldwide. Today, UPLC-MS-based metabolomics coupled to multivariate statistics constitutes breakthrough approaches to harness the chemical diversity of large grape germplasm collections including hybrid interspecific producers (*V. vinifera* × *V*. sp.) [3].
In this context, polyphenol-rich grape cane extracts from 24 Euro-American interspecific hybrids were analyzed by UPLC-MS. Metabolic phenotypes based on the relative concentration in phenolics acids, flavonols, flavan-3-ols and stilbenoids have been established and the cosmetic potential of the corresponding extracts was investigated using several biological assays including antioxidant activities (DPPH, FRAP, CUPRAC, ABTS and chelation) and tyrosinase inhibition. Pairwise correlations were used to explore the relationship between relative levels of single compounds and biological activities.

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P.3.04 - SCREENING BASED ON THE (POLY)PHENOLIC PROFILE OF MEDITERRANEAN VEGETABLES

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There is increasing evidence that the adherence to the Mediterranean diet could improve overall health being. Fruits and vegetables have been extensively investigated as natural sources of phytochemicals. Among these, (poly)phenols exert a preventive effect against aging and several chronic diseases, such as cardiovascular diseases, cancer, metabolic syndrome, etc. Since their chemical structures influence bioactivities, a comprehensive characterisation of these key constituents is essential [1].
The aim of this research was to screen three green Mediterranean vegetables based on their (poly)phenolic profile, celery, chicory and mint, as a preliminary step towards selecting these vegetables as potential ingredients to enrich the polyphenol content of fruit-based beverages. High-performance liquid chromatography coupled to mass spectrometry (LC-MS) was selected for the detection of these structurally highly diverse compounds. An initial full scan analysis was conducted to identify the most characteristic (poly)phenolic compounds of each sample, scanning from 100 to 1000 m/z. Subsequently, multiple reaction monitoring (MRM) acquisition mode was applied to analyse a total of fifty-eight individual (poly)phenolic compounds. Among these, a total of forty (poly)phenols were found in, at least, one of the three plant-based matrices.
Mint was dominated by flavanones, specifically, rutinoside derivatives. Celery and chicory were abundant in hydroxycinnamic acids, found mainly as organic acid esters. Among the flavonoids, the predominant forms in celery were apigenin and luteolin glycosides, whereas chicory was abundant in kaempferol derivates.
Celery, chicory and mint are appropriated for the enrichment of fruit-based beverages due to their variety of (poly)phenols. Additionally, this work contributed to a qualitative and quantitative in-depth characterisation of their (poly)phenolic profile.
Acknowledgements: This research was funded by the Government of Navarra through the program “Industrial Doctorates 2021-2024”. I.A. Ludwig is supported by the Gobierno de Navarra (Departamento de Universidad, Innovación y Transformación Digital); Grant name and Reference: "Talento senior 2021 ANDIA”, 0011-3947-2021-000034.

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P.3.05 - MODEL POLYPHENOLS IN DEDUCTION OF THE PLANT AND POLYPHENOL EVOLUTION USING QUANTITATIVE AND STRUCTURAL TOOLS

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The diversity of tannins and other polyphenols is enormous, but we are lacking a reliable picture of their distribution in the plant kingdom and in the evolutionary plant tree of life. Obviously, the majority of species are chemically completely unknown but especially the true quantitative data is difficult to obtain among the structural data. Compound-specific quantitation standards should be used but either purification of compounds from complex mixtures, lack of commercial standards or structural complexity of the compounds are making this difficult. However, quantitative information might be essential for drawing the line between the phylogenetic classification and chemotaxonomy of plants, since only structural differences between i.e. hydrolysable tannins produced by diverged plant orders cannot explain the whole picture of the evolution of chemistry in the plant kingdom [1].

Compound-specific quantitative data from plant extracts is at least difficult if not even impossible to obtain utilizing classic quantitation assays of tannins or other polyphenols. Fortunately, modern liquid chromatographic, spectrophotometric and mass spectrometric methods can be used in both compound characterization and quantitation. [1] Although researcher needs to be aware of its limitations, such as ionisation characteristics of compounds, when comparing results between compound groups or single compounds.

A rough qualitative grouping of how hydrolysable tannins are distributed in plant kingdom in respect to Cronquist's phylogenetic classification system, instead of up-to-date flowering plant classification (APG IV), is already available [2]. However, our aim is to yield a new quantitative dimension, which is useful when tracking back the evolution of plants and polyphenol biosynthesis. In addition, structural details, such as species-specific linkages between hydrolysable tannin monomers, might clarify which plant species are the most advanced in polyphenol biosynthesis, how the most complex hydrolysable tannins are synthesised in plants or what kind of a polyphenol palette plants had 100 million years ago? In our poster, we show selection of model polyphenols per each central biosynthetic branch of different polyphenol groups (Figure 1).

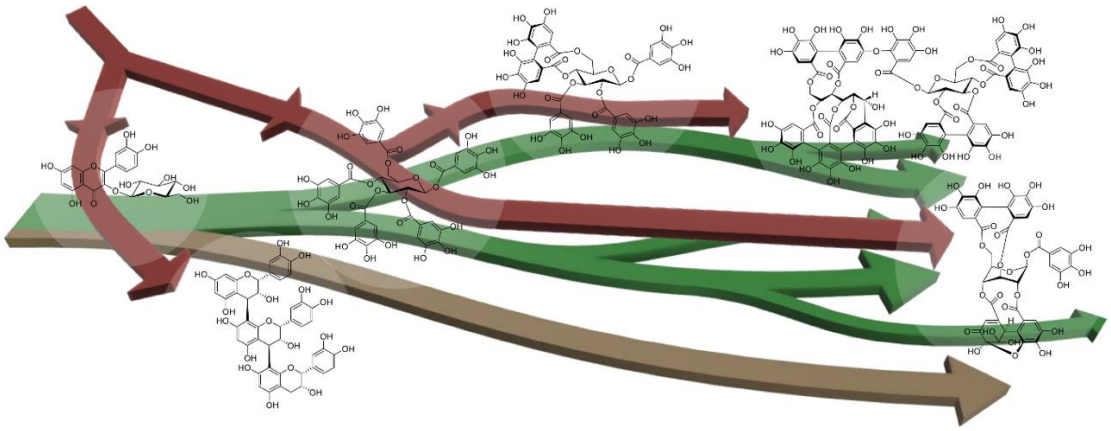

Figure 1: Quantitative data of model compounds along biosynthetic pathways (red arrow) and structural information improves our knowledge of evolution of plants (green and brown arrows) and polyphenol biosynthesis.

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P.3.06 - AN ELICITATION STRATEGY TO IMPROVE HEMP SECONDARY METABOLITES ACCUMULATION

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Hemp, *Cannabis sativa* L., is a commercial crop, well known for industrial, food, medical, and recreational applications. Its female inflorescences accumulate a large number of bioactive secondary metabolites (SMs), including cannabinoids, terpenoids and phenolic compounds. The content ratio between ∆-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) is conventionally used to distinguish between drug and fiber types. Nowadays, there is also a growing interest in compounds other than cannabinoids, which are associated with powerful health benefits and may act synergistically, contributing to the "entourage effects" of cannabis-based medicines. In plants, SMs usually occur at low concentrations and their synthesis is induced by adverse stimuli. A well-documented strategy to raise SMs content is the use of elicitors, molecules able to trigger the plant defence response. Specifically, the jasmonate-related phytohormones (jasmonic acid, JA, and methyl jasmonate, MeJA), have been reported to positively stimulate secondary biosynthetic pathways workflows, leading to increased production of various SMs.
The aim of this work was the optimization of SMs hemp productivity (total phenols, cannabinoids, terpenoids) by the use of two MeJA dosages, namely 1 mM and 10 mM, applied to female plants of a dioecious variety. Moreover, since the effect of the elicitor could even be increased by the use of nanoparticles as carriers, the MeJA treatment was also applied using chitosan nanoparticles (CHTnp). To this purpose, a small-scale field experiment has been organized with the following treatments: control, 1 mM MeJA, 10 mM MeJA, CHTnp (Mock) and CHTnp charged with 1mM MeJA. The inflorescence samples were harvested at two different sampling times, at early flowering and at full development. A biochemical characterization of inflorescences in terms of total phenol content, antioxidant capacity, CBD, THC and terpenoids content has been carried out.
The results showed that all the above cited biochemical parameters were affected by the elicitation treatments and by the sampling time. The elicitation with 10 mM MeJA resulted the most effective on all the parameters. In particular, this treatment gave rise to 17% increase CBDA content in early flowering inflorescences and to 78% increase in full developed inflorescences. Moreover, an interaction between treatment and sampling time was observed. A parallel RNA-Seq analysis looking at global gene expression changes is in progress, focusing on both biosynthetic and regulatory genes.

The research was supported by Regione Lombardia, MITICAL project, Bando 2018 per Progetti di ricerca in campo agricolo e forestale, d.d.s. n. 4403 del 28/03/2018.

P.3.07 - NMR METABOLOMICS AND DNA SEQUENCING OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* CULTURES TREATED WITH HYDROLYSABLE TANNINS

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Hydrolysable tannins (HTs) are a highly bioactive specialized metabolite group that are found widely in the plant kingdom including many nutritionally important food items. Promising activity data has been reported about the antibacterial, antiviral, anti-inflammatory, and antitumor capabilities of these compounds. HTs have shown efficient inhibition capacity against bacterial strains such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) [1], which are among the most prominent bacterial strains responsible for antibacterial resistance caused deaths. In our current study, we expanded the knowledge on what bacterial metabolome changes occur when *E. coli* and *S. aureus* cultures are inhibited by structurally different HTs after various culture growth times. Further effort was also placed on studying a more biomimetic bacterial setting with fecal samples included in the *S. aureus* growth medium. This setting was studied also via bacterial DNA sequencing to observe possible changes in sequenced bacteria proportions due to the tannin treatments.
We utilized NMR spectroscopy to perform untargeted metabolomics on the HT treated bacterial culture samples (*E. coli* and *S. aureus*) and the fecal fermentation samples [2]. Observable differences between the tannin treatments were witnessed in all three sets through multivariate analysis (Figure 1) and significant individual metabolites explaining the largest proportion of the variance were found. The bacterial metabolome changes caused by different HT structures were also in line with plated inhibition results. The most effective HT structures were found to contain hydrophobic as well as hydrophilic regions, i.e. both galloyl and HHDP type substructures, and also a larger molecular size was observed to increase effectiveness.

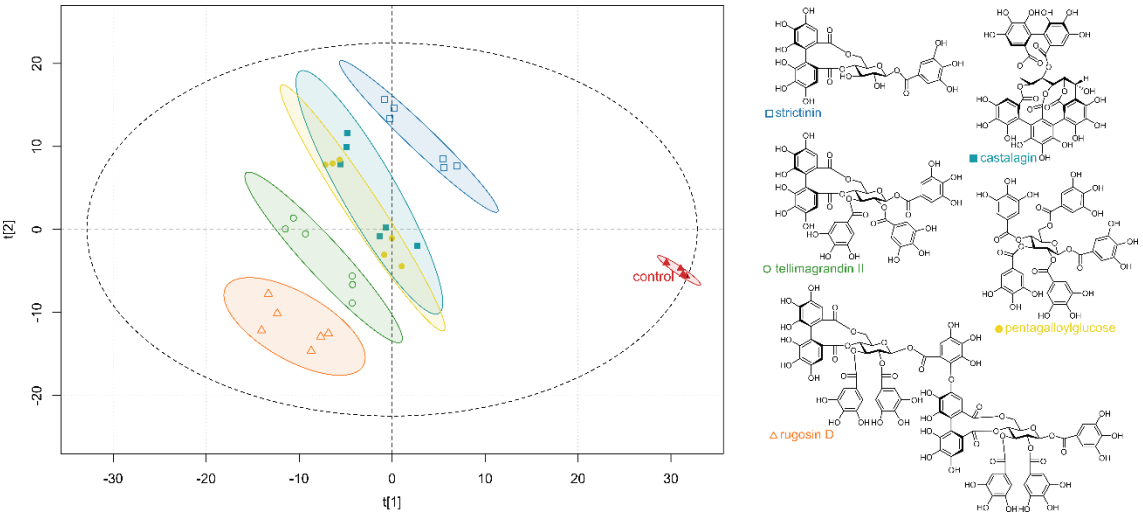

Figure 1: PCA score plot from the NMR metabolomics data of *E. coli* culture samples treated with different hydrolysable tannins after 24 hours of incubation. Groups are colored according to hydrolysable tannin treatment and controls.

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P.3.08 - CHEMICAL CHARACTERIZATION OF PROPOLIS SAMPLES COLLECTED IN BENIN AND CONGO GUIDED BY ^{13}C-NMR DEREPLICATION

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Bees use propolis, mixed with beewax and salivary enzymes, to seal and smooth out the internal walls of hives, as well as a protective barrier against fungal and bacterial infections. Propolis is a resinous natural substance collected by honeybees from buds and exudates of various trees and plants. Therefore, its chemical composition is geographically dependent. Propolis are generally classified as "poplar-type" in temperate zones and "green Brazilian- ", "Clusia-", "Macaranga- " or either Mediterranean-type in tropical zones. While flavonoids and phenolic acids are the major classes of compounds in propolis from temperate areas, tropical propolis, especially those from Africa, are often less well known. Though previous studies on West-African propolis associated new polyprenylated stilbenes with anti-trypanosomal activities (Ghana, [1]) or showed the presence of bioactive prenylated isoflavonoids in Nigerian samples [2] there was no report on chemical composition of Beninese propolis.
The aim of this study was to determine the chemical composition of several propolis samples collected in three different zones of Benin and Congo and to evaluate their antioxidant and/or anti-AGEs activities.
The phytochemical composition of EtOH extracts from eight batches collected in different regions of Benin and Congo was studied using coupled chromatographic methods (GC-MS, HPLC-DAD-ELSD and HPLC-MS). The association of a ^{13}C-NMR dereplication process using MixONat software and adapted databases allowed to characterize straightfully products in mixtures.
In addition to triterpenoids, one Beninese propolis sample exhibited an original composition with antioxidant methoxylated stilbenoids/phenanthrenoids when another contained anti-AGEs prenylated and geranylated flavanones. Finally, resorcinols and phenols derivatives were identified in the Congolese sample [3].

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P.3.09 - AUTHENTICATION OF SPANISH HONEYDEW AND BLOSSOM HONEY BASED ON THE POLYPHENOLIC CONTENT AND ANTIOXIDANT CAPACITY

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Honey produced by *Apis mellifera* is very popular in the human diet, not only because of its appreciated taste and nutritional characteristics but also because of their beneficial properties for human health, comprising antioxidant, anti-inflammatory, antifungal, and antibacterial activities. Although carbohydrates and water are the major components, most bioactivities are related to minor components, such as phenolic compounds, amino acids, or vitamins.

Honey attributes depend on a diversity of factors, including the type (honeydew and blossom honey), the botanical origin (rosemary, thyme, eucalyptus, heather, etc.), and the geographical origin. For instance, honeydew and blossom honey display different physicochemical properties, with the total phenolic content (TPC), the total flavonoid content (TFC), and the antioxidant capacity being some of the most important.

In this work, TPC, TFC, and antioxidant capacity by ferric reducing antioxidant power (FRAP) were determined for a set of 73 honeys (50 blossom and 23 honeydew honey). TPC values ranged from 0.1 to 0.7 mg gallic acid equivalents (GAE) per gram of sample, with honeydew honey displaying values significantly higher ($p < 0.05$) than those found for blossom counterparts. TFC indexes were above 1.5 mg quercetin equivalents per gram of sample for honeydew honey and heather honey. The maximum FRAP values were observed for thyme honey (2.2 mg Trolox equivalents g^{-1}), followed by honeydew and heather honey.

None of these indexes individually was able to differentiate all the honey classes considered in this study. Anyway, they presented different cross-sensitivities which provided complementary information to discriminate and authenticate honey based on the type and the botanical variety. For this purpose, chemometric methods, such as principal component analysis (PCA) or partial least square-discriminant analysis (PLS-DA), can be applied for a comprehensive and more efficient honey characterization using TPC, TFC, and FRAP data. Both honeydew and blossom honey types were satisfactorily discriminated, with an overall prediction error below 5%. In addition, all blossom honey kinds were assigned correctly according to their botanical origin by PLS-DA based on a two-class modeling classification tree approach.

P.3.10 - ASSESSMENT OF THE PLASMA AND URINARY METABOLITES AND PHENOLIC ACID CATABOLITES FROM MANGO PUREE POLYPHENOLS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY

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Mango (*Mangifera indica* L.) is a tropical fruit that attracts interest from commercial and nutritional perspectives as a rich source of bioactive compounds [1]. Mango (poly)phenols have relevance to intestinal health and in the prevention of chronic inflammatory diseases [2], including inflammatory bowel diseases [3]. However, as yet, little is known about the bioavailability of mango (poly)phenols or the metabolites/colonic catabolites form in vivo after ingestion nor how these effects are mediated by such bioactive compounds. Following acute consumption of 300 g of mango puree by 10 healthy volunteers, 0-24 h plasma and urine samples were analysed by targeted high-performance liquid chromatography−high resolution mass spectrometry in order to identify mango (poly)phenol metabolites and phenolic acid and aromatic catabolites. The UHPLC-HRMS method was validated for specificity, linearity, limit of detection and quantification, intra-day and inter-day precision, recovery and matrix effect, which were determined for 34 compounds in both urine and plasma matrices. After method validation, a total of 97 and 42 metabolites were determined in urine and plasma samples, respectively, over a 24 h collection period post ingestion of 300 g of mango puree (poly)phenols containing 193 µmol of which gallotannins together with gallic acid derivatives comprised the 36.2% (70.5 µmol) and other phenolic acids accounting for 55.4% (107.3 µmol). Phenolic acids derivatives including those from cinnamic acids, phenylhydracrylic acids, phenylpropanoic acids, phenylacetic acids, benzoic acids, mandelic acids, hydroxybenzenes and hippuric acids structures together with their sulfate and glucuronide derivatives were identified or partially identified in plasma and/or urine samples. These data provide a detailed evaluation of the fate of mango (poly)phenols as they pass through the gastrointestinal tract and are absorbed into the circulatory system prior to renal excretion.

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P.3.11 - APPLIED CHEMOMETRICS IN AN ACTIVE MOLECULAR NETWORK AS A COMBINED STRATEGY FOR THE RAPID IDENTIFICATION OF BIOACTIVE COMPOUNDS

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Natural products (NPs) are known for a wide range of very interesting biological effects. To avoid the repetitive isolation of previously described compounds and time-consuming bioassay-guided fractionation strategies, the early identification of active metabolites from complex mixtures has become a key element in NPs research [1].

Recently, the concept of bioactivity-based molecular networks (BMN), combining various statistical analysis methods with the mass spectrometry (MS) data, has been developed [2]. In this way, nodes representing potentially active molecules are quickly identified. Various dereplication tools are then used to determine whether these metabolites are known and have been described in the literature.

Based on this concept, a BMN workflow considering the variable importance of projection (VIP) score and the regression coefficient (RC) of each variable from a PLS model, was applied for the analysis of 14 fractions from a *Garcinia parvifolia* bark extract. It allowed the rapid identification of inhibitors of advanced glycation end-products (AGEs) [3]. The combined use of ^{13}C-NMR based dereplication (MixONat software) and MS data eventually improved the confidence level of the NPs annotation.

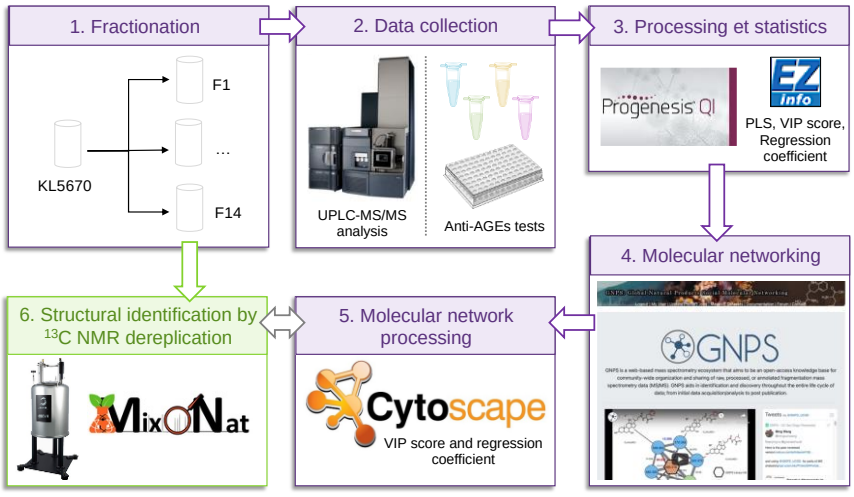

Figure 1: The workflow of the BMN approach integrating statistical data (VIP score and RC). (1) The crude bark extract KL5670 was fractionated into 14 fractions. (2) Fractions were analysed by UPLC-MS/MS and tested by an anti-AGEs assay. (3) Data were pre-processed by ProgenesisQI and exported to EZinfo for statistical treatment. (4) Pre-processed data were imported to the GNPS molecular networking web-platform. (5) The obtained MN was then formatted and merged with statistical data by using Cytoscape. (6) Based on this information metabolites of interest were analyzed by the MixONat ^{13}C NMR dereplication software. Finally target metabolites were isolated in order to confirm/disconfirm predicted structures and anti-Ages activities.

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P.3.12 - CHARACTERIZING THE UNIQUE PHENOLIC PROFILES OF DISEASE-RESISTANT GRAPES AND WINE

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In the context of climate change and based on their disease resistance and cold hardiness, there is a need for increased understanding of the chemical composition of interspecific hybrid grapes and wine in order to optimize winemaking strategies. Phenolic compounds play a particularly important organoleptic and quality determination role in wine, but can vary significantly in interspecific hybrid wines compared to wines produced from *Vitis vinifera* cultivars [1, 2, 3].
In this study, the phenolic composition of interspecific hybrid red winegrapes and wines (*Vitis* spp.), including both economically-relevant cultivars from regions around the United States and cultivars sourced from France, were characterized. These phenolic profiles were compared in the context of Pinot noir (*Vitis vinifera)* sourced from the USA and France, which is a wine cultivar known for its unique anthocyanin and phenolic composition.
Targeted fractionation of subfamilies of phenolics, including anthocyanins, low molecular weight phenolics, and polyphenolics was performed on the skins of winegrapes from cultivars (*Vitis* spp.) Vidoc, Coliris, Artaban, and Chambourcin, while targeted anthocyanin profiling was performed on wines from the cultivars (*Vitis* spp.) Maréchal Foch, Chambourcin, Norton, Baco noir, Léon Millot, Marquette, Frontenac, Noiret, Corot noir, Petite Pearl, St. Croix, Fredonia, Regent, and Ives. Samples were analyzed for total phenolic content, total tannin content, total anthocyanin content, and CIEL*a*b* color values. Phenolics extracted from grape skins using methanolic extraction were subsequently fractionationated via solid-phase extraction. Profiles of individual fractions were characterized using ultra-high pressure liquid chromatography (UPLC) and liquid chromatography-mass spectrometry (LC-MS) techniques. Preliminary results show a wide variety of phenolic compounds within each hybrid cultivar; notably, high contents of diglucosidic anthocyanins, non-malvidin anthocyanins, and acylated anthocyanins, and a complexity in non-anthocyanin phenolic fractions.
A more thorough understanding of the differences between the phenolic compositions of interspecific hybrid cultivars and those of *Vitis vinifera* sets the groundwork for further exploration of phenolic interactions over wine aging and the sensory impact of the unique phenolic profile within hybrid wines.

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P.3.13 - POLYPHENOLIC PROFILING BY LIQUID CHROMATOGRAPHY WITH UV DETECTION FOR TEA AUTHENTICATION AND ADULTERATION ASSESSMENT

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Tea (*Camellia sinensis*) is an aromatic plant native to East Asia that is currently cultivated in many other tropical areas of the world. The inflorescences and tender sprouts are collected and processed to obtain a range of products consumed worldwide to make infusions and refreshing drinks. A diversity of teas can be found in the market, often classified into five types according to their fermentation process as follows black, green, oolong, red, and white tea. Tea is quite susceptible to adulteration, simple, fast, and cheap control mechanisms are needed to detect possible adulterations and minimize fraudulent practices. Adulterations of a select tea may be due to the addition of other teas of lower quality or other types. In some cases, however, products from other plants, such as cereal starch, legume husks, and chicory (*Chicorium intybus*) are even added to increase the product volume at a reduced cost.

This work aims to develop a simple and effective methodology for polyphenolic profiling by liquid chromatography with UV detection (HPLC-UV), combined with chemometric methods to detect and quantify tea samples adulterated with chicory. For that purpose, seventeen phenolic acids and flavonoids typically occurring in tea were selected as the tea markers; their peak areas were used as the data of chemical descriptors. 120 samples belonging to the five varieties of tea (black, green, red, oolong, and white) and chicory were analyzed chromatographically, and exploratory studies were performed using principal component analysis (PCA). Besides, classification models were assessed by partial least square-discriminant analysis (PLS-DA). Finally, several adulteration cases were addressed by partial least square (PLS) regression based on adding different chicory percentages to each tea variety; Cross-contaminations of teas (e.g., red tea adulterated with an oolong tea) were also studied. Excellent results were obtained in all cases, with calibration, cross-validation, and prediction errors below 2.0%, 4.2%, and 3.9%, respectively. These results supported the performance of our proposal for the evaluation of tea fraud.

P.3.14 - CHEMICAL CHARACTERIZATION OF A WATER-SOLUBLE POLYPHENOL-RICH EXTRACT FROM CAROB PODS

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Carob trees (*Ceratonia siliqua* L.) are an endemic cultivar in the whole Mediterranean area. They are very important for animal and human nutrition because of their high nutritional value and the presence of many bioactive compounds (i.e., inositols, insoluble fiber, polyphenols, etc.) in their fruits, beside the relevant and very well-known carob seeds which are prime matter for locust bean gum production. Spain and Valencia are the main production areas of Carob fruits worldwide. Actually, the food and nutraceutical industries are producing a wide diversity of functional ingredients based on carob fruits and their health benefits on many diverse target functions, cardio-vascular protection, glucose-insulin metabolism, type 2 diabetes, fertility, gut health, anti-inflammatory activity, etc.

Carob pods are the seedless part of the carob fruit which contain 40-60 % of sugars, 42-52 % of total dietary fibre (of which 39-47 % is insoluble fibre), 16-21 % of insoluble highly-polymerized proanthocyanidins), 5-6 % proteins, 3-4 % of cyclic polyols, 2-3 % minerals, 0.5 - 1.5 % of soluble polyphenols and up to 1 % of fats (data expressed on dry matter basis). The phenolic fraction is much less known with respect to the rest of the carob pod constituents because of their very complex composition, as well as the botanical diversity/variation of the carob fruit (geographical locations, climate, soil composition, agricultural techniques, etc). Therefore, the aim of this work was chemical characterization of phenolic compound present in the ADM product Carob Soluble Polyphenols.

In this work, seeds-free carob pods were submitted to extraction with water at real industrial conditions of production at the factory of ADM in Valencia (Spain). The crude carob pod extract was clarified using membrane separation techniques. The obtained clarified carob extract did contain from 1.3 to 2.0 % of total polyphenols (expressed as gallic acid, DM basis, Folin-Ciocalteu method). Next, the polyphenol fraction was purified by using chromatographic tools that reduced drastically the presence of sugars and other interfering substances. The final polyphenol-enriched carob product was analysed by RP-HPLC-PAD-MS for its phenolic composition.

A total of 62 molecules were identified, of which 25 were identified unambiguously, 29 tentatively with very high confidence and the rest, with lower confidence. The main groups of identified phenolic compounds were (from higher to lower contents): flavonols, gallotannins and benzoic acid derivatives, flavanones, ellagitannins, cinnamic acid and derivatives, and flavones.

These analytical findings provide relevant information on the potential usefulness of the studied Carob Soluble Polyphenol product.

P.3.15 - IDENTIFICATION AND QUANTIFICATION OF MAJOR PHENOLIC COMPOUNDS FROM DIFFERENT STRAWBERRY GENOTYPES

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Strawberry (*Fragaria* × *ananassa*) is the most widely consumed berry fruit worldwide, with its consumption rising annually due to its exceptional sensory and nutritional characteristics. Numerous studies have been conducted on non-volatile compounds in strawberry, which contribute to its exceptional taste, its unique red colour and high nutritional value [1,2]. Among the wide variety of non-volatile compounds, polyphenols stand out, which have the ability to act as antioxidant and anti-inflammatory agents against many chronic diseases, highlighting the benefits of strawberry consumption for human health. In the present work, the major phenolic compounds present in different strawberry genotypes were identified and quantified using liquid chromatography coupled with mass spectrometry (LC-MS). A total of 26 phenolic compounds were detected after refining their separation on a polar C18 column testing a wide range of chromatographic conditions, of which 21 could be identified. The main phenolic categories included anthocyanins, proanthocyanidins, flavan-3-ols and hydroxycinnamic acid derivatives. Quantification of polyphenols was performed by constructing calibration curves with representative external standards for each category, *i.e.*, pelargonidin-3-*O*-glycoside, coumaric acid and epicatechin. The compounds present in the largest concentrations in most of the strawberry genotypes were found to be: cinnamoyl glucose, *p*-coumaroyl hexose and its isomer, pelargonidin 3-*O*-glucoside, catechin and two proanthocyanidins, which are dimers of catechin. Two additional peaks of high intensity were also detected in the chromatograms; however such compounds remained unidentified. Repeatability was acceptable (<15% relative standard deviation). The established method has been successfully applied for the evaluation of the polyphenolic composition of various strawberry genotypes.

Acknowledgment: This research has been co-financed by the European Union and Greek national funds through the Operational Program “Competitiveness, Entrepreneurship and Innovation”, under the call “RESEARCH – CREATE – INNOVATE” (T2EΔK - 01924).

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P.3.16 - KAFFIR LIME (*CITRUS HYSTRIX*) PEEL AS A SOURCE OF FUNCTIONAL INGREDIENTS

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Kaffir lime (*Citrus Hystrix)* is a popular citrus in Southeast Asia. The interest in the fruit is constantly growing, however mainly in the case of its very aromatic leaves, which are commonly used in Thai cuisine. Kaffir lime juice is not directly consumed because of its pungent taste. The peel of the fruit is also food waste. The study aimed to determine the main by-products of the peel, that may be used as functional ingredients in various branches of industry.
The contents of bioactive compounds such as: polyphenols (22.63 ± 2.12 mg GAE/g DW), flavonoids (2.72 ± 0.25 mg CE/g DW, vitamin C (2.43 ± 0.19 mg Asc), xantoproteins + carotenes (53.8 ± 4.24 ug), anthocyanins (24.8 ± 1.8 mg CGE/kg DW), chlorophylls A and B (188.5 ± 8.1, 60.4 ± 3.23 µg/g DW) were evaluated. CUPRAC and DPPH assays were also provided with the results of 76.98 ± 8.1, and 12.01 ± 1.02 µmol TE/g DW, respectively, to assess the antioxidant capacity of the peel. Determination of essential oil, obtained by hydrodistillation, showed the content of: sabinene (31.93%), β-pinene (26%), and limonene (19%). With the use of the MP-AES technique, nine microelements (Fe, Zn, Cu, Mn, Co, Ni, Cr, Mo, V), four macroelements (Mg, Ca, K, Na), and seven ballast substances (Cd, Hg, Pb, Al, V, Sr, Pt) were determined. The pectins extracted with the use of citric acid were characterized by the degree of methylation, DPPH free radical scavenging, galacturonic acid content, and DSC analysis.
Kaffir lime peel, as a citrus waste, is a promising raw material in many branches of the industry due to its high content of valuable by-products whose presence increases its dietary and therapeutic value.

P.3.17 - SHADES OF FINE DARK CHOCOLATE: POLYPHENOL METABOLOMICS AND MOLECULAR NETWORKING TO ENLIGHT THE BROWN FROM THE BLACK

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Fine-quality dark chocolates (70% cocoa content) have a dark brown color [1] that is partially influenced by phenolic compounds [2]. However, some of those chocolates have a light brown color, which is a challenge for manufacturers that opens new marketing strategies.
The aim of this work was to evaluate the phenolic profiles of dark chocolates having a black and brown color to reveal discriminating compounds. Sixteen fine chocolate samples, containing 70% cocoa, provided by Valrhona from thirteen different *Theobroma cacao* clones and years (2019 and 2020) were selected according to their dark black or light brown color. Non-targeted metabolomics approach based on UHPLC−HRMS/MS experiments, univariate and multivariate statistical analysis, as well as Feature-Based Molecular Networking analysis (FBMN) [3] was conducted (Figure 1). The analysis of differentially accumulated metabolites between brown and black chocolates showed an overaccumulation of 27 metabolites in black chocolates. Among them glycosylated flavanols and small glycosylated A-type procyanidins (dimers and trimers) were highly representative. On the other hand, 50 overaccumulated metabolites were found for brown chocolates. Among them, 27 larger B-type procyanidins, from trimers to nonamers, were present. *C*-glycosylated or oxidized dimers or trimers and dehydrodicatechins B also participated in the discrimination of the two sets of samples. Phenolic and color profiles were more related to genetic factors, *i.e.* the studied cocoa clones. However, environmental conditions may also have influenced the black chocolate color. This study provides new insights on the phenolic profiles of black and brown chocolates that may be useful to better understand the color variations of dark chocolates. Moreover, owing to their different phenolic profiles, these two types of chocolates may have different potential functional properties for human health.

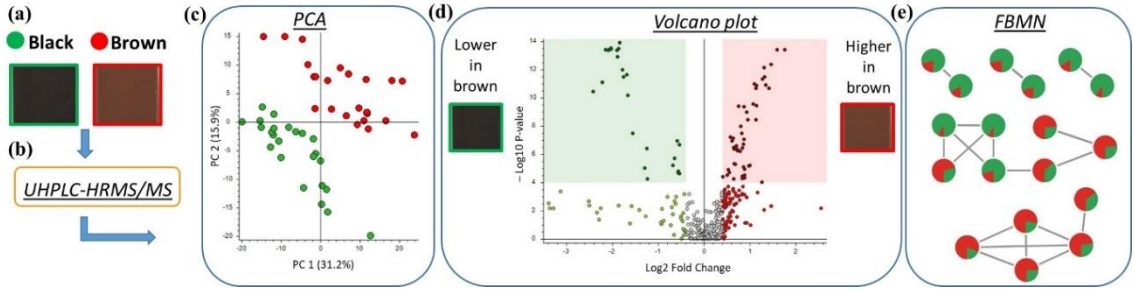

Figure 1: Chocolate samples (a), type of instrumental analysis (b), multivariate (c) and univariate (d) statistical analysis, as well as FBMN analysis (e) to reveal discriminating compounds of black and brown chocolates

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P.3.18 - THE SUGAR PUZZLE IN THE ANALYSIS OF HONEY PHENOLIC COMPOUNDS

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Honey is a sweet substance that bees, mainly *Apis mellifera* species, elaborate from blossom nectar and/or honeydew (exudates of plants or plant-sucking insects). Honey is a natural foodstuff produced worldwide, largely used as food and a source of antioxidants thanks to its phenolics. Carbohydrates are the major constituents of honey, comprising about 80 % (w/w) of honey. Honey also contains water (15–20%) and minor components such as minerals, proteins, free amino acids, vitamins, organic acids and phenolic compounds (flavonoids and phenolic acids). Honey composition depends strongly on the plant species from which the nectar or the honeydew is collected, and other factors such as pedoclimatic conditions and beekeeping practices (handling, processing and storage). Regarding that phenolic compounds are plant taxonomic markers, the analysis of honey phenolic profile is considered a very promising tool to determine the botanical and geographical origins of honey.

In general, the analytical procedure for the determination of the individual phenolic compounds in honey involves the dilution of the honey sample in acidified water (pH 2), the extraction of the analytes from the sample matrix and the separation, identification and quantification of the phenolic compounds by liquid chromatography coupled to a diode-array detector and a mass spectrometer (LC-DAD-MS). Direct injection of the filtered diluted honey sample into the chromatographic system has been suggested, but it is not recommended for routine analysis because the high sugar contents dirty the ionization source and inaccessible parts of the mass detector, leading to the loss of sensitivity, and gradually clog the chromatographic column, which is revealed by the increasing system pressure recorded after a relative small number of injections. Solid-phase extraction (SPE) is the most commonly used technique for honey sample preparation in order to remove matrix components such as carbohydrates. Different solid phases, i.e. Amberlite XAD-2, C18, polymeric Strata-X, Oasis HLB, etc., have been tested, and several SPE procedures have been optimised and proposed in literature to isolate and concentrate phenolic compounds from honey to be determined by LC-DAD-MS. These studies were focused on determining the optimal conditions for the extraction of the phenolic compounds, which were quantified by LC-DAD, but the content of sugars in the final phenolic extract was not evaluated. Sugars do not interfere in the UV-visible spectrophotometric detection of phenolics ($\lambda > 230$ nm) because carbohydrates have no chromophores in their molecules and they absorb UV light only at wavelengths below 200 nm. However, those SPE extraction procedures do not remove efficiently the sugars from the matrix, which results in the obstruction of the chromatographic column in the short-term.

In this study, a new method for the analysis of phenolic compounds in honey based on liquid-liquid extraction (LLE) and LC-DAD-MS was developed and validated. The LLE procedure was optimised to maximize the phenolic extraction and the elimination of sugars in the final phenolic extract. Sugars were monitored along the extraction procedure by ^{1}H-NMR and FT-IR. The extraction yield for phenolic compounds was higher than 80% and 99% of the sugars were removed. The proposed methodology allowed the extraction of phenolic compounds throughout the entire range of polarity, and is simple and can be operated easily.

P.3.19 - COMPARISON OF MACERATION, ULTRASOUND AND MICROWAVE ASSISTED EXTRACTION OF STILBENOIDS AND FLAVAN-3-OLS FROM GRAPE CANES USING DESIGN OF EXPERIMENTS

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Grape canes, as abundant viticultural byproducts, represent a rich source of bioactive polyphenols making them of high interest for human health, cosmetics and for the development of biocontrol agents in sustainable agriculture [1]. Previous studies describing the optimization of polyphenol extraction from grape canes were based on unspecific colorimetric method such as total polyphenol content (Folin−Ciocalteu test) or focused only on *E*-resveratrol and/or *E*-ϵ-viniferin [1]. However, grape canes contain many structurally diverse polyphenols including flavan-3-ols and numerous stilbenoids [2]. The goal of this study was to compare three techniques of extraction namely: maceration, ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE) using UPLC-DAD-MS/MS for an accurate quantification of metabolites as well as design of experiment (DOE) to optimize extraction conditions for each method. The studied factors of the different DOE were the ethanol/water proportion (25-95% of ethanol) in the solvent of extraction and the solid/liquid ratio (29.3 to 170.7 mg/mL). The present range of solid/liquid ratio was chosen to cover conventional extraction conditions up to industrial scale. The response variables were the nine major polyphenols accumulated in grape canes (i.e. catechin, epicatechin, *E*-piceatannol, *E*-piceid, *E*-resveratrol, ampelopsin A, *E*-ϵ-viniferin, hopeaphenol and vitisin B) as well as their total. Optimal temperature (80°C) and time of extraction (30 min) were previously determined.
Whatever the extractive technique, and according to the response surfaces, highest metabolite and total concentrations were obtained using 71-95% ethanol, except for *E*-piceatannol that was better extracted at 48-55% ethanol. Regarding the solid/liquid ratio, best results were obtained with 29.3 mg/mL for the three techniques, except for *E*-resveratrol that was better extracted with a solid/liquid ratio of 170.7 mg/mL using maceration and UAE. Among the three extraction techniques, best results were obtained using maceration and UAE especially with 84.7% ethanol and 50 mg/mL solid/liquid ratio.
In conclusion, despite the structural diversity of grape cane polyphenols, the present study showed that most of these compounds present close optimal conditions of extraction, regardless of the extraction technique. Additionally, we observed that maceration and UAE were more effective than MAE. For an industrial perspective of grape cane extraction, maceration has the advantage to be commonly used, low cost and less energy-consuming.

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P.3.20 - AN ORIGINAL NON-TARGETED LC-MS APPROACH FOR POLYPHENOL OXIDATION PRODUCTS ELUCIDATION IN APPLE JUICE.

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Apples and derivate products are rich in polyphenols, the consumption of those specialized metabolites has potential health benefits. Through apple juice processing (crushing pressing), the oxidation naturally occurs caused by the contact of the plastidial polyphenol-oxidase (PPO) and its vacuolar phenolic substrates. Coupled to oxygen presence, the PPO catalyzes the oxidation of apple native polyphenols to numerous oxidation products (OPs) with specific structures and functional properties. Organoleptic (color, bitterness, astringency, turbidity) and nutritional apple juice qualities might therefore be modified by oxidation processes. To better understand its mechanism and consequences on juices and ciders, it is necessary to know the molecules formed by oxidation.

In contrast to previous works dealing with targeted analysis of OPs on the basis of oxidative reactivity of polyphenols [1,2], the present study aims to set up a standardized non-targeted approach for detecting OPs in juices of different apple varieties depending on the amount of oxygen consumed.

Juices from five cider apple varieties were prepared under anoxic conditions. They then underwent a controlled oxidation by cumulative additions of oxygen reached thanks to an experimental prototype. Thus, five oxidation levels (null to maximum) were analyzed for each experiment by HPLC-UV-MS. Samples were injected before and after depolymerization by phloroglucinolysis to characterize both native and oxidized procyanidins. Raw mass spectrometry data were processed using Galaxy Workflow 4 Metabolomics (W4M) to obtain the full ions list.

An original workflow was developed using a four steps data filtering to retain OPs related ions. A linear regression model (polynomial 2nd order) was applied to each ion, variety by variety to fit the intensity of each ion to the cumulative injected oxygen. The model enabled (i) the collection of all ions that responded to oxidation, (ii) the removal of weak oxygen dependent ions, (iii) the selection of ions that increase with the supply of oxygen (OPs). Then the last step (iv) used a hierarchical agglomerative clustering to annotate ions (retention time, molecular ion, isotopes, fragments, adducts, stacking) into “molecular signals”. This approach enabled the identification of 32 OPs previously identified by targeted approaches and 22 potential OPs that had not been detected before.

The presented non-targeted approach associated to quantitative sorting is strengthened through the detection of already known OPs and even provided supplemental information about OPs. Ultimately, the approach will contribute to the deep understanding of oxidations processes and their effects on the juice quality and composition.

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P.3.21 - VALIDATION OF A QUANTIFICATION METHODS FOR THE CROWN PROCYANIDIN: TETRAMERS, PENTAMERS AND OTHERS OLIGOMERS

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Grapes condensed tannins play a major role in the organoleptic properties and quality of red wine. Recently, a new sub-family of macrocyclic condensed tannins has been identified in red wine and name crown tannins. Indeed, the first compound identified and characterized by NMR was the crown procyanidin tetramer which is composed of a macrocyclic structure composed of four (-)-epicatechins with a central cavity in the molecule [1]. Moreover, this crown procyanidin tetramer exhibit of very specific characteristics and physico-chemical properties compare to the non-linear condensed tannins [2]. Regarding their location, the crown tannins found in wine come only from the skin of grape berries. Furthermore, these crown tannins showed a certain stability during red wine ageing due to some specific resistance to oxidation, which drastically differs from non-cyclic tannins which decrease over time and are sensitive to oxygen. Moreover, together with the crown procyanidin tetramer, a lot of other crown tannins has been identified by UPLC-UV-QTof such as galloylated tetramer, pentamer, ….

In view of the different physico-chemical properties of crown tannins and non-cyclic condensed tannins, a quantification method for all the crown tannins derivative (tetramers, pentamers and other oligomers) was developed by UPLC-UV-Q-Tof in order to study and quantified then in various matrix and estimate their involvement in the red wine colloidal matrix of. Several parameters were taken into account and evaluated for the validation of the method based on the OIV validation guide, namely: linearity, minimum detection limit, limit of quantification, precision and accuracy. In this study, the validated quantification method was then applied on several wines samples from different origins in the Bordeaux region and from different vintages.

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P.3.22 - A METABOLOMIC INSIGHT INTO THE PHENOLIC PROFILE OF CEREAL CROPS

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Phenolic compounds (PC) have been highlighted due to their potential bioactivity in health [1]. These compounds are naturally present in plants, such as the Gramineae family. Cereal grains are one of the main staple foods worldwide and represent a rich source of nutrients and PC [2]. The objective of this work was to investigate the phenolic diversity in whole cereal grains and their coproducts based on an untargeted metabolomic approach. In this work, a total of 69 samples were analysed consisting of four milling fractions (husk, bran, flour, wholemeal), 23 genotypes, and 7 cereal grains (barley, millet, oat, rye, sorghum, triticale, wheat). Free and bound PC were extracted from ethanolic solution, and then after alkaline and acid hydrolyses, respectively [3]. Samples were analysed in an UHPLC-ESI-QTOF-MS (Waters) using a C18 column (Waters). The data were acquired in DDA mode (data-depending acquisition) using MassLynx (Waters), processed by MZmine v. 2.53, and exported to Sirius, GNPS (Feature-Based Molecular Networking, FBMN), and Cytoscape for annotation and construction of the molecular networking. Overall, 7,674 nodes have been found by FBMN analysis, divided into single nodes or clusters. Among them, 16 clusters were found presenting 4 nodes or more and from these, 11 clusters were noticed as the main molecular networking (the first line, Fig. 1). Different classes of PC and their derivatives have been characterized, in glycosylated or aglycone form, such as hydroxycinnamic acids (e.g.: ferulic acid, diferulic acids, chlorogenic acids), hydroxybenzoic acids (e.g.: 4-hydroxybenzoic, dihydroxybenzoic acids), flavanols (e.g.: catechin and its dimers, procyanidins), and flavonols (e.g.: kaempferol and quercetin). Sorghum, millet, barley, and oat were the most noticed species in some clusters, especially related to the bran fraction. However, depending on the molecular networking, all the species shown a similar profile. A comprehensive insight into the phenolic profile of milling fractions of different cereal crops was provided through the untargeted metabolomic approach. These findings corroborate the potential of whole grains and coproducts as a source of bioactive compounds and can contribute to update the MS-based databases.

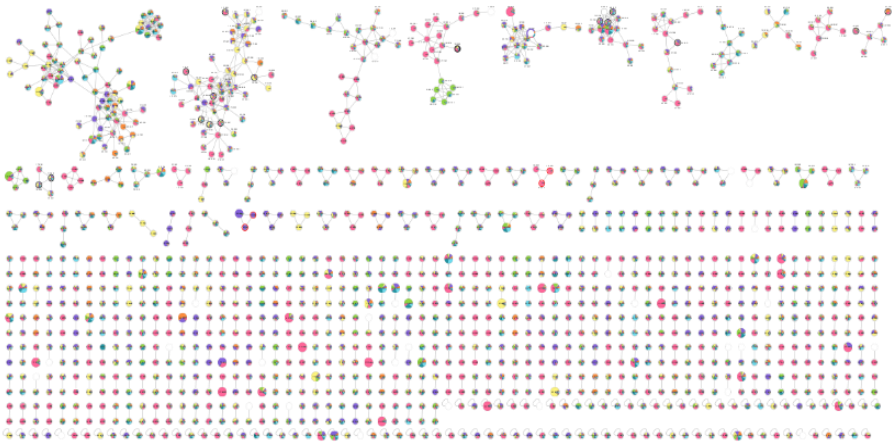

Figure 1: FBMN Clusters corresponding to compounds of cereal samples.

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P.3.23 - UHPLC-MS/MS-BASED METABOLOMICS TO ASSESS GENOTYPE AND CROP EFFECTS IN THE SYNTHESIS OF PHENOLIC COMPOUNDS DURING SORGHUM GRAIN MATURATION

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Sorghum [*Sorghum bicolor* (L.) Moench] ranks as the fifth important cereal crop, is drought tolerant and represents a gluten-free and rich source of phytochemicals, especially phenolic compounds (PC) [1]. PC concentration and composition may vary during grain development, crop and genotype [2]. Therefore, the aim of this study was to apply a high-resolution metabolomics tool to analyze how genotype and crop influences the synthesis of PC during sorghum grain growth. Sorghum grains from two genotypes: IS15752 and Macia, respectively rich (R) and poor (P) in tannin were field-grown in Montpellier (France) in two crop years (2017 named as C1 – for sorghum R and P; and 2018 named as C2 – for sorghum P). All samples were harvested at five different stages of grain growth (from cellular division, grain filling, physiological maturity and mature, S1-S5). Free (FPC) and bound (BPC) phenolic compounds were sequentially extracted and analyzed by UPLC-ESI-QTOF-MS^E in negative ion mode [3]. Globally, 97 PC were annotated, being 36 PC found in common to both genotypes. Rich-tannin sorghum (R) showed 43 exclusives PC, against 18 exclusively found in poor-tannin sorghum. The free/bound PC ratio (r=2.6) was higher in the P than in R sorghum (r=1.9), indicating that this rich-tannin genotype presents more complexed phenolics. Flavonoid was the major class in R (59%) while P included more phenolic acids (up to 41%), indicating the genetic variability of the PC traits in sorghum. Throughout the sorghum grain maturation, the contrast between genotypes was also observed. Although P presented higher values of FPC, these compounds showed a decrease (-60%) from S3 to mature, while R showed a sharp increase by 15-fold times from S3. Crop effect was also observed for P genotype, from 54 annotated PC: 36 were common to both crops, while 15 PC were exclusive to C2 and only 3 to C1. Total ion abundance of annotated PC was higher for C2 than for C1, but there was no change in phenolic classes when comparing the crops. Finally, principal component analysis was able to explain 72% of data variability and showed a clear distinction between the genotypes. The y-axis (23%) was able to separate the P genotype samples into immature (S1-S3, upper quadrant) and mature (S4-S5, lower quadrant). There was a low variability in the phenolic profile between the studied crops, the genotype and tannin effects were more evident. This study revealed for the first time the phenolic accumulation based on metabolomics during sorghum grain development and confirmed the hypothesis that genetic variability influences the PC content. This work can contribute to the input of open databases of phenolic compounds and to stimulate the selection of sorghum genotypes based on their bioactive potential.

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P.3.24 - CHEMICAL CHARACTERIZATION OF PROPOLIS SAMPLES COLLECTED IN BENIN AND CONGO GUIDED BY ^{13}C-NMR DEREPLICATION

Azonwade F.[a], Mabanza-Banza B.[b], Le Ray A-M.[c], Bréard D.[c], Blanchard P.[c], Goubalan E.[a], Baba-Moussa L.[a], Banga-Mboko H.[b]; Richomme P.[c], Derbré S.[c], Boisard S.*[c]

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Bees use propolis, mixed with beewax and salivary enzymes, to seal and smooth out the internal walls of hives, as well as a protective barrier against fungal and bacterial infections. Propolis is a resinous natural substance collected by honeybees from buds and exudates of various trees and plants. Therefore, its chemical composition is geographically dependent. Propolis are generally classified as "poplar-type" in temperate zones and "green Brazilian- ", "Clusia-", "Macaranga- " or either Mediterranean-type in tropical zones. While flavonoids and phenolic acids are the major classes of compounds in propolis from temperate areas, tropical propolis, especially those from Africa, are often less well known. Though previous studies on West-African propolis associated new polyprenylated stilbenes with anti-trypanosomal activities (Ghana, [1]) or showed the presence of bioactive prenylated isoflavonoids in Nigerian samples [2] there was no report on chemical composition of Beninese propolis.
The aim of this study was to determine the chemical composition of several propolis samples collected in three different zones of Benin and Congo and to evaluate their antioxidant and/or anti-AGEs activities.
The phytochemical composition of EtOH extracts from eight batches collected in different regions of Benin and Congo was studied using coupled chromatographic methods (GC-MS, HPLC-DAD-ELSD and HPLC-MS). The association of a ^{13}C-NMR dereplication process using MixONat software and adapted databases allowed to characterize straightfully products in mixtures.
In addition to triterpenoids, one Beninese propolis sample exhibited an original composition with antioxidant methoxylated stilbenoids/phenanthrenoids when another contained anti-AGEs prenylated and geranylated flavanones. Finally, resorcinols and phenols derivatives were identified in the Congolese sample [3].

[1] Almutairi S., Eapen B., Chundi S.M., Akhalil A, Siheri W., Clements C, Fearnley J., Watson D.G. & Edrada-Ebel R., *Phytochemistry Letters*, 10, 35-39, 2014.
[2] Zhang T., Omar R., Siheri W., Al Mutairi S, Clements C., Fearnley J., Edrada-Ebel R. & Watson D.G., *Talanta*, 120, 181-190, 2014.
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TOPIC 4: PROCESSING, SENSORY PROPERTIES, SAFETY & REGULATORY

PL.4.01 - OXIDATION OF PHENOLICS IN FOOD PROCESSING: BLESSING OR CURSE?

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Enzymatic browning, catalyzed by polyphenol oxidase (PPO) is a well-known phenomenon upon processing of plant materials. In recent years, with the transition from animal-based to more plant-based proteins, it has gained relevance as oxidation of phenolics by PPO might also affect protein functionality besides discoloration of the protein isolates. In addition to enzymatic oxidation, also autoxidation of phenolics (i.e., in ready-to-drink green teas) and complexation of phenolics by metal ions (i.e., upon iron-fortification of foods) can occur, both leading to undesirable colors in food products. Nevertheless, there are also examples indicating that oxidation of phenolics in food materials is actually desirable. For instance, the conversion of monomeric green tea leaf catechins to dimeric (e.g., theaflavins) or oligomeric phenolics by PPO is responsible for the color of black tea. Other examples might be the oxidative coupling of phenylalkenoic acid amides to hordatines (barley) or avenanthramides (oat) by peroxidase. These oligomerized phenolics potentially have antimicrobial activity, and might be used as clean label food preservatives, once their potency as antimicrobials and toxicity profiles have been established. Finally, laccases can be used as valuable tools for oxidative degradation of lignin, which facilitates better utilization of plant biomass. The challenges in mitigating the undesirable oxidation reactions, and directing the desirable ones, are discussed against a backdrop of industrial applications.

PL.4.02 - POLYPHENOLS IN HUMAN NUTRITION: REGULATORY ASPECTS AND RISK OF NOVEL FOOD CLASSIFICATION

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Polyphenols are commonly found in botanicals and explain at least in part the beneficial health effects of plants. For instance, among the most well-known polyphenols, curcumin is involved in the anti-inflammatory and anti-arthritic properties of turmeric (*Curcuma longa* L.), whereas the main bioactives of green tea (*Camellia sinensis* L.) are polyphenols, and notably epigallocatechin gallate (EGCG). Due to their nutritional or therapeutic benefits, polyphenols have been well-studied. However, the regulatory aspects of the use of polyphenols for human nutrition must be considered. Polyphenols and plant extracts may be considered in Europe as Novel Foods, i.e. ingredient which have not been consumed in Europe before the 15 May 1997. Novel foods can be authorized only after the evaluation of a scientific application by the European Food Safety Authority (EFSA) and the publication of a regulation indicating the conditions of uses and the main characteristics of the ingredient by the European Commission. Even if polyphenols are consumed daily through fruits and vegetables, purified polyphenols may be considered as new ingredients. This has been the case for instance of *Ecklonia cava* phlorotannins, purified EGCG from green tea leaves, or flavonoids from licorice. Polyphenols may also be used as food additives, either for their colouring properties, or as antioxidants. In the US, the situation is identical, with GRAS, NDI or food additive applications needed for these compounds.

Authorization of polyphenols as active food ingredient or as food additive requires specific applications, according to the current European Regulations. Such applications should contain all data related to the production process and characterization of the polyphenol(s), but also safety data substantiating the lack of safety concerns in the target population. Only a complete safety evaluation by EFSA can open the door to a use of new polyphenol(s) as foods ingredient.

O.4.01 - INFLUENCE OF GRAPE FLESH ON THE RETENTION OF TANNINS FROM SKINS AND SEEDS

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One of the main challenges in red wines made from cold-hardy hybrid grapes (*Vitis* spp.) is their low tannin concentration, which negatively affects wine quality. Although the extraction of tannins from grapes can be facilitated by the disruption of cells, the adsorption of tannin to macromolecules (such as proteins and pectins) affects their retention rate in finished wines. The goal of this study was to determine the impact of grape flesh, on the extractability and retention of tannins from skins and seeds. Red grape cultivars, Pinot noir (*V. vinifera*), and Marquette (*V. spp*.), were selected because of the different composition and concentration of tannins and anthocyanins. After being peeled, each respective grape tissues (skin with/without flesh, and seed with/without flesh, and a combination of skin, seed, and flesh) were soaked in a wine-like solution for five days. Tannins and monomeric anthocyanins in supernatants of each condition and time point were quantified by HPLC-DAD/FLD. The composition of tannin was analysed by HPLC-DAD after acid-catalysis in the presence of an excess of phloroglucinol.

After five days of soaking, the tannin concentration was the highest in the supernatant of Pinot noir seed condition (Fig. 1). This concentration significantly decreased in the presence of flesh, up to 91 and 93% decrease in Pinot noir skin and seed conditions, respectively, and up to 66 and 81% decrease, in Marquette skin and seed conditions, respectively. A lower mean degree of polymerization of tannins in supernatants of Pinot noir skin, seed, and flesh compared to skin, suggested that larger tannins bind to macromolecules from flesh, as previously observed [1]. Marquette grapes are rich in anthocyanins mono- and di-glucoside, and this characteristic is being explored to explain the decrease of tannins in skin with flesh condition. The composition of anthocyanins, cell wall polysaccharides, and soluble proteins, and binding affinities between those macromolecules need to be evaluated to deeply understand the factors affecting tannin retention in wines made from non- *V. vinifera* grape cultivars.

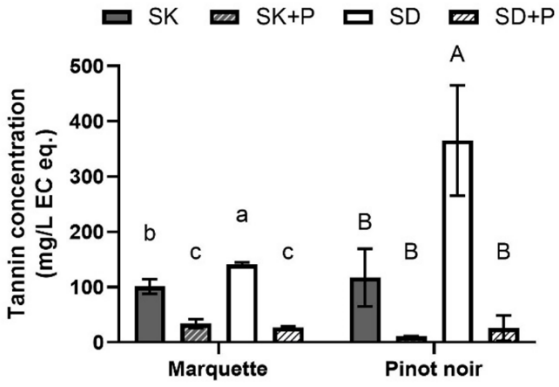

Figure 1: Tannin concentration (mg/L) in supernatants of skin (SK), skin with flesh (SK+P), seed (SD), and seed with flesh (SD+P) after five days of soaking in a wine-like solution. Lowercase and uppercase letters indicate a significant difference ($p < 0.05$) among treatments when compared in Marquette and Pinot noir tissues, respectively.

[1] Bindon K., Kassara, S. & Smith, P. *Australian Journal of Grape and Wine Research*, 23, 22–32, 2017.

O.4.02 - REVERSIBLE HAZE CAUSED BY SELF-AGGREGATION OF OXIDISED PROCYANDINS IN AN APPLE-BASED BEVERAGE

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Physicochemical hazes that appear during storage of clear apple-based beverages are a concern for producers. Since the turbidity are among determinant quality parameters of this beverage, there is a need to better understand the aggregation phenomena that lead to the formation of visible aggregates.

Among apple-base beverages, pommeau is a traditional mistelle obtained by adding apple spirit to apple must, subject to haze formation. Pommeau is also a good example of polyphenols and ethanol rich system but poor in proteins and polysaccharides and, in these respects, similar to Port wines.

The effect of thermal stress was first investigated on turbidity of Pommeau. Samples were subjected to temperature drop-jump cycles and studied by DLS. Results showed that the formation of Pommeau haze was reversible upon heating, revealing a non-covalent nature of the interactions.
The composition of pommeau haze was analysed in order to determine which families of compounds are responsible for their formation. The results revealed that procyanidins were the main polyphenols quantified in pommeau hazes [1]. A part of the haze remained unexplained by the analyses. However, several markers of the oxidised polyphenols were identified in haze suggesting that oxidation of phenolic compounds contributes to its formation in pommeau.
Model solutions were designed to mimic pommeau hazes in order to decipher the mechanisms by which oxidised phenolic compounds contribute to the formation of haze [2]. Oligomeric procyanidins alone have the ability to self-aggregate after oxidation in model solution and the reversibility upon heating was very similar to that observed for real pommeau. The degree of polymerization as well as the level of oxidation determine the kinetics of aggregation. Chemical markers of procyanidins oxidation were also investigated in the model system using LC-MS analyses revealing that both intra and intermolecular oxidative coupling were involved in the formation of aggregates.
These findings allowed to support the dominant role of oxidized phenolic compounds in formation of haze in Pommeau, and probably in other similar drinks [3]. This knowledge has been also used to improve curative or preventive treatments for colloidal instability.

[1] Millet M., Poupard P., Le Quere J.-M., *et al.*, *Journal of Agricultural and Food Chemistry*, 65, 6404-6414, 2017.
[2] Millet M., Poupard P., Guilois-Dubois S., *et al.*, *Food Chemistry*, 276, 797-805, 2019.
[3] Bauduin R., Poupard P., Cottereau, P., *et al.*, *Innovations Agronomiques*, 79, 49-59, 2020.

O.4.03 - REACTIVITY OF HYDROXYCINNAMIC ACIDS IN NON-ENZYMATIC BROWNING REACTIONS – INHIBITING OR PROMOTING EFFECT ON COLOR FORMATION?

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Food browning results from enzymatic- or non-enzymatic conversion of food ingredients such as carbohydrates, amino components, and phenolic compounds. It is well known that these reactions significantly impact the quality of food and thus, consumer acceptance. *Enzymatic browning* is caused by enzymatic oxidation and polymerization of phenolic compounds, mostly leading to regular polymers, namely *melanins*. The enzymatic conversion of phenol-rich ingredients is critical during harvesting, storage, and processing, because it leads to undesired changes regarding the food quality of fresh fruits and vegetables [1]. On the other hand, heat-induced *non-enzymatic browning* reactions are vital in modern food processing, primarily resulting in desired changes of sensory properties, texture, and color of food [2]. These comprise a vast number of possible reaction pathways that lead to the formation of complex copolymers [3]. Consequently, exact chemical structures of high-molecular-weight end products formed by non-enzymatic browning of carbohydrates and amino compounds, defined as *melanoidins*, are mostly unknown. Studies investigating the involvement of phenolic compounds in such reactions are limited, even though these were also identified as constituents of melanoidins in plant-based goods, most prominently, coffee.

The present study aimed at characterizing heat-induced reactions of the prominent hydroxycinnamic acids caffeic acid and ferulic acid in combination with key α-dicarbonyl intermediates of the MAILLARD reaction, namely glyoxal, methylglyoxal, and diacetyl. The reactivity of these compounds was characterized by analyzing the color formation (*Vis* photometry) and the conversion of the reactants (HPLC-DAD) after heat treatment under roasting conditions at 220 °C for up to 10 min. The structural composition of heterogenous reaction products was elucidated by (multiple stage) high-resolution mass spectrometry.

Overall, the highest reactivity resulted for binary model systems containing carbonyl compounds carrying an aldehyde group, more precisely, glyoxal and methylglyoxal. This was reflected in a synergistic color formation and a highly increased conversion rate of the reactants. Aromatic electrophilic substitution reactions, nucleophilic additions, and aldol reactions were identified as key reaction steps leading to the formation of colored, heterogenous oligomers. The present study provides novel insights into the heat-induced, non-enzymatic formation of phenol-containing colorants which could be considered as precursors of phenol containing melanoidins formed in roasted plant-based goods like coffee or cocoa.

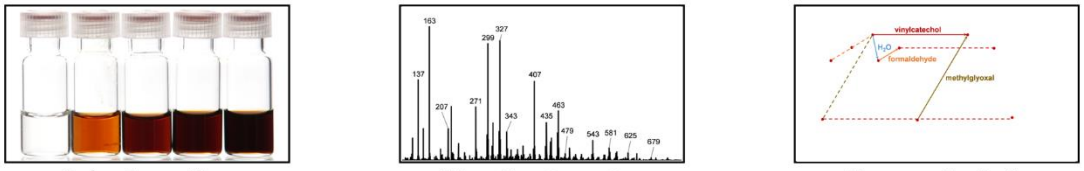

Figure 1: Characterization of Novel Colorants formed by incubation of phenolic acids with α-dicarbonyl compounds.

[1] Martinez M. V. & Whitaker J. R., *Trends in Food Science & Technology*, 6, 195–200, 1995.
[2] Liu S., *et al.*, *Frontiers in Nutrition*, 9, 973677, https://doi.org/10.3389/fnut.2022.973677, 2022.
[3] Kanzler C., Wustrack F. & Rohn S., *Journal of Agricultural and Food Chemistry*, 40, 11960–11970, 2021.

O.4.04 – CONTINUOUS FLOW HIGH-PRESSURE HOMOGENIZATION RETAIN ANTHOCYANINS AND MAINTAIN BLUEBERRY JUICES QUALITY

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Blueberry is one of the rich dietary sources of diverse bioactive compounds including anthocyanins that significantly contribute to its antioxidant activity. Several studies have shown that dietary intake of blueberry can provide protective effects against tumorigenesis, inflammation, and cardiovascular diseases. Consumers desire minimally processed foods enriched with nutrients, flavor, free of additives, and safe to consume. Therefore, fast and efficient processing techniques are well accepted by consumers to maintain the sensory and nutritive qualities of food products. The impact of high-temperature short-time (HTST) and continuous flow high-pressure homogenization (CFHPH) on blueberry juice quality parameters were investigated. In CFHPH, extracted juice was treated at different pressure, inlet temperature, and flow rates. The quality of processed blueberry juices was analyzed periodically at 0, 15, 30, and 45 days of storage for physicochemical parameters, and polyphenol oxidase (PPO) and peroxidase (POD) enzyme activities. Results demonstrated that a significant reduction was observed in POD and PPO enzyme activities in CFHPP treatment juices. Characterization of anthocyanins was performed by ultra-high-pressure liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-HR-QTOF-MS). Results demonstrated that blueberry juice comprises thirteen different major anthocyanins that were predominantly mono-glucosides and galactosidase of five anthocyanidins, namely, delphinidin, cyanidin, petunidin, peonidin, and malvidin. The total anthocyanins content on the first day of treatment ranged from 43 to 168 mg/100 g of blueberry juice depending on the treatment type. In general, individual anthocyanins increased with pressure and the retention of anthocyanins was higher in CFHPH treatment compared to HTST blueberry samples during storage. This presentation will also include the evidence for health-promoting effects of polyphenol-enriched plant extracts/ products and purified compounds.

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OY.4.01 - EFFECT OF ENDOGENOUS TANNINS ON LIPID OXIDATION IN OIL-IN-WATER EMULSIONS STABILIZED BY SORGHUM PROTEIN INGREDIENTS

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Plant-based foods are highlighted nowadays driven by the perceived health and environmental benefits, in line with the “clean label” tendency. Sorghum is a versatile grain and has the ability to adapt to dry and hot climates, being a good natural alternative for use in foods. Some sorghum cultivars present tannins in their composition and, although these molecules can bind proteins, making them considered antinutritional factors, their presence could inhibit or delay the oxidation of polyunsaturated lipids, a major deterioration process in multiphase food systems. The oxidative stability of model systems such as oil-in-water (O/W) emulsions is thus a research topic of great importance, but the relationship between lipid oxidation and the effect of endogenous polyphenols present in plant protein extracts used to stabilize such emulsions remains unclear.

In this context, this work aimed to identify and quantify the flavanols present in sorghum proteins extracted from tannin-rich cultivars and their effect on lipid oxidation in O/W emulsions in comparison to tannin-free ones. Simple polyphenols were extracted from freeze-dried sorghum protein extracts using acidified methanol, and analyzed by HPLC. Quantification of total flavanols, including condensed tannins (*i.e.* procyanidin oligomers and polymers), and the calculation of their average degree of polymerization (DPn) were performed after acidolysis in the presence of phloroglucinol and analysis of the reaction media by UHPLC. The formation of primary (conjugated dienes and hydroperoxides) and secondary (thiobarbituric reactive substances, including malondialdehyde) oxidation products, and the tryptophan fluorescence were monitored during incubation of emulsions at 40 °C in the presence of iron.

Total polyphenol concentrations in sorghum protein extracts amounted to 1317 and 2011 mg/100 g of dry powder for tannin-rich samples BRS305(T+) and SC782(T+), respectively. In contrast, polyphenols were almost totally absent in tannin-free samples BR501(T-) and BRS310(T-). Flavanol monomers (*i.e*. (+)-catechin and (–)-epicatechin) and some procyanidin dimers were clearly detected in BRS305(T+) and SC782(T+) samples. However, the main polyphenolic constituents in these samples were procyanidins oligomers and polymers presenting a DPn of 13.0 for BRS305(T+) and a lower DPn value (10.6) for SC782(T+), the later showing higher concentrations of procyanidins and flavanols. For both samples, (+)-catechin and (–)-epicatechin were observed as terminal and extension subunits, in which (–)-epicatechin was the most abundant form as extension subunit, whereas (+)-catechin was the most abundant form as terminal subunit. The oxidative stability of the emulsions formulated with protein extracts rich in tannins (BRS305(T+) and SC782(T+)) was largely superior compared to that of emulsions made with the tannin-free ones, showing that the presence of endogenous tannins greatly prevents lipid oxidation.

These results reveal a strong relationship between the oxidative stability of O/W emulsions and the presence of endogenous polyphenols in sorghum protein extracts. Such results highlight the great potential of tannins as natural antioxidants, even when complexed with proteins.

OY.4.02 - PHENOLIC COMPOUNDS COULD INTERFERE WITH SECONDARY AMINE N-NITROSATION IN CURED MEAT FORMULATION AND DIGESTION.

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Nitrite is a common additive in cured meat formulation that provides microbiological safety, lipid oxidation management and typical organoleptic properties. However, nitrite is also associated with the formation of nitrosamines, some of them being associated with colon carcinogenesis. Nitrite addition is thus pointed at and strategies should be established in order to control the formation of nitroso-compounds during meat processing and digestion. This study aimed to evaluate the antinitrosating capacity of phenolic compounds representing plant diversity and to investigate the mechanisms underlying their antinitrosating capacity.
N-acetyltryptophan (AcTrp) was used as a model of secondary amine and its corresponding nitrosamine, *N*-acetyl-*N*-nitrosotryptophan (NO-AcTrp), was followed in an aqueous solution modelling ham. Kinetics were run at pH 5 to simulate secondary amine nitrosation during product storage and the beginning of the digestion and at pH 2.5 in order to mimic the end of gastric digestion. AcTrp nitrosation and reactivity of pure phenolic compounds were followed by UPLC-DAD-MS.
In the absence of phenolic compounds, AcTrp nitrosation was found to be five times higher at pH 2.5 compared to pH 5 suggesting that the pH decrease occurring during gastric digestion can favor secondary amine nitrosation. Additionally, all the phenolic compounds evaluated were able to limit NO-AcTrp formation at both pH 2.5 and pH 5. The antinitrosating capacity decreased in the same following order for both pH: caffeic and ferulic acids > epicatechin > chlorogenic acid ≈ rutin. Hydroxytyrosol proved to be slightly more efficient than chlorogenic acid and rutin to limit nitrosation at pH 5 while those three compounds exhibited the same antinitrosating capacity at pH 2.5. UPLC-DAD-MS analyses highlighted the unique ability of each phenolic compound to react with nitrite undergoing mostly C-nitration, C-nitrosation and oxidation as shown below for epicatechin (Figure 1). Phenolic compounds are able to scavenge part of the nitrite thus reducing residual nitrite available for N-nitrosation of AcTrp.
This major finding suggests that introducing phenolic compounds either as natural ingredients in meat products or through the diet could be an efficient way to manage nitrosamine formation during cured meat processing, storage and digestion. Studies are now underway to test these hypotheses with real cured meats.

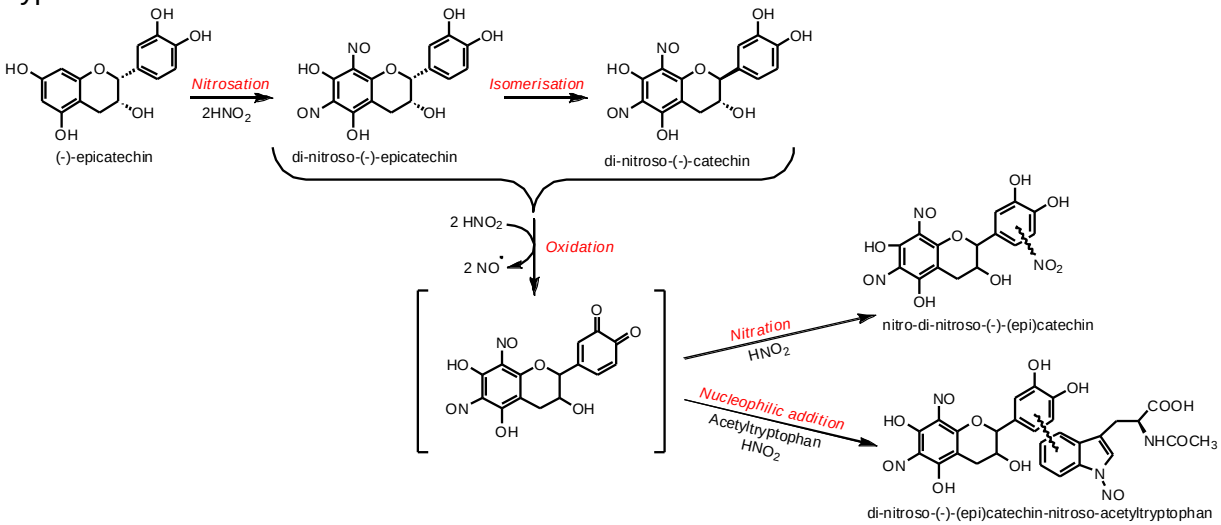

Figure 1: Proposition of mechanisms occurring behind epicatechin antinitrosating capacity

OY.4.03 - NOVEL AFFINITY-BASED PURIFICATION OF PLANT PPOS AND ENZYMATIC CHARACTERIZATION OF STRAWBERRY PPO ISOFORMS

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Brownification in juices is a frequent phenomenon that degrades their quality and palatability for the customer, causing big profit losses for the industry. Red fruit juices e.g., from strawberry, raspberries and grape are known for their low colour stability. The causes are still not clear, but the oxidation of polyphenols is thought to be at the root of the issue and so far, four players are known to be involved: polyphenol oxidase as the major one (PPO), peroxidase (POX), ascorbate oxidase (AOX) and β-glucosidase [1]. Polyphenol oxidases are multicopper enzymes that can catalyse a broad array of polyphenolic substrates and are suspected to be majorly involved in the oxidation process. Their role is speculated to be cell defence against pathogens or wounding, as PPOs oxidise polyphenols to reactive *o*-quinones by means of molecular oxygen, which can quickly polymerise and thereby seal the cells. *In vivo* the enzyme is expressed in its latent pro-form, in which its C-terminal domain shields the active site and inhibits enzymatic reactions. However, it was shown that the latent forms can be activated *in vitro*, e.g. by addition of sodium dodecyl sulphate (SDS) or fatty acids. During fruit maturation or once the fruit is crushed, proteases are released and cleave this shielding domain, releasing the active PPO. Characterization of fruit enzymes can be performed via two main strategies, using either native enzyme preparations obtained from raw material, or recombinant enzymes. For PPO, however, in both cases obtaining active, rather than the latent form is the main challenge.

We here present a novel purification method of active PPOs from plant sources based on affinity chromatography with a wide range of potential applications. A crude protein extract from apple leaves was applied to the designed column material, and elution of the affinity tag yielded in the co-elution of purified active PPO. This novel method might be used for the purification of any active plant PPOs for their enzymatic characterization, or, more importantly, for the removal of active PPOs during food production processes.

Furthermore, we recombinantly expressed strawberry (*Fragaria* x *ananassa*) PPOs to enable homogeneous enzyme purification without undesired matrix effects or isoenzyme interference. We identified four PPOs to be abundantly expressed at ripe fruit stages by screening transcriptomic databases and their codon optimised sequences were cloned into a pET-28(+) vector containing a C-terminal 6-His-tag. After heterologous expressions in *E. coli,* however, only one of the four initial constructs was successful. Redesigned constructs with a N-terminal 6-His-tag to alleviate the energy at the beginning of the mRNA allowed us to obtain soluble latent protein of the other three PPOs. All four enzymes, purified by affinity chromatography using a nickel resin, possessed monophenolase activity, which was tested with tyramine in the presence of SDS. The substrates catechol, L-DOPA, dopamine as well as selected polyphenolic compounds belonging to the groups of flavanols, flavonols and hydroxybenzoic acids were also used for their enzymatic characterisation.

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OY.4.04 - UNDERSTANDING A SECONDARY MECHANISM IN ASTRINGENCY: ADSORPTION OF DIFFERENT FAMILIES OF POLYPHENOLS TO SALIVARY ORAL MODELS

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Astringency is described as a tactile sensation of puckering, tightening and dryness in the oral cavity, commonly induced for by phenolic compounds [1]. The major mechanism attributed to this phenomenon is the interaction between salivary proteins and polyphenols and respective formation of insoluble complexes that precipitate in the oral cavity. However, more recently, this research line is growing curious about the importance of secondary mechanisms (salivary film disruption, polyphenol-membrane interactions, and mechanoreceptors) in the perception of different subqualities of astringency. However, there is still little to no proof that can substantiate these theories. A recent study from our team as already shown some first evidence that, depending on their structure, polyphenols may bind in different ways to the oral constituents and therefore elicit different mouthfeels [2]. In this study, the total adsorption of compounds to salivary cellular models was evaluated (Figure 1). Overall, Alum, a strong astringent standard, has shown a higher adsorption potential to the oral models compared to the other mixtures and higher affinity to oral epithelial models. Since alum can't precipitate salivary proteins, these results hint that compound adsorption may be an important mechanism to elicit astringency for certain compounds. Analysing the interaction of Grape Seed Extract (GSE) and Tannic Acid (TA) with oral epithelial cell models, the resultant adsorbed compounds of GSE had up to an eight-fold decreased adsorption when compared to Alum (0.016 mg/cm2), at similar initial concentrations. For Green Tea Infusion (GTI), a higher adsorption was achieved in models where saliva was not present, up to 2-fold. To further substantiate these results a correlation with a certified sensorial analysis is currently being performed.

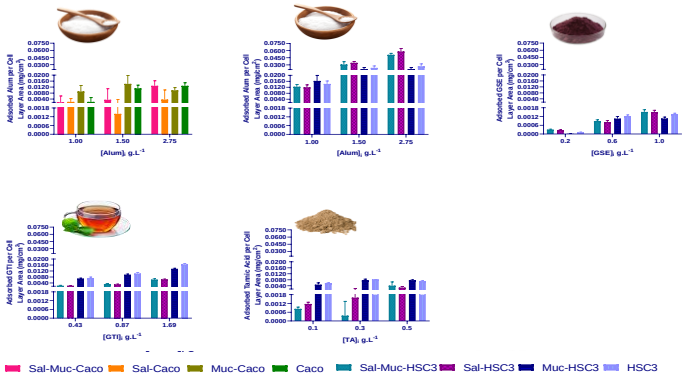

Figure 1: Total adsorption of compounds to salivary cellular models per monolayer area. Oral models constituted by epithelial cells (HSC-3 or Caco-2), whole saliva and mucin, were applied to interact with three concentrations of a sensorial standard and different families of polyphenols: Alum (sensorial astringent standard; at 1 g.L^{-1}, 1.5 g.L^{-1} or 2.75 g.L^{-1}), Grape seed extract (representative of procyanidins; at 0.2 g.L^{-1}, 0.6 g.L^{-1} or 1 g.L^{-1}), Tannic acid (representative of hydrolysable tannins; at 0.1 g.L^{-1}, 0.3 g.L^{-1} or 0.5 g.L^{-1}) and Green Tea Infusion (astringent food matrix rich in flavan-3-ols; at 0.43 g.L^{-1}, 0.87 g.L^{-1} or 1.69 g.L^{-1}). After interaction, total adsorption of polyphenols to the oral models was evaluated by colorimetric methods using Aluminon (for Alum), DMACA (for Grape Seed Extract and Green Tea infusion) and Folin-Ciocalteu (for Tannic acid).

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OY.4.05 - POLYSACCHARIDES FROM BANANA AND ORANGE PEELS: FROM WASTE TO ASTRINGENCY MODULATION OF POLYPHENOL-RICH PRODUCTS

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The most appreciated beverages worldwide such as wine, beer and tea can often elicit some negative reactions among consumers due to their high polyphenol content. Indeed, sensory properties like color and taste, are the key drivers of food consumption and acceptability. Astringency is probably one of the utmost taste properties linked to beverages quality. If one hand, a balanced level of astringency is desirable for their quality, on the other hand a high level can lead to a rejection of these polyphenol-rich beverages [1]. Although the most studied mechanism to explain astringency onset relies on polyphenol interaction and precipitation with salivary proteins, recent advances showed that polyphenols can also bind different oral constituents, such as oral cells and mucosal pellicle pointing out their contribution for the molecular perception of astringency [2]. Polysaccharides have been emerging as possible modulators for astringency perception, due to their ability to disrupt salivary proteins-polyphenol interactions [3].

The aim of this work was to unveil the effect of different polysaccharides on the disruption of polyphenol interactions with several oral constituents (tongue epithelia cells, salivary proteins and mucosal pellicle). For this purpose, different polyphenol-rich extracts were used, such as tea (green and black), red wine and grape skin. These extracts were chosen to represent the main composition of several polyphenol-rich products as well as to cover a wide range of polyphenol families. Concerning polysaccharides, they were obtained by sequential extraction with different solvents (water, imidazole solution and sodium carbonate solution) from banana and orange peels.

The present work showed evidence that polysaccharides from both fruit peels were able to decrease the interaction between polyphenols and different oral constituents. Preliminary results showed that the polysaccharide inhibitory effect is dependent of the fruit source. For instance, in the case of banana peels, water and imidazole polysaccharide fractions seem to be the most effective, while for orange peels seem to be imidazole and sodium carbonate polysaccharide fractions. Furthermore, PRPs and cystatins were the less precipitated proteins in the presence of polysaccharides from orange peels. The observed polysaccharide´s effect can be explained by the structural features of the compounds involved: polysaccharides, polyphenols and salivary proteins. In summary, fruit peels can be a valuable source of bioactive compounds, such as polysaccharides, to produce high-value final products with enhanced sensory properties. Moreover, reusing these peels could promote a much more efficient management of wastes by Food Industry.

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OY.4.06 - WHOLE WHEAT BREAD SUPPLEMENTED WITH POLYPHENOL-RICH EXTRACTS OF LOGGING RESIDUES FROM *PICEA ABIES, PINUS DENDIFORA*, AND *ABIES ALBA*

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There is an increasing interest in added-value food obtained with environmentally sustainable practices. Phenolic compounds extracted from non-traditional sources or by-products were employed as functional ingredients for bakery products. Unlike isolated vitamins, different compounds bring synergistic effects, enhancing the bioavailability compared to regular food intake. However, phenolic compounds have structure diversity and can interact with macromolecules, which may influence the baking process, bread's sensory and nutritional properties [1]. This pilot study investigates an innovative application of forestry residues (green twigs) from tree species (i.e., *Picea abies* harvested in Sweden, *Pinus dendifora* harvested in Japan and *Abies alba* harvested in Italy) (Figure 1). The objective is to develop bread prototypes enriched with aqueous logging residue extracts obtained through a green technology from different species and examine their effects on the bread's rheology, bioactivity (e.g., antioxidant and antimicrobial activity), quality parameters, and sensory characteristics. In addition, the interaction between wheat gluten proteins and polyphenols will be assessed.
Green twigs samples were treated equally regarding harvesting, drying, and milling. Since the extraction was conducted using the hydrodynamic cavitation technique at different times and temperatures [2], the most promising extract in terms of total dissolved solids and bioactivities, will be selected for the bread-making supplementation. Also, detailed chemical composition and characterisation of extracts and bread prototypes will be analysed. We hypothesise that a new bread supplement from a sorted underutilised fraction of conifers logging residues, with a high content of pine needles, may provide a significant amount of bioactive compounds. Such components may ease the baking process and enrich whole wheat bread's functionality and bioactivity without considerably impacting its sensory and rheological properties. The bread prototypes with added biomass extracts may help maintain an individual's nutritional status. Therefore, this pilot study approaches a broader utilisation of the wood and logging residues, creating value through innovative use of the bio-resources. The results will be discussed at the conference presentation.

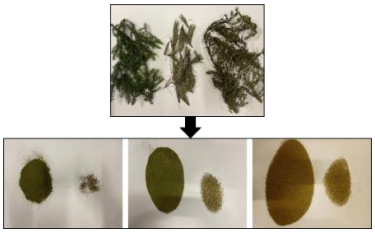

Figure 1: Plant materials (Spruce, Pine and Fir)

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OY.4.07 - CHARACTERISATION OF PHENOLIC COMPOUNDS AND POLYSACCHARIDES IN STRAWBERRY: HARVEST EFFECTS AND THEIR CORRELATION WITH COLOUR STABILITY.

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Consumer's decision regarding fruit products like juices and nectars is highly influenced by the perceived colour, which mostly results from a high phenolic content. The acceptance rate of strawberry nectar has been linked to colorimetric parameters through an Acceptance Factor (AF) [1-2]. However, this correlation has been limited to pH, Brix, titrable acidity and sensory perception of consumer (*fresh, healthy, juicy, aged, etc…)*. Moreover, macromolecules like polysaccharides and/or proanthocyanidins, and their interaction with anthocyanins may influence the colour stability of strawberry products [3]. Hence, a physicochemical study was implemented to evaluate the potential effect of these molecules on colour stability. A correlation between variety, ripening stage, harvest time and chemical composition was established regarding the Acceptance Factor (AF) [2]. These new parameters can give an accurate description of the most adequate raw material for nectars and juices with high colour stability.

The content and composition in phenolic compounds and polysaccharides of 13 strawberry varieties at 2 ripening stages and 2 harvest times were characterised. Colour stability was evaluated by monitoring the colour change of strawberry nectar after production, calculated as the change in the acceptance factor after 12 weeks (AFΔ12), based on the CIELAB parameters [1-2]. Phenolic compounds were identified by HPLC/ESI-MS^2 and quantified via HPLC-DAD with or without depolymerization by menthofuranolysis. In addition, polysaccharides were extracted and characterised regarding their composition (neutral sugars, galacturonic acid) and structure (degrees of methylation, linearity and branching).

Samples were successfully correlated with 12 out of 40 variables analysed (individual anthocyanin concentration, degree of polymerization and composition of proanthocyanidins, degree of methylation and composition of pectin…*).* Overall, significant differences were found for these variables depending on variety, ripening stage and harvest time. Distinctively, overripe samples from the late harvest showed a higher colour stability. Concerning variety, more stable samples often had higher concentrations of the main strawberry anthocyanin pelargonidin-3-glucoside (P3G) (i.e. Variety Faith). By contrast, varieties like Rendezvous and Mailing Centenary displayed high colour stability despite their low content in P3G. This suggests that other variables, such as cyanidin-3-glucoside, proanthocyanidins rich in epiafzelechin, and pectin characteristics (xylose, galactose, degree of methylation of 70-80%), have a positive influence on the colour of strawberry nectars. This mainly shows that the mechanisms of colour stabilization of strawberry products during storage are multifactorial and still incompletely understood.

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P.4.01 - EFFECT OF GRAPE VARIETY AND MACERATION TIME ON RESISTANCE AGAINST OXIDATION OF ROSÉ WINES

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Rosé wine is one of the most consumed wines worldwide, especially during summer time. Colour is one of the main organoleptic characteristics that consumers base their purchase choice, therefore rosé wine colour is of the outmost importance to control from an oenological point of view. Anthocyanins, extracted from a short period of time (usually some hours) are responsible for the pinkish-salmon desired hue of rosé wine.
Rosé wines have a small amount of tannin, that usually prevents wines from oxidation, and are also rich in phenolic acids [1], which are easily oxidized into yellowish pigment and could cause the undesirable browning of rosé wines. Therefore, the use of oenological practices (besides the addition of SO_2) that could prevent the oxidation and browning of the wine are highly desired. Thus, the aim of this work is to assess the effect of different grape varieties and extraction times on the capability of rosé wines to resist oxidation process. To do this, rosé wines were made using grapes from *V. vinifera* cv Syrah (SY) or cv Tempranillo (TE), grown in Condado de Huelva D.O. (Southwestern Spain) and employing two different maceration times for each grape variety: short (S, 10 min) and long (L, 2 hours). Thus, four different wines were elaborated (SYS, SYL, TES and TEL). After maceration, the mash was drawn off to remove the solid parts and the free run musts were submitted to alcoholic fermentation spontaneously occurred. When the fermentative processes finished, the wines were again racked and maintained for a stabilization period of 1 month. Then, wines were submitted to an oxidation process by reaching oxygen saturation level in the solution. Wines were studied before oxidation process and then the evolution of both, colour and pigment composition was monitored during the next days. Colour was determined from the absorption spectra (380-770nm) of wines, from which the CIELAB parameter were calculated. Also, differential colorimetry was employed to assess the role of copigmentation in colour. The pigment composition of wines was determined by means of HPLC-DAD-MS. The amount of dissolved oxygen in wine was also monitored during the process by direct measurements of oxygen using optic probes. From the data obtained different kinetics of evolution of colour, pigment composition and dissolved oxygen during the oxidation process were built for the four studied wines. Results allowed us to assess the importance of grape variety and maceration time to modify the ability of rosé wines to resist oxidation, which can be considered in order to increase the self-life of this type of wines.

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P.4.02 - EXTRACTION AND CHARACTERIZATION OF ENZYMES INVOLVED IN THE ENZYMATIC BROWNING OF STRAWBERRY FRUIT PRODUCTS

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Enzymatic browning of fruit and vegetables is a natural phenomenon that occurs after exposure of the tissues to the atmosphere when peeled, cut, processed or damaged in another way. It can also be a sign of diseases or abnormal conditions. The color change comes from the enzymatic oxidation of polyphenols, and the degradation of anthocyanins, and thereby a loss of red hues. In industry, the browning effect is highly undesired, as it signifies quality and nutritional loss. Several enzymes were suggested as the causative agent for this phenomenon, and were targeted for research, with a view to inhibiting their activity [1]. Polyphenol oxidase (PPO) is considered the most recognized and studied agent of the browning effect. PPO is a copper-containing enzyme that catalyzes the hydroxylation of monophenols to diphenols and diphenols to quinones.[1] Peroxidase (POD) also contributes to enzymatic browning; POD catalyzes the oxidation of multiple phenolic and non-phenolic compounds, with hydrogen peroxide (H_2O_2) as a co-factor [1]. β-Glucosidase is an important enzyme that hydrolytically cleaves the β-glucosidic bonds of many compounds found as their glycosidic precursor in fruits and vegetables.[1] Lastly, ascorbate oxidase (AOX) is a blue copper enzyme that uses ascorbic acid as an electron donor to reduce oxygen to water. AOX's role in plants and fungi is not fully understood [2] To study these enzymes and their activities, strawberries (*Fragaria* x *ananassa)* were selected as a role model for berries and fruits with limited color stability of their processed products. Strawberries and their products, such as puree and nectar, are considered of high value in the food industry, due to the popularity of strawberry as a fruit, and its use in many different food products.

To analyze the influence of cultivar and ripening stage on enzymatic browning, a rapid and reliable protein extraction protocol maintaining the enzymes' functional activity is needed. Fruits are, however, recalcitrant plant tissues for enzymatic studies, due to their low protein content and the presence of interfering substances, such as pigments, polyphenols, polysaccharides, and starch, and the total protein amounts extracted strongly vary with the protocol [3]. Strawberry fruits were ground into fine powder in liquid nitrogen. The targeted enzymes were extracted with citrate buffer at varying parameters, such as the time of extraction, pH of the buffer, and in the presence of different additives with varying detergent to solvent ratios. Best results were obtained with Triton X-100 as a detergent and polyvinylpolypyrrolidone (PVPP) as a protective agent to remove endogenous phenolic compounds. The total protein concentration obtained, however, was as low as 1.8 µg per 10 g of strawberry powder. Activities of the targeted enzymes were tested with artificial substrates; catechol as a substrate for PPO, guaiacol and hydrogen peroxide for POD, *p*-nitrophenyl-β-D-glucopyranoside for β-glucosidase, and ascorbic acid for AOX. We here present the analysis of 10 cultivars at two fruit ripening stages.

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P.4.03 - EFFECT OF ULTRAVIOLET LIGHT-EMITTING DIODES AND PULSED LIGHT ON POLYPHENOL OXIDASE DEGRADATION AND *ESCHERICHIA COLI* INACTIVATION IN APPLE JUICE

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Pulsed light (PL) and ultraviolet light-emitting diodes (UV-LED) technology are powerful ultraviolet light germicidal methods that have raised significant attention recently. The main mechanism of both treatments photoinduced modifications in the double bonds of the biomolecules causing chemical alterations. Enzymes are one of the major targets, due to the abundance of endogenous chromophores in their structure [1]. Polyphenol oxidase (PPO) is one of the enzymes involved in food spoilage, affecting the phenols, resulting in the browning of cut fruits and vegetables, leading to a less attractive appearance, and a loss of bioactive compounds and nutritional quality. The present research aims to study the inhibition of the polyphenol oxidase enzyme, the retention of total polyphenols and antioxidant activity in apple juice treated by PL and UV-LED. Furthermore, the inactivation of *Escherichia coli* as a microorganism of interest in food is also addressed. The PL (200-1100 nm) and UV-LED (255, 265, and 280 nm) equipment was used to treat fresh apple juice (*Malus pumila*) (thickness=0.5 cm, lamp distance= 5 cm) with treatment times between 10 to 70 s and 25 to 177 J/cm^2 for PL and to 20 to 60 min and from 0.40 to 1.32 J/cm^2 for UV-LED. After each treatment, the polyphenol oxidase enzyme, total polyphenol content, and antioxidant capacity of apple juice were evaluated by spectrophotometry, and the result were compared with the inactivation of *E. coli* for each treatment (Figure 1). Inhibition of the polyphenol oxidase enzyme was observed in both treatments along all the treatment conditions. A more effective inhibition of polyphenol oxidase was observed in the treatment by pulsed light, achieving a reduction of 27.7 U/mL of polyphenol oxidase and inactivating 3.2 log cycles of *E. coli* at 70 s. Compared to the sample treated by UV-LED at 60 min, where polyphenol oxidase was reduced up to 18 U/mL, while achieving an inactivation of up to 6.2 log cycles of *E. coli*. In addition, both technologies increase the concentration of polyphenols and antioxidant capacity, compared to thermal pasteurization. Overall findings suggested that PL and UV-LED technology are effective treatments with the potential to be industrially scalable, being an alternative to replace thermal pasteurization, inhibiting the polyphenol oxidase enzyme without counteracting the bioactive properties of the juices.

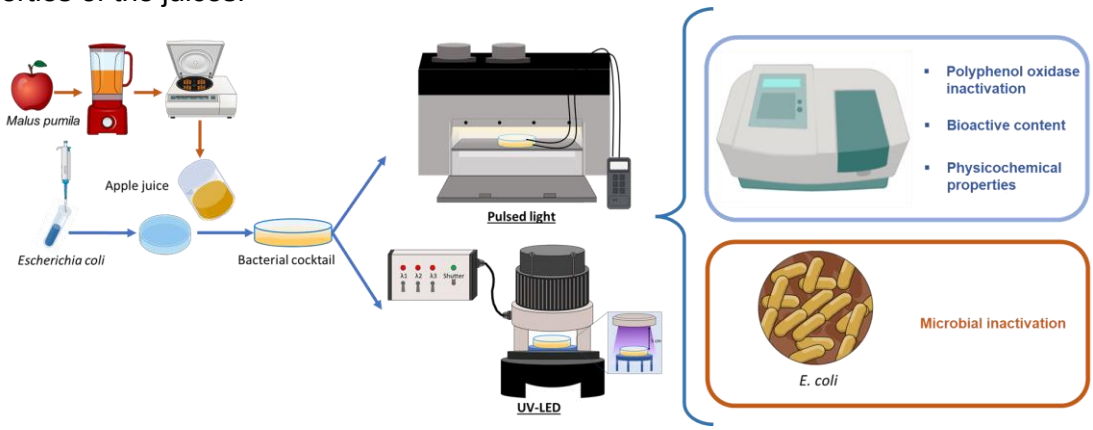

Figure 1: Scheme of the methodology for apple juice treatment by PL and UV-LED

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P.4.04 - THERMAL PROCESSING OF EITHER FRUITS OR VEGETABLES DIFFERENTLY INFLUENCES POLYPHENOL STABILITY

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Polyphenols are the major microconstituents in fruit and vegetables (F&V) and contribute to their organoleptic and nutritional properties. Epidemiological studies demonstrated that the consumption of fruit and vegetables is inversely associated with the development of cardiovascular diseases [1]. On the other hand, cardioprotection has been correlated to polyphenol consumption [2]. As fruit and vegetables are mainly consumed processed, preservation of polyphenols and vitamins is sought during thermal processing. To this end, a better understanding of their stability during thermal processing is essential to improve the nutritional quality of processed F&V products. The aim of our study is to evaluate the stability of common F&V polyphenols and ascorbic acid during conventional thermal processing. Mechanisms occurring behind the reactivity will also be discussed.

We designed two aqueous model systems containing soluble pectins, an apple polyphenol extract [3], cyanidin-3-glucoside, rutin and ascorbic acid either at pH 4 or pH 6 to study polyphenol stability for fruit or vegetable, respectively, in both hot break (95 °C) and cold break (65 °C) processings. Concentrations in polyphenols and ascorbic acid were as in fruit and vegetables. Kinetics for polyphenols were followed by HPLC-DAD-MS. Degree of polymerization and composition of proanthocyanidins were assessed after acidic depolymerization in the presence of menthofuran.

In the vegetable model at pH 6 and 95 °C, the polyphenol stability over 8 h was: rutin > 5-caffeoylquinic acid > catechin ~ epicatechin > cyanidin-3-glucoside > ascorbic acid. In the fruit model at pH 4 and 95 °C, polyphenols proved to be largely more stable although with a similar order: rutin ~ 5-caffeoylquinic acid > epicatechin ~ catechin > cyanidin-3-glucoside > ascorbic acid. At 65 °C, all compounds were mainly stable at both pH except cyanidin-3-glucoside and ascorbic acid.

This study highlighted a larger evolution of 5-caffeoylquinic acid at pH 6 than at pH 4 for both temperatures tested. Indeed, 5-caffeoylquinic acid led to an equilibrium with 3-caffeoylquinic and 4-caffeoylquinic acids through intramolecular trans acylation without any further degradation. At 65 °C, this equilibrium was slower and not achieved after 8 h. Additionally, (+)-catechin concentration peaked after 3 h at 95 °C and pH 6 with a two-fold higher content whereas (-)-epicatechin decreased by a larger amount at the same time suggesting both epimerization and degradation of monomeric flavanols.

These results suggest a greater stability for polyphenols in fruit processing than in vegetable processing for both temperatures. In the vegetable model, the major reactions are regioisomerization of caffeoyl- and coumaroylquinic acids, epimerization of monomeric flavan-3-ols and depolymerization of proanthocyanidins.

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P.4.05 - POTENTIAL USE OF *TORULASPORA DELBRUECKII* AS A NEW SOURCE OF MANNOPROTEINS OF OENOLOGICAL INTEREST

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Global climate change is having a profound impact on vine phenology and can lead to changes in the viticultural suitability of some traditional winemaking regions, particularly, of the Southern European regions. In this sense, the increasing temperatures and altered rainfall patterns can modify biochemical and physiological processes of the vine, thus, affecting the accumulation in the grapes of sugar, acids and phenolic compounds. In consequence, the gap between technological and phenolic maturity of the grapes is increasing, which can result in wines with altered color and unbalanced astringency [1]. The addition to wine of mannoproteins (MPs) has been proposed as a possible way of mitigating some of the undesirable climate change consequences on wine quality. In fact, there are studies that show that MPs can be able to modulate wine astringency [2] and protect wine color [3]. However, the effect of MPs on the sensory properties of wine seem to be variable due to the high heterogeneity of this class of biomolecules and, at present day, no clear relations are established between the composition and structure of the MPs and their techno-functional properties.

MPs are glycoproteins located in the outer layer of the yeast cell wall and are composed of one major glycosidic moiety (50-95%) linked to one minor protein moiety. MPs are naturally released to the wine by yeast during fermentative and post-fermentative processes [2]. Currently, most of the studies are focused on MPs derived from *Saccharomyces cerevisiae*, the main oenological yeast. However, some studies point out that MPs derived from other yeast species and, particularly, MPs derived from *Torulaspora delbrueckii* could be more effective for the modulation of some wine organoleptic properties [3].

Therefore, the objective of this study was to evaluate the effect of different MPs extracted from *T. delbrueckii* on wine color and phenolic composition. In order to obtain MPs with different structure and composition, three treatments were applied to the yeast: induced autolysis, enzymatic extraction and alkaline extraction. Then, the protein and polysaccharidic moieties of the extracted MPs were characterized by SDS-PAGE, Lowry method, HRSEC-RID and HPLC-DAD-MS. The obtained MPs were added to a Tempranillo red wine and changes in the phenolic composition and color were analysed before and after the stabilization of the wine (involving cold treatment followed by filtration) as well as 1 month after bottling. Wine color was analysed spectrophotometrically by triestimulus colorimetry and the detailed phenolic composition was evaluated by HPLC-DAD-MS.

The obtained results showed that the extracted MPs presented important structural and compositional differences. Furthermore, the addition of the MPs to red wine resulted in changes in color and phenolic profile that depended on the type of MP added. Overall, these results point out to the potential oenological use of the MPs obtained from *T. delbrueckii*.

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P.4.06 - COOKING INFLUENCE ON COLOR ATTRIBUTES, TOTAL PHENOLIC CONTENT, PHENOLIC PROFILE (LC-UV-MS) AND POLYPHENOL OXIDASE ACTIVITY IN NEW HYBRIDS OF YAM (*DIOSCOREA ALATA*)

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Yam is a major staple food which provides both energy and bioactive compounds. In the French West Indies, over the past ten years, yam production declined by 70%, which was partly due to a fungal disease leaf anthracnose. New hybrids resistant to anthracnose were created but some of them have quality flaws. Some hybrids are susceptible to browning when cut [1]. We showed recently that this susceptibility is positively correlated to total phenolic and catechin contents and negatively correlated to procyanidins with high degree of polymerization. Moreover, an extra change in color is observed after cooking. Darkening appearing after cooking is highly undesirable in potatoes [2] but has not been previously reported in yams. We determined the influence of boiling on color attributes, total phenolic content, phenolic profile (LC-UV-MS) and polyphenol oxidase activity in three varieties of yam contrasted for their susceptibility to browning.

The new hybrids “INRA15”, highly susceptible to browning, and « Caribinra » with low susceptibility to this defect, were compared to “Kabusah”, which exhibited moderate susceptibility. Boiling during 22 min led to darkening in all varieties. It also induced an average 42 % decrease in the total phenolic, flavanols and total procyanidins contents of the pulp without any significant modification in the structure of procyanidins. The decrease in the contents of phenolics compounds after cooking could be due either to oxidation or to leakage in the boiling water. Polyphenol oxidase activity was the highest in « INRA15 » and was inhibited only after 11 min in boiling water when the temperature of the core of the pulp attained 60 °C. The leakage of flavanols in water was on average of 2 % and was linked to compounds with a low degree of polymerization. Cooling at ambient temperature during one hour induced no significant change in color attributes or total phenolic content.

For the first time, the phenolic profile of flavanols and the polyphenol oxidase activity in the pulp of yam were determined sequentially throughout cooking. The phenolic profile of flavanols in boiling water had not yet been examined. Cooking darkening in yam occurs during boiling and not during cooling, unlike what was reported in potatoes [2]. Further studies are needed to determine how to prevent cooking darkening in yam.

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P.4.07 - EFFECT OF COMMERCIAL MANNOPROTEINS ON THE MODULATION OF OXIDATIVE PROCESSES IN ROSÉ WINES

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Rosé wine consumption has increased greatly in the last years, reaching over 10% of the global market of wines. The colour of rose wine, like that of red wines, is due to the anthocyanins extracted from red grape skins and to the derived pigments formed through reactions involving other must and wine components. Because of the lack of an important presence of other polyphenols, anthocyanins in rosé wines are quite exposed to oxidative reactions leading to an irreparable loss of the bright rosé colour. The addition of SO_2 is one of the strategies used by wineries to prevent the oxidation processes; but this is one of the most controversial additives and the demand for wines with low sulfur dioxide content is increasing. The aim of this work is to study the effect of oenological mannoproteins on preventing oxidation processes in rose wine affecting colour. To do this, six commercial mannoproteins (issued from yeast) were characterized by SDS-PAGE, HPLC-RID and HPLC-MS. Rosé wines were made using grapes from *V. vinifera* cv Tempranillo. The mannoproteins used presented a wide range of average molecular weights, between 34.2 kDa and 53.0 kDa and their protein content was ranging from 5 to 35% and had notable differences in their monosaccharide composition. Each individual mannoprotein was added to the rosé wine that was treated with an oxidizing agent. The colour of the oxidized sample-wines in presence and in absence of the mannoproteins was analysed using tristimulus colorimetry using CIELAB parameters and the formation/disappearance of phenolic compounds was studied by HPLC-MS. Differences in the phenolic profile and on colour of the different wine-samples were observed, that could be attributable to the effect of the mannoproteins and related to the differences presented in their structure.

P.4.08 - ADDITION OF OAK WOOD ALTERNATIVE PRODUCTS: QUALITATIVE AND SENSORIAL EFFECTS FOR A WHITE WINE OF ALIGOTE.

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Wines matured in contact with wood are extremely popular with consumers all over the world. Oak wood allows the organoleptic characteristics of wine to be modified. Wines are enriched with volatile and non-volatile compounds extracted from the wood. The aromas extracted from oak wood contribute to the construction of the wine's aromatic profile and the main polyphenols extracted can modify taste perceptions such as astringency and bitterness. All the compounds extracted from the wood thus contribute to the balance and quality of the wines.
The maturation of wine in vats with the addition of alternative oak products has become increasingly popular in all wine producing countries of the world. The main reasons for the development of such products are the optimisation of their production, the reduction of the cost of wine ageing as well as the increase of the level of hygiene in the production. This study is part of this context and focuses on oak chips: an alternative wood product to barrels. It aims to evaluate the optimum dose and the best level of toasting of the oak wood for the addition of these chips during alcoholic fermentation in a white Aligoté wine.
During our experiment, the white Aligoté must before alcoholic fermentation was added with different doses of chips (1-2-3-4-5 g/L) at different toasting levels (5 levels: fresh, light toasting, medium toasting, medium + toasting, strong toasting). A control wine could was also made without the addition of chips for comparison. In order to determine the optimal dose and toasting of the oak chips used, the classic oenological parameters (Foss: pH, Alcoholic Strength, Total Acidity, Volatile Acidity, Sugars), colour (A_{420nm} and CIELAB parameters), total phenolic compounds (TPI, total tannins and Folin index), monomeric and dimeric proanthocyanidin, phenolic acid and ellagitannin composition (HPLC-UV/MS), as well as fruity and woody aroma markers (GC/MS) were analysed. Sensory analyses were also carried out for each wine.

P.4.09 - BLACKCURRANT LEAVES AS A DONOR OF POLYPHENOLIC COMPOUNDS IN THE PRODUCTION OF FRUIT SORBETS

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In recent years, much attention has been paid to the possibility of using new, alternative sources of bioactive compounds in the food industry for fortification and the design of functional products. Interesting in this regard are blackcurrant leaves, which contain a number of polyphenolic compounds, essential oils, and minerals. So far, they have been perceived as waste materials, although they can be a potentially attractive addition to the design of functional foods.
Therefore, the aim of the study was to develop innovative, fruit sorbets with attractive sensory properties and health-promoting properties using blackcurrant leaf infusions as a donor of polyphenolic compounds. In the obtained products, the basic characteristics of the chemical composition according to PN were analyzed. In addition, the qualitative (LC/MS-QTof) and quantitative (UPLC-PDA-FL) content of polyphenolic compounds [1] was determined, and the pro-health potential of the obtained formulations was determined, including the ability to inhibit α-amylase, α-glucosidase, and pancreatic lipase by *in vitro* [2]. Finally, the sensory attractiveness of the obtained products and their glycemic index *in vivo* conditions were also determined.
As a result of the conducted research, it was shown that blackcurrant leaves can be an attractive addition to the production of fruit sorbets. They not only have a beneficial effect on the quality of the finished products, fortifying their composition in flavan-3-ols and flavonols, thus increasing their health-promoting potential, but also creating their sensory qualities. In addition, it has been shown that the positive health-promoting effect in vitro is reflected in the human model in vivo, which indicates the functionality of the obtained formulations.

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P.4.10 - DIVERSE COLOR PERFORMANCE OF HYDROXYPHENYL PYRANOANTHOCYANINS ACROSS PH

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Color is an influential sensory attribute, altering product perception and liking at first glance. With consumer preference for clean-labels and rising concerns about ingredient safety, product developers are looking for nature-derived colorants for foods and consumer goods. Pyranoanthocyanins (PACNs) derived from anthocyanins are a promising option. Found in aged wine, aged juices, and several plants, PACNs can produce vibrant colors with high stability to storage and bleaching [1,2]. Hundreds of PACNs are possible with unique chemical structures and coloring characteristics. As a flavylium compound, their chemical structure and color change with pH, further imparting diversity [2]. Our goal was to evaluate the role of PACN chemical structure on color performance and solubility across pH.

Eight PACNs were formed from cyanidin-3-glucoside (from *Sambucus nigra)* or malvidin-3-glucoside (from *Berberis boliviana*) and 4 hydroxycinnamic acids to yield: 10-*p*-hydroxyphenyl-, 10-catechyl-, 10-guaiacyl-, and 10-syringyl-PACNs (Figure 1). Identities were verified by uHPLC-PDA-ESI-MS/MS. PACNs were isolated (~90% pure), dried, and color was evaluated (40 µM) in pH 1 and 3 KCl buffers and aqueous solutions at pH 3.5–10 adjusted with HCl or NaOH. Spectra were measured from 260–700 nm over 2 hours and converted to CIELAB coordinates.

At acidic pH (1–3.5), hydroxyphenyl-PACNs produced yellow to red colors ($\lambda_{vis\text{-}max}$ 493–511 nm, Figure 1), with molar absorptivities up to 2.1x (pH 1) and 7.5x (pH 3) greater than anthocyanins. At these pH values, more hydroxyl and methoxy substitutions on the B and E ring resulted in a bathochromic $\lambda_{vis\text{-}max}$ and lower hue angle (Figure 1). At mild acid pH (4.5–7), PACNs remained colored, albeit at a shifted $\lambda_{vis\text{-}max}$ and lower intensity (Figure 1). 10-*p*-Hydroxyphenyl-PACNs lost more color than the other PACNs. Cyanidin-derived 10-catechyl and 10-guaiacyl-PACNs stayed at or became more yellow orange (hypsochromic $\lambda_{vis\text{-}max}$) while those from malvidin became more purple (bathochromic $\lambda_{vis\text{-}max}$). At basic pH, solutions were pink to indigo with hue angles reaching 286° and $\lambda_{vis\text{-}max}$ at pH 10 from 529–583 nm, suggesting formation of (anionic) quinoidal base. Aqueous solubility was best with 10-*p*-hydroxyphenyl- and 10-syringyl-PACNs while 10-guaiacyl and 10-catechyl-PACNs could precipitate in acidic solutions. Hydroxyphenyl-PACNs exhibited diverse and versatile color performance, and the identified trends may aid in PACN selection to give the desired sensory attributes.

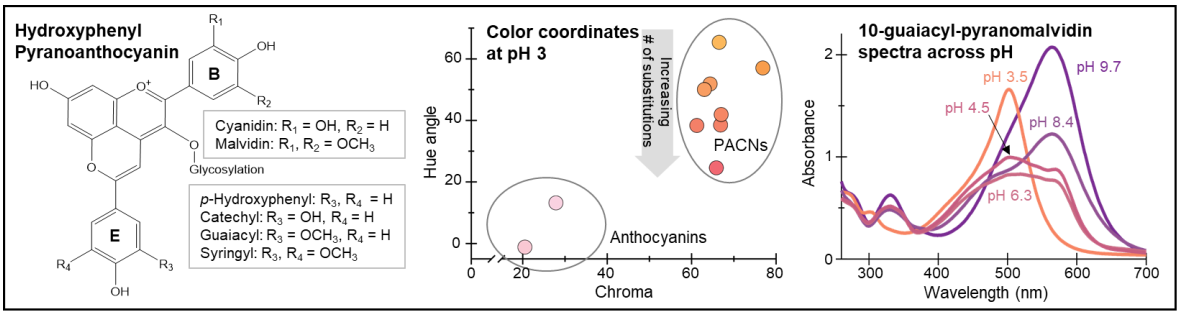

Figure 1: Chemical structure of the 8 pyranoanthocyanins (PACNs) evaluated, effect of number of B and E ring substitutions on color of PACN, and spectral changes across pH for 10-guaiacyl-pyranomalvidin-3-glucoside. Color of circles and spectra represent the solution color.

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P.4.11 - OPTIMIZED ISOLATION OF PHENOLIC COMPOUNDS FROM AQUEOUS EXTRACT OF THE HALOPHYTE *SALICORNIA RAMOSISSIMA*

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Phenolic compounds isolated from an aqueous extract of a residual fraction of the edible halophyte *Salicornia ramosissima* could induce high antioxidant capacity and a series of health-promoting effects in feed, food, or nutraceutical applications. *S. ramosissima* have been found to contain a high concentration of phenolic compounds, and the biomass has been an agricultural challenge as the biomass is high in salts. The isolation of phenolic compounds is therefore of interest from an environmentally friendly extraction method excluding toxic and volatile solvents. The isolation of phenolic compounds was investigated using membrane filtration, liquid-liquid extraction, resin adsorption, and centrifugal partition chromatography (CPC) and analysed by the Folin-Ciocalteu assay for total phenolics, DPPH assay for antioxidant capacity, and finally analysed on UPLC-MS/MS in negative ion-spray mode for detection of individual phenolic compounds. The phenolic compounds analysed by UPLC-MS/MS were protocatechuic acid, p-coumaric acid, vanillic acid, caffeic acid, ferulic acid, syringic acid, quercetin, isorhamnetin, neochlorogenic acid, cryptochlorogenic acid, chlorogenic acid, astragalin, hyperoside, and isoquercetin. Sequential membrane filtration and weak acid hydrolysis before a sequential membrane filtration indicated the phenolic compounds forming complexes with other compounds, retaining the phenolic compounds in the retentate fraction of large molecular weight cut-off membrane sizes. Conventional liquid-liquid extraction using sequential ethyl acetate and n-butanol showed to extract most phenolic compounds, apart from compounds with low partition coefficient in a octanol:water liquid-liquid system, indicating a low isolation efficiency of polar phenolic compounds, e.g. chlorogenic acid. Analysis of the extract after resin adsorption by Amberlite XAD-4 hydrophobic resin showed high efficiency for separation, with 100 % of phenolic compounds adsorped to the resin, and 96.7 % eluated from the resin using ethanol. CPC isolation was carried out to fractionate the concentrated extract after treatment by resin in the main classes of molecules. Different solvent mixtures were tested to determine the best partition ratio between both phases. Trials were done using an organic or aqueous phase as a mobile phase. In both cases, a good separation of hydroxycinnamic acids, flavonoids and chlorogenic acids was observed, with better efficiency in the process using the aqueous phase as the mobile phase. Using these easily scalable methods it was possible to make a complete separation of the phenolic compounds of interest. The compounds of the highest concentration were isoquercetin, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid and ferulic acid. Further studies on the purification of isolated fractions are needed to produce pure compounds.

P.4.12 - PRESERVATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY DURING CACTUS PEAR JUICE CONCENTRATION BY OSMOTIC MEMBRANE DISTILLATION PROCESS

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Hydrophobic polymeric (0.45 and 0.20 µm polytetrafluoroethylene (PTFE) and 0.10 µm polypropylene (PP)) membranes were efficiently applied for cactus pear juice by using Osmotic Membrane Distillation (OMD) process. Using 0.45 µm PTFE membrane, the final concentration of the filtred juice (FJ) was 16.4 °Brix ± 0.1 (at 20°C, after 5h) and 23.4 °Brix ± 0.2 (at 35°C, after 18h). In order to determine the effect of the membrane concentration process on the juice quality, four different juice stocks were analyzed. The juice quality was assessed by determining the total phenolic compounds level (by the Folin–Ciocalteu method), total flavonoids content (using the aluminium trichloride method), phenolic acids content (using HPLC system) and antioxidant activity.

Based on the statistical analysis, the comparison of the results revealed that the TPC content increased significantly (at $p<0.05$) after each OMD process, except the case of the concentrated juice at 30°C, where a slight decrease of TPC was noticed. On the other hand, the increase of process time and temperature, led to an increase of the TPC content from 38.05 ± 1.02 mg/100g to 67.13 ± 1.69 mg/100g with the increase of the juice concentration from 16.4 ± °Brix to 23.4 ± 0.15°Brix. Moreover, the concentrated juice did not loose phenolic compounds. Furthermore, the statistical analysis shows that the juices after OMD process possess higher TFC in comparison to the juice prior to OMD. However, It was noticed that caffeic acid content was preserved after OMD process.

The determination of the gallic acid and chlorogenic acid content indicate that the juice processed at 35°C present a higher content of gallic acid and chlorogenic acid compared with the concentrated juice at 30°C (at $p<0.05$). Finally, the antioxidant activity determined by DPPH method, indicate that OMD process causes an increase in the juice capacity to reduce DPPH free radical, especially, with prolonged process time and at higher temperature. This can be explained by an increase in phenolic compounds, knowing that the antioxidant activity is related to phenolic compounds, such as flavonoids and phenolic acids. Furthermore, it was found that OMD process allowed to obtain a juice semi-concentrate, with a concentration of 23.4 ° Brix, and with high content of antioxidants compounds. Indeed, the obtained product can be used in food products and nutritional complements formulations.

P.4.13 - EFFECT OF SOLUBLE POLYSACCHARIDE ADDITION AGAINST OXIDATION OF ROSÉ WINES

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Rosé wine is rapidly increasing its popularity worldwide, especially during summertime. The colour of this type of wine is of crucial importance in oenology, as it is especially relevant for consumers, whose purchase choice is highly dependent on the attractiveness of the wine's colour. Short-time macerations with the red skin of the grapes cause the partial extraction of anthocyanins, which are responsible for the pinkish-salmon hue of rosé wines. However, the low quantity of tannins (antioxidants) and richness in phenolic acids [1], which can be easily oxidized into yellowish pigments, tend to predispose rosé wines to an undesirable browning. Although the use of SO_2 for the prevention of oxidation is highly extended, this practice is expected to be reduced due to its possible adverse effects in human health. Therefore, the search for alternative oenological adjuvants that prevent the oxidation and browning of rosé wines is highly desired.

Thus, the aim of this work is to assess the effect of the addition of soluble polysaccharides, issued from grape pomace on the oxidation process. To do this, rosé wines were made using grapes from *V. vinifera* cv Syrah, grown in Condado de Huelva D.O. (Southwestern Spain) and employing two different maceration times: short (S, 10 min) and long (L, 2 hours). Thus, two different wines were elaborated (SYS and SYL). Soluble polysaccharides were extracted, purified and characterized (by means of HPLC-DAD-MS and HPLC-RID) from white grape pomace and added to the rosé wines. Then, wines were submitted to an oxidation process by reaching oxygen saturation level in the solution. Wines' phenolic composition was studied before oxidation process and then the evolution of both, colour and pigment composition was monitored. Colour was determined from the absorption spectra (380-770nm) of wines, from which the CIELAB parameter were calculated. The phenolic composition of wines was determined by means of HPLC-DAD-MS. The amount of dissolved oxygen in wine was also monitored during the process by direct measurements of oxygen using optic probes.

The extract of polysaccharides presented three main fractions: F1 (25%) with a MW of 104 kDa; F2 (13%) with a MW of 8 kDa and F3 (62%) with a MW of 2 kDa. Pigments composition analysed by HPLC-DAD-MS showed native and some derived anthocyanins (pyranoanthocyanins among others). Total anthocyanin content (45.75 mg/L and 35.82 mg/L), as expected was slightly higher in SYL.

From the data obtained different kinetics of evolution of colour, phenolic composition and dissolved oxygen during the oxidation process were built for the studied wines. Results allowed us to assess the importance of polysaccharide addition to modify the ability of rosé wines to resist oxidation, evaluating the possible application of a natural polysaccharide obtained from wine's by-product as an oenological adjuvant.

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P.4.14 - EVALUATION OF A PHENOLIC EXTRACT FROM OLIVE VEGETATION WATER AS A CLEAN-LABEL INGREDIENT IN FRESH BASIL PESTO SAUCE

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Lipid oxidation and microbial alteration affect food safety, nutritional value and sensory properties and shorten the shelf life of food. To delay these negative changes, additives are currently commonly added to foods. However, synthetic additives are usually perceived negatively by consumers, as recent studies have shown that their long-term intake may lead to harmful effects. Therefore, replacing synthetic additives with natural ones is of great interest today, both for consumers and the food industry. The new EU directives and the global turn towards circular economy models are also pressing the agricultural industry to valorize their by-products as a valuable source of polyphenols with antioxidant and stabilising effects. Olive fruit is a rich and exclusive source of phenols (especially secoiridoid) with recognised biological (antimicrobial, antioxidant and health-promoting) and sensory properties. These bioactive compounds are largely found in virgin olive oil and olives as well as in the leaves, pomace and olive vegetation water (OVW). The recovery of bioactive compounds from OVW with an environmentally friendly system allows obtaining various products in different formulations tailored for a wide range of applications as functional ingredients, additives, etc. in the cosmetic and/or food industry and, on the other hand, the reduction of the pollutant load of OVW.
The aim of this study was to investigate the effect of OMV's phenolic extract (PE) as an additive of natural origin to improve oxidative stability and extend the secondary shelf life (SSL) of fresh basil pesto sauce (FBPS) under simulated retail storage conditions for a product served loose in deli counters (a common sales method in Italian food stores). For this purpose, two different formulations of fresh basil pesto sauce (with a ratio of 250 and 500 mg of phenols/kg pesto) were characterized in terms of physicochemical and microbiological parameters, phenolic and volatile compounds, antioxidant activity and sensory profile and compared with a control formulation (mixture of ascorbic acid and sorbic acid) (FBPSPE1, FBPSPE2 and FBPSCA, respectively). The evolution of the main quality parameters was evaluated during 0, 1, 2, 3, 6 and 7 days of storage after opening the pack. The addition of PE led to a reduction in the primary and secondary oxidation products responsible for the ‘rancid' off-flavour, in a way that was proportional to the amount of PE used for most parameters analyzed. Notably, the peroxide levels in FBPSPE1 and FBPSPE2 were 1.1- and 1.3- fold lower, respectively, than in the control samples; a similar trend was also observed for the concentration of C_6-C_9 aldehydes (1.9- and 2.9- fold lower, respectively, in the pesto sauces enriched with PE). After 7 days of storage after opening, PE showed a higher ability to protect α-tocopherol and other bioactive molecules of pesto (rosmarinic, caffeic and chicory acids) than ascorbic acid. In addition, FBPSPE1 and FBPSPE2 still contained bioactive molecules responsible for protecting blood lipids from oxidation according to Commission Regulation (EU) 432/2012. Both concentrations of PE effectively improve the antioxidant activity and the sensory quality of the fresh pesto until the end of storage. Our results suggest that PE could be a promising 'clean label' ingredient to address the growing consumer awareness of safety and health-promoting quality aspects in food choices. Overall, the use of PE from OMW also represents a sustainable tool for reducing food waste and adding value to a highly polluting waste product.

TOPIC 5 - BIOGENESIS AND FUNCTIONS IN PLANTS & ECOSYSTEMS

PL.5.01 - DECIPHERING THE BIOSYNTHESIS AND REGULATION OF HYDROLYZABLE TANNINS.

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Hydrolyzable tannins (HTs) are polyphenolic compounds derived from the shikimate pathway. HTs play important roles in plant defense against microbes and herbivores. More attention has been devoted to HTs due to their human health benefits; in addition to being potent antioxidants, HTs exhibit cancer chemopreventive, cardioprotective, anti-inflammatory, antimicrobial, and antiviral activities. Despite the documented functions of HTs in plant protection and their bioactivities in the human diet, the biosynthetic pathway leading to HT production in plants has only begun to be elucidated recently. Addressing knowledge gaps in our understanding of HT biosynthesis can open the door to improved plant chemical composition and human health. The progress and outstanding questions in HT metabolism and regulation will be discussed in this chapter.

PL.5.02 - TANNINS AND CLIMATE CHANGE: ARE TANNINS ABLE TO STABILIZE CARBON IN THE SOIL?

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Interaction between tannins and proteins has been studied for more than half of a century due to its significance for numerous fields of study. In chemical ecology, tannins are involved in response to environmental stress, including defense against pathogens, herbivores, and changing environmental conditions. The newest insights show that tannins may interact in similar way as with proteins also with other organic compounds, including fungal cell wall component, chitin. Considering that soil microorganisms, especially fungi have crucial role in accumulation and stabilization of organic matter, interaction of tannins with proteins and chitin may be of crucial importance for understanding the mechanisms behind soil carbon (C) stabilization. The newest insights in the field revealed that plant root-derived tannins interact with fungal necromass, rich in proteins and chitin. This interaction leads to fungal necromass stabilization in the soil. Thus, this novel explanation for C stabilization may be a hitherto overlooked mechanism that stabilizes microbial-derived C in boreal forest soils. This knowledge may be used in planning land use and management to mitigate climate change.

O.5.01 - DISRUPTION OF PROANTHOCYANIDIN BIOSYNTHESIS IN POPLAR ROOTS AND LEAVES BY CRISPR/CAS9

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Forest trees and other woody plants contain high concentrations of proanthocyanidins (condensed tannins) in leaves, bark, and roots; these compounds function in defense and as in vivo antioxidants during environmental stress. Trees in the genus Populus (poplars and aspens) including the widespread aspens P. tremula and P. tremuloides, are often ecologically important as keystone species. Hybrids of these two species grow rapidly and are excellent research tools. Furthermore, they are genetically transformable and their genomes can be edited using CRISPR/Cas9 technology. In this work, we used CRISPR/Cas9 to knock out two previously characterized regulators of PA biosynthesis, MYB115 and MYB134 [1], in a P. tremula x tremuloides hybrid. Multiple transgenic lines including both single knock-out and double knock-outs were generated. Successfully disrupted MYB genes had small insertions or deletions in both parental alleles, causing frameshift mutations and leading to inactive gene products. Phytochemical characterization of the single and knock-out lines revealed that in leaves, disruption of either gene caused a reduction of approximately 60% in PA concentration; in roots however, only the double knock-out plants showed a significant reduction of PAs. Analysis of catechin content corroborated this pattern. Likewise, analysis of gene expression for the key enzymes anthocyanidin reductase (ANR1) and leucoanthocyanidin reductase (LAR2) indicated that expression of both enzymes was reduced, paralleling the proanthocyanidin content for both the single and double knock-out plants. Surprisingly, the salicinoid phenolic glycosides of poplar including salicortin and tremulacin were also downregulated in leaves of double knock-outs; however, these compounds were upregulated in roots. Our work demonstrates that i) CRISPR in an effective strategy to modify secondary metabolites in poplar, ii) MYB134 and MYB115 transcription factors both contribute to control of PA biosynthesis, but additional regulators are also required, iii) the roles of these MYBs are distinct in roots compared to leaves, and iv) additional regulatory connections of proanthocyanidins with other branches of phenolic metabolism in poplar remain to be elucidated. Our knockout poplars plants will be important tools for studying the physiological and ecological roles of proanthocyanidins.

[1] James A.M., Ma D., Mellway R., Gesell A., Yoshida K., Walker V., Tran L., Stewart D., Reichelt M., Suvanto J., Salminen J.-P., Gershenzon J., Séguin, A. & Constabel C.P., *Plant Physiology*, 174, 154-171, (2017).

O.5.02 - MUTAGENESIS OF A DOUBLE BOUND REDUCTASE (MPDBR) GENE TRIGGERS AN ALTERNATIVE STILBENE PATHWAY IN *MARCHANTIA POLYMORPHA*

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Marchantia polymorpha (Marchantia) is a liverwort, belonging to the bryophyte division of land plant. The plant represents an emerging platform for synthetic biology due to its small genome size and ease of culture in both soil and axenic conditions. Marchantia is notably a rich source of phenolic compounds, including flavonoids (auronidins and flavones) and bis-bibenzyls (Figure 1). While significant progress has been made in understanding the biosynthetic and regulation of flavonoids in Marchantia [1], the bibenzyls (dihydrostilbene derivatives) have so far received little research attention, although they exhibit a wide range of biological activities, with potential for use as pharmaceuticals, agrochemicals and cosmetics. We have recently identified the enzyme responsible for the first step in bibenzyl biosynthesis in Marchantia (MpDBR) *i.e.,* a carbon-carbon double bound reductase producing a dihydro-precursor from p-coumaroyl-CoA, in an unusual reaction occurring only in a few plant species such as apple [2]. The impact of the CRISPR/Cas9-mediated mutagenesis of MpDBR at the metabolomics and transcriptomics level will be presented, revealing the synthesis of new-to-Marchantia stilbene derivatives.

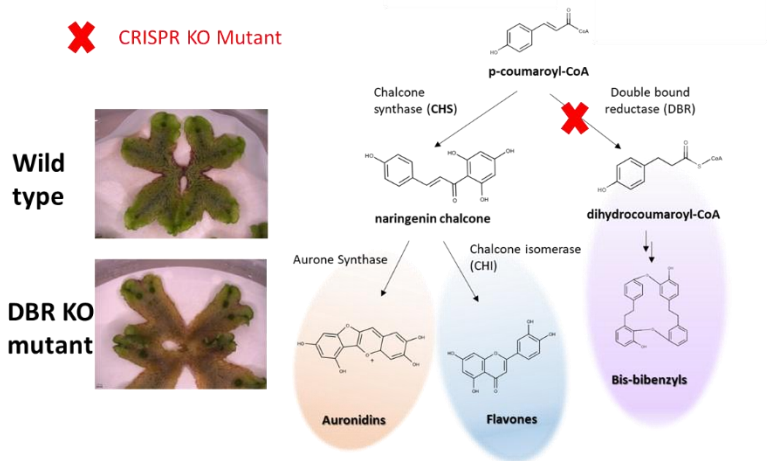

Figure 1: Phenylpropanoid pathway in the liverwort *Marchantia polymorpha. Mutagenesis of a double bound reductase (DBR) gene drastically affected the Marchantia metabolite profile.*

[1] Albert N., *et al.*, *New Phytologist*, 218, 554-566, 2018.
[2] Ibdah M., *et al.*, *Phytochemistry*, 107, 24-31, 2014.

O.5.03 - HETEROLOGOUS GENE EXPRESSION ENABLES BIOSYNTHESIS OF HYDROLYZABLE TANNIN PRECURSORS IN HERBACEOUS MODEL PLANTS

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Aluminum (Al) toxicity is the main factor limiting the elongation of plant roots on acid soils that cover about 30% of the total land area worldwide. The tree species *Eucalyptus camaldulensis* shows a 200–1000 times stronger Al resistance than herbaceous model plants. This extraordinary resistance level is caused by the detoxification capacity of hydrolyzable tannins (HT) like oenothein B accumulating in roots [1]. To elucidate the biosynthesis of oenothein B, we established a method for pathway reconstitution in the HT-nonaccumulating model plant *Nicotiana benthamiana*.

In *E. camaldulensis*, shikimate dehydrogenases (EcSDH2 and EcSDH3), which catalyze the reaction to synthesize gallic acid, and UDP glycosyltransferases (EcUGT84A25 and EcUGT84A26), which catalyze the reaction from gallic acid to β-glucogallin, have been identified as enzymes after branching from the shikimate pathway (Figure 1). Here, we describe *Agrobacterium*-mediated co-expression of *E. camaldulensis* genes *EcSDH2*, *EcSDH3*, *EcUGT84A25* and *EcUGT84A26* in leaves of *N. benthamiana*. Results showed that transgenic leave areas of *N. benthamiana* synthesized β-glucogallin, the universal metabolic precursor of HTs including oenothein B. In the future, we will use *N. benthamiana* leaves for transient co-expression with other candidate genes supposed to act more downstream in HT biosynthesis.

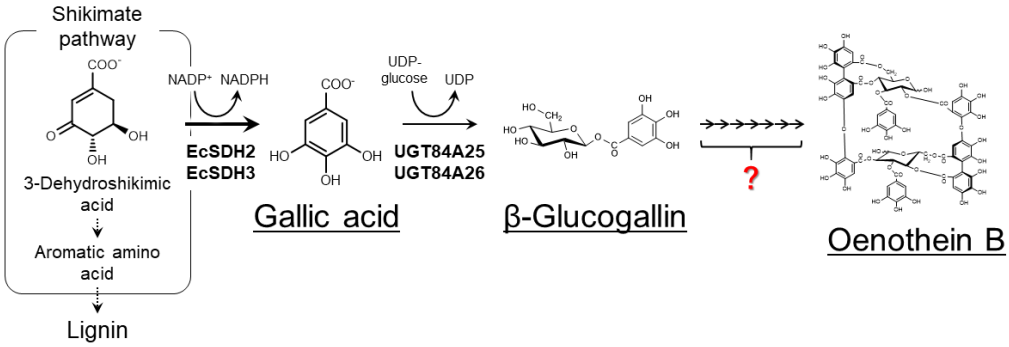

Figure 1: Biosynthetic pathway of hydrolyzable tannins in *Eucalyptus camaldulensis*.

[1] Tahara K., *et al.*, *Plant Physiology*, 164, 683-693, 2014.

O.5.04 - EXPLORATION OF SALVIGENIN BIOSYNTHESIS IN 'SAGE' PLANTS SALVIA

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Among the *Salvia* taxa widespread all over the world, 23 species are known to be present in Greece with *S. fruticosa* and *S. officinalis* mostly used as spices, beverages and in traditional medicine [1]. Due to their bioactivities (antimicrobial, antioxidant, neuro-protective, antiproliferative, anti-cholinesterase *etc.*), both species have been revealed as important aromatic/medicinal and commercial plants. Flavones are among the most abundant polyphenolic compounds identified in many Lamiaceae species. Characteristic flavone derivatives described in *Salvia* are salvigenin (SAL; 5-hydroxy-6,7,4'-trimethoxyflavone) and its glycosides. The putative biosynthetic pathway leading to SAL was described in basil (*Ocimum basilicum* L.) [2]. While apigenin, appears to be the general flavone precursor, multiple routes with varying orders of hydroxylation and methylation seem to be involved in the synthesis of SAL in basil. Despite the reported bioactivities of SAL and its derivatives and the occurrence of the metabolites in *Salvia*, studies on quantitative targeted metabolomics are still scarce and the validation and elucidation of the biosynthetic pathway remains unknown yet.
From our in-house available RNA-seq dataset of *S. fruticosa,* we were able to retrieve blast hits with high homology to orthologous genes of the predicted SAL biosynthetic pathway, namely, flavone synthase II (FNSII), flavone-6-hydroxylase (F6H) and two methyl-*O*-transferases (OMTs). The open reading frames of the four genes from *S. fruticosa* were cloned in appropriate vectors and overexpressed in *Saccharomyces cerevisiae*, and the gene products were identified as apigenin and genkwanin suggesting the functional characterization of two genes, *FNSII* and *7-OMT,* respectively. On-going experiments are in process to characterize the remaining genes.
Apart from the importance of unraveling the biosynthetic steps through single gene characterization, our work is currently focused on the reconstruction of the entire pathway of SAL biosynthesis using *de novo* synthesis and validation via multi-step combinatorial (bio)synthesis in yeast. Additionally, to functionally validate the activity of the SAL genes *in planta,* the sophisticated tool of the gene editing in non-model plant species of *S. fruticosa* is undertaken for the first time.

[1] Karousou R., Hanlidou E. & Kokkini S., The sage plants of Greece: distribution and infraspecific variation, In *Sage: the genus Salvia* (Kintzios S.E., Ed), Taylor & Francis, Chapter 2, 27-53, https://doi.org/10.1201/9780203304556, 2000.
[2] Berim A. & Gang D.R., *Journal of Biological Chemistry*, *288*, 1795-1805, 2013.

O.5.05 - DIRIGENT PROTEIN SUBFAMILY MEMBERS AS GATEWAYS TO POLYPHENOL PTEROCARPAN AND ISOFLAVENE PLANT DEFENSES

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Dirigent protein (DP) biochemical activities, a class of proteins we discovered, give rise to distinct complex classes of plant polyphenols. DPs apparently emerged during the aquatic-to-land transition of plant life, with phylogenetic analyses revealing the presence of numerous DP subfamilies in the plant kingdom. The vast majority (>95%) of DPs in these large sub-families still await discovery of their biochemical functions. Here, we solved structures of isoflavene- and pterocarpan-forming proteins with dirigent-like domains, PsPTS2, PsPTS1 and GePTS1, by X-ray crystallography to high resolution (Figure 1). PsPTS2 produces both plant defense pterocarpans and isoflavenes, with the latter perhaps undergoing subsequent metabolism to generate the opposite diastereomeric plant defense pterocarpan analogs, e.g. (+)-pisatin. PsPTS1 and GePTS1, by contrast, stereospecifically convert diastereomeric chiral isoflavonoids into chiral pterocarpans [1,2].

The PTS structures enabled comparisons with stereoselective lignan-forming DPs DRR206 and AtDIR6, and an aromatic terpenoid-forming DP ortholog GhDIR4, given that each DP subfamily member can provide entry into distinct plant polyphenol natural product classes. Our experiments suggest a common biochemical mechanism in binding and stabilizing distinct plant phenol-derived mono- and bis-quinone methide intermediates during different C–C and C–O bond forming processes. [Apart from PTS, however, the intermediate in couplings mediated by lignan-forming DPs is a resonance-stabilized quinone methide radical whose formation *in vivo* requires an oxidase.] These observations provide key insights into both DP emergence and functional diversification during land plant evolution/adaptation, and also important clues as to how additional physiological polyphenol roles for DPs and proteins harboring dirigent-like domains can now be rationally and systematically identified.

Supported by a grant from the National Institute of Food and Agriculture, United States Department of Agriculture: 2022-67013-37046.

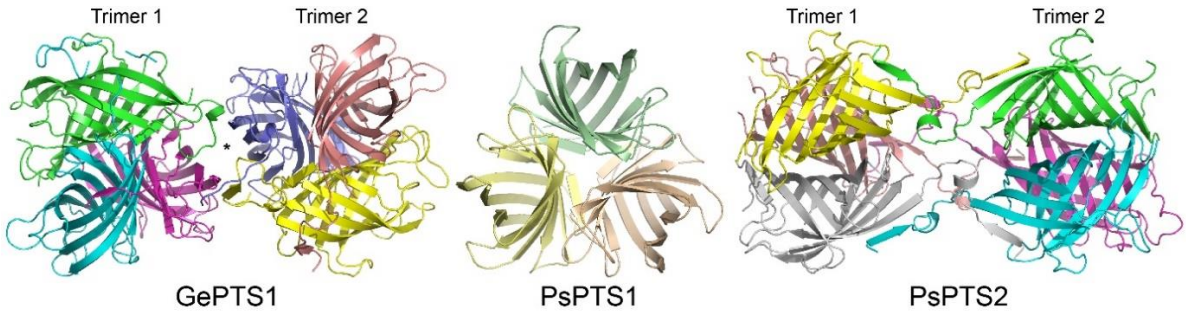

Figure 1: Ribbon representations of DIR-b/d sub-family DP (PST) structures.

[1] Meng Q., Moinuddin S.G.A., Kim S.J., Bedgar D.L., Costa M.A., Thomas D.G., Young R.P., Smith C.A., Cort J.R., Davin L.B. & Lewis N.G., *J. Biol. Chem.*, 295, 11584-11601, 2020.
[2] Davin L.B., Cort J.R., Smith C.A., Meng Q.M., Moinuddin S.G.A., Costa M.A., Bedgar, D.L. & Lewis N.G., Dirigent Protein Roadmap to Lignans and Other Vascular Plant Phenol Classes, In *The Lignan Handbook* (Lewis N.G., Davin L.B., Munasinghe V.R.N. & Roberts A.D., Eds), Taylor & Francis, Chapter 4, 57-77, https://doi.org/10.1201/9781315152523, 2022.

OY.5.01 - DISCOVERY OF A DIHYDROCHALCONE 3-HYDROXYLASE (DHC3H) FROM WILD *MALUS* SPECIES THAT PRODUCES SIEBOLDIN *IN VIVO* BY *DE NOVO* TRANSCRIPTOME ASSEMBLY AND FUNCTIONAL ANALYSIS

Miranda S.*[a,b,c], Lagrèze J.[a,b], Knoll A.S.[a], Angeli A.[a], Espley R.[a], Dare A.[a], Malnoy M.[a], Martens S.[a]

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Dihydochalcones (DHCs) are specialized metabolites with a limited natural distribution, found in significant amounts in *Malus* × *domestica* Borkh. (apple) and *Malus* species. Among them, *M.* × *domestica* accumulates significant amounts of phloridzin, whilst trilobatin and sieboldin are abundant in wild relatives. DHCs have demonstrated a wide range of bioactive properties in biomedical models, such as anti-diabetic and antioxidant. According to the currently proposed DHC pathway, 3-hydroxyphloretin, the key precursor of sieboldin in *Malus* species, would also be an intermediate compound towards the biosynthesis of the flavour enhancer neohesperidin DHC. However, little is known about how these metabolites are biosynthesised and what is their function *in planta*.

DHC pathway diverts from the main phenylpropanoid pathway from *p*-coumaroyl-CoA by the action of a postulated double bond reductase (DBR). Then, chalcone synthase (CHS) catalyses the condensation of *p*-dihydrocoumaroyl-CoA to phloretin. Phloretin can be directly glycosylated at position 2′- or 4′ to produce phloridzin or trilobatin, respectively. PGT1 and PGT2 have been identified as 2′- and 4′-*O*-UDP-glycosyltransferases responsible for the respective synthesis of phloridzin and trilobatin. However, sieboldin has been postulated to derive from hydroxylation in position 3 of phloretin before been glycosylated. In this study, we aimed to identify candidate genes that may account for the 3-hydroxylation of phloretin (DHC3H), involved in DHC formation of wild *Malus* species. To this end, we combined transcriptomic analysis and a *de novo* transcriptome assembly to characterise two putative 3-hydroxylases in two wild *Malus* species (*Malus toringo* (K. Koch) Carriere syn. *sieboldii* Rehder, *Malus micromalus* Makino) whose DHC profile is dominated by sieboldin. We assessed the *in vivo* activity of putative candidates to produce 3-hydroxyphloretin and sieboldin by *de novo* production in *Saccharomyces cerevisiae*. We found that CYP98A proteins of wild *Malus* accessions (CYP98A195, *M. toringo* and CYP98A196, *M. micromalus*) were able to produce 3-hydroxyphloretin, leading to sieboldin production by co-expression with PGT2. CYP98A197-198 genes of *M.* × *domestica*, however, were unable to hydroxylate phloretin *in vivo*. CYP98A195-196 proteins exerting 3-hydroxylase activity co-localised with an endoplasmic reticulum marker. Using phloretin for protein docking modelling, CYP98A protein model from wild accessions showed mutations in key residues close to the ligand pocket predicted. These mutations were located within known substrate recognition sites of cytochrome P450s, which could explain the acceptance of phloretin in CYP98A protein of wild accessions. In addition, we developed a HRM marker for CYP98A isoforms to screen a *Malus* germplasm collection by HRM marker analysis, and identified three clusters that corresponded to the alleles of domesticated and wild species. Moreover, CYP98A isoforms identified by HRM analysis correlated with the accumulation of sieboldin in other wild and hybrid *Malus* genotypes. Taken together, we provide the first evidence of an enzyme isolated from wild *Malus* accessions producing sieboldin that could be involved in the key hydroxylation step towards the synthesis of sieboldin in *Malus* species.

OY.5.02 - CROWN PROCYANIDINS: LOCALISATION, EVOLUTION AND A NEW BIOSYNTHESIS APPROACH?

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Polyphenols plays an important role in plants with specific properties [1]. They are widely distributed all over the plant kingdom and in the plants themselves. Their evolution during ripening and their biosynthesis have been widely studied. In the grapevine, condensed tannins are presents all around the plant from the roots to the leaves. Condensed tannins localization and accumulation during development are directly linked with the biosynthesis activities in the different organs of the plants. Recently, a new sub-class of condensed tannins have been identified and characterized in the wine named crown procyanidins. The macrocyclic structure with an inside cavity has been determined by NMR [2]. Their properties, such as the neuroprotective activities or their resistance to oxidation [3], make this molecule unique. Crown procyanidins properties in wine was studied previously. Our investigation was to localize crown procyanidins, tetramer and pentamer, in the entire grapevine, to follow their evolution and finally to understand their origin in the grapevine. Various grape vine samples and grape skin samples at different maturity stages have been analysed. Detection and quantification have been realised by UPLC-UV-Q-TOF. Crown procyanidins have been detected in various organs of the grapevine such as vine branch, roots, leaves and skins of the grape berries. Different concentration and proportion of crown tetramer and pentamer have been reported all over the plant (Figure 1). Crown procyanidins exhited the highest concentration in the skin of the grape berries. During the ripening, in grape skin, crown procyanidins accumulated whereas non-cyclic tannins, with a similar mDP, decreased. As conclusion of all the analysed samples, a crown procyanidins biosynthesis have been hypothesized with a combination of enzymatic and chemical reaction in the biosynthesis pathway.

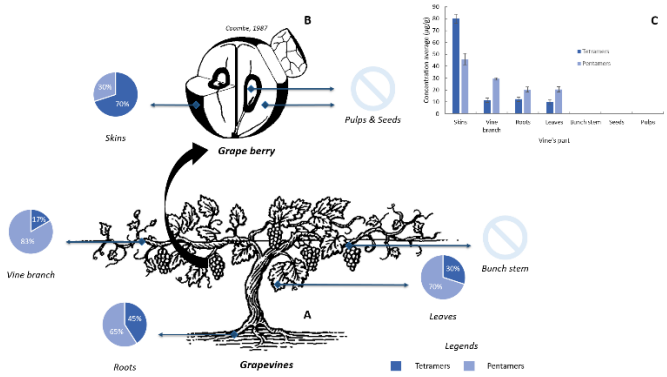

Figure 1: Localisation of the crown procyanidins in the grapevine.

[1] Beecher G.R., *Pharmaceutical Biology*, 42, 2–20, 2004.
[2] Zeng L., Pons-Mercadé P., Richard T., Krisa S., Teissèdre P.-L. & Jourdes M., *Molecules*, 24, 1915, 1-10, 2019.
[3] Jouin A., Zeng L., Canosa M.R., Teissedre P.-L. & Jourdes M., *Foods*, 11, 3194, 1-11, 2022.

OY.5.03 - EFFECTS OF WOUNDING AND WATER LOSS ON THE ACCUMULATION OF CAFFEOYLQUINIC ACIDS IN FORCED CHICORY ROOTS

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Forced chicory roots (FCR) are a highly interesting biomass because these least valued by-products of Belgian endive culture are also a valuable source of phenolic compounds such as caffeoylquinic acids (CQAs). The two main CQAs identified are chlorogenic acid (5-CQA) and dicaffeoylquinic acids (diCQAs). These two molecules have many interesting biological activities (antioxidant, antibacterial, anti-inflammatory and anti-UV). However, the limited content of these compounds in the plant is an issue for their extraction. An alternative to reduce these disadvantages is to increase the concentration of these metabolites during the post-harvest phase. The increase of metabolites can be made by the application of abiotic stress such as wounding, UV or drought [1]. The objective of this work is to identify the mechanisms during drying and wounding that explain the variations in CQAs content and to use it to maximise the content of CQAs. To maximise diCQAs content, water loss must be avoided. Thus, after a wounding treatment, FCR should be stored at room temperature and the extraction should be performed when water loss begins. To maximise 5-CQA content, a slow water loss after a wounding treatment should be performed. So, FCR should be dried at room temperature to achieved a slow water loss and the extraction should be performed at the end of the drying. In all cases, water loss and temperature should be carefully managed to avoid degradation of CQAs by biological activities. Furthermore, two main competitive mechanisms are highlighted to regulate the CQAs content in the biomass: the biosynthesis of CQAs by the phenylpropanoid pathway and the consumption by the oxidation/oligomerisation of CQAs.At optimal condition, wounding and water loss treatment significantly ($p<0.05$) increased the concentration of CQAs in FCR. In particular, 5-CQA content was 3.3-fold higher after wounding and 244 hours of drying at room temperature. For diCQAs, the content was 2.3-fold higher after wounding and 71 hours of drying at room temperature (Figure 1).

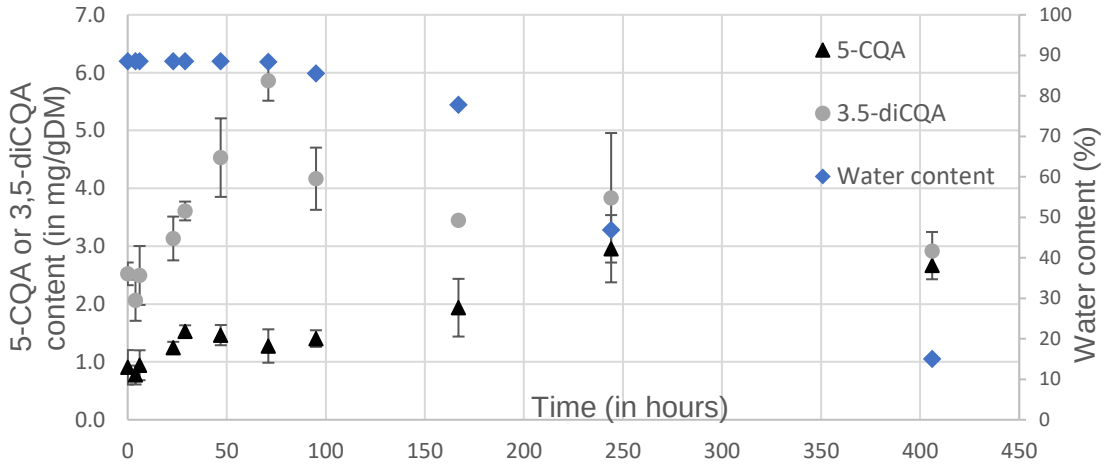

Figure 1: Evolution of CQAs content during drying at room temperature

[1] Becerra-Moreno A., Redondo-Gil M., Benavides J., Nair V., Cisneros-Zevallos L. & Jacobo-Velázquez D.A., *Frontiers in Plant Science*, 6, 837, https://doi.org/10.3389/fpls.2015.00837, 2015.

P.5.01 - STUDY OF GENISTEIN METABOLON STRUCTURE IN GLYCINE MAX: A DUAL EXPERIMENTAL AND THEORETICAL APPROACH

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Isoflavonoids are specialized metabolites; they could be phytoalexins and attractants serving plant ecological fitness; they were reported to play a role in root nodulation [1]. Among them, genistein is of major interest because of its potential application in estrogen-dependent cancer chemotherapy. It can, in particular, be found in high concentration in soya (Glycine max), which has high genistein content per gram of dry mass. Genistein, as well as other metabolites of the isoflavonoid family, are produced via enzymes of the isoflavonoid branch of the phenylpropanoid pathway. It is now admitted that these proteins aggregate to form a metabolon – an enzymatic association that facilitates the substrate channeling and accelerates the metabolic flux (Figure 1).

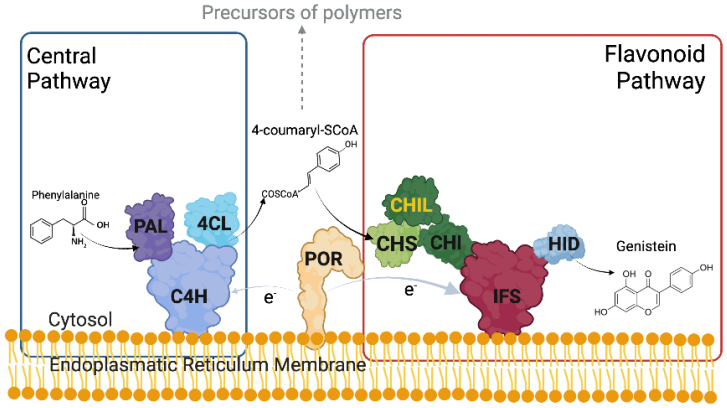

Figure 1: Schematic representation of the phenylpropanoid pathway and the proteins involved in the genistein biosynthesis.

The essential components of genistein functional biosynthetic pathway in soya are known and have been previously investigated independently [2]. Nevertheless, the exact architecture of the metabolon remains obscure. In this project, we aim to build the first model of enzyme organization within the metabolon involved in genistein biosynthesis taking benefits from the synergy between biochemical, molecular biology, and computational methods. Moreover, the project investigates non-catalytical protein candidates potentially playing an allosteric role in biosynthesis. The candidate genes of the metabolon proteins will be expressed in a heterologous system, and the interaction between the proteins will be verified by protein-protein interaction assay. The theoretical computer model of the metabolon structure will be further built based on the experimental results. At the same time, the laboratory experiments are guided by the results of molecular dynamics simulations and protein docking obtained *in silico*.

This project has received financial support from the CNRS through the MITI interdisciplinary programs.

[1] Yonekura-Sakakibara K., Higashi Y. & Nakabayashi R., *Front. Plant Sci.*, 10, 943, https://doi.org/10.3389/fpls.2019.00943, 2019.
[2] Dastmalchi M., Bernards M.A. & Dhaubhadel S., *The Plant Journal*, 85, 6, 689-706, 2016.

P.5.02 - GROUP-SPECIFIC MASS SPECTROMETRY MAPPING OF PLANT POLYPHENOLS

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Every plant species produces a unique combination of polyphenols for their own protection against UV radiation, pathogens and herbivores. The history of chemical plant research has revealed nearly ubiquitous occurrence of polyphenols in vascular plants, but the plant kingdom is still full of unexplored species and unrevealed polyphenol combinations.
As polyphenols' value has stabilized due to their anti-herbivore activity, health benefits and medicinal applications, new biologically active compounds are studied and searched constantly. There is a growing need to screen increasing amount of plant species and their polyphenol content. Simultaneously, new efficient analytical methods and automatization enable faster analysis of very large data sets, highlighting the importance of the data handling and interpretation. New tools to handle and visualize large data sets are needed.
We have created a new mass spectrometric fingerprint mapping tool to visualize the distribution of different polyphenol classes and related bioactivities in plants. Our tool creates discriminating contrast for every selected variable at any concentration level. This allows us to compare different polyphenol groups and activity types at the same scale in one chart.
To test our tool we studied the polyphenols and two important bioactivities within chemically diverse plant populations. We screened 30 plant species with diverse growth forms and evolutionary ages, and additionally monitored 10 of these species three times over three seasons. Eight major polyphenol groups were measured using the group-specific UPLC-DAD-MS/MS method [1, 2]. The bioactivities were tested with the oxidative activity assay and the radial diffusion assay.
The results revealed unique MS-fingerprints for each species and allow us to estimate the variation level within populations and seasons. Our tool enables the addition of variables and can be applied for example to different compound groups, taxonomic levels, ecological treatments and time series.

[1] Engström M.T., Pälijärvi M., Fryganas C., Grabber J.H., Mueller-Harvey I. & Salminen J.-P., *J. Agric. Food Chem.*, 62, 3390–3399, 2014.
[2] Engström M.T., Pälijärvi M. & Salminen J.-P., *J. Agric. Food Chem.*, 63, 4068–4079, 2015.

P.5.03 - INSECT HERBIVORES AS MODIFIERS OF PROANTHOCYANIDIN POLYMERS

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The plant kingdom contains a huge variety of oligomeric and polymeric proanthocyanidins that vary in their stability and bioactivity depending on their structural details. Previously we have shown how especially the polymer size and the high prodelphinidin rather than procyanidin share are important for e.g. anthelmintic and antimethanogenic activity, protein precipitation capacity, and oxidative activity of proanthocyanidins [1, 2]. Furthermore, we have attempted to improve the chemical properties of plant proanthocyanidins by their chemical modification in vitro, thus trying to turn inactive compounds into more active ones [3].
Insect herbivores are attractive sources of new compounds not found in plants, as they may modify plant defences and use them for their own benefit. On the other hand, plant metabolites may be modified also by plant oxidative enzymes and the alkaline gut found especially with the lepidopteran larvae. These modifications may yield e.g. larger proantocyanidin polymers by oxidative coupling between the natural oligomers and polymers thus potentially increasing their bioactivities linked to especially their protein precipitation capacity.
Here we used tens of insect species in their larval stages to study how they modify plant proantocyanidins in vivo. We fed the larvae with leaves of their respective host plant species, and analysed both leaves and frass for their bioactivities with two well-plate assays and proanthocyanidin profiles with the 2D fingerprinting tool developed for ultrahigh performance liquid chromatography triple quadrupole mass spectrometry. To study the possible sources of proanthocyanidin modification, we estimated both the enzymatic oxidative activity of leaves and the pH of the larval gut. In our poster, we show the chromatographic and mass spectrometric profiles of different types of proanthocyanidins before and after feeding and give plausible explanations for their observed chemical modifications in each specific case.

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P.5.04 - IMPACT OF FUNGAL AND PLANT LACCASES ON PLANT CELL WALL PROPERTIES AND STRUCTURE

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Plant cell walls are made up of complex assemblies of biomolecules that largely determine the biological functions and properties of plants. Among these molecules, lignins are key bio-polymers in the structure of plant cell wall because they confer rigidity to fibers and hydrophobicity to vessels. However, these features impede biomass deconstruction and the industrial processes used to remove lignins are polluting and expensive. Lignins are obtained from the polymerization of three types of phenolic compounds called monolignols, the *p*-coumaryl, coniferyl and sinapyl alcohols, which once assembled give rise to the *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units. They are linked by various chemical bonds. While monolignols are synthesized in the cytosol, spontaneous reactions governed by oxidizing enzymes (peroxidase and laccase) take place in the cell wall. Plant laccases belong to a multigene family and contribute to the unique composition of lignin due to their location in different tissues, plant species and their possible substrates specificity for monolignols [1]. Interestingly, it seems that lignin depolymerization by fungi is also under the control of laccases. This opposite biological role is questioning. Considering that fungal laccases are often used in industrial processes and might be used for biomass deconstruction [2], it remains unclear whether fungal laccases can impact lignification *in planta* or not. In this project, we propose to test this hypothesis.

In order to answer this question, we expressed a laccase from *Pycnoporus cinnabarinus* (white-rot decomposer) in *Arabidopsis thaliana* both in wild-type and in *Atlac4xlac17* double mutant genetic backgrounds. Thanks to the addition of the mCherry fused to the fungal laccase, we were able to detect the presence of the chimeric protein in vessels with confocal imaging. Preliminary results show a lower lignin content in genetically modified plants. Other compositional cell wall analyses as well as multi-scale and multimodal spectral imaging approaches are currently carried out. Results will be shown on this poster.

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P.5.05 - STRUCTURAL STUDY OF STRAWBERRY ALLERGENS WITH LIGAND FLAVONOIDS

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Fra a 1 is one of strawberry allergen that causes oral allergic syndrome (OAS), also known as pollen food allergy syndrome. Fra a 1 belongs to pathogenesis-related proteins (PR-10), which is involved in plant biological defense [1], known as major allergen of OAS. PR-10 proteins have been reported to have a hydrophobic region which can bind various type of polyphenols. Fra a 1 has been reported to bind flavonoids from the result of isothermal titration calorimetry [2]. Although crystallization of Fra a 1 and flavonoid complex has been tried, X-ray crystallography has not been performed with some isoforms probably due to their instability. For investigation of protein-ligand complex, NMR is powerful tool and potentially applicable to unstable Fra a 1 proteins. In this study we performed NMR analysis for the mixture of Fra a 1 and flavonoids.

For the preparation of Fra a 1 isoforms, using previously prepared cDNAs, expression vectors were constructed. To obtain the proteins with ^{15}N, the constructed vectors were transformed into *E. coli* BL21(DE3) and were inoculated into M9 minimal medium with $^{15}NH_4Cl$ as the only source of nitrogen. The protein samples were purified by the standard method. Quercetin, myricetin and other flavonoids were purchased as reagent grade and used without further purification. NMR experiments were performed at 25℃ using Bruker Avance Ⅲ 600 MHz spectrometer equipped with a cryoprobe prodigy. ^{1}H-^{15}N HSQC spectra were obtained with 100 μM ^{15}N-labelled Fra a 1 proteins in 10mM sodium phosphate (pH 7.4), 10mM NaCl, 10% D_2O.

NMR analysis was targeted to two Fra a 1 isoforms, 1.01 and 1.02. The HSQC of Fra a 1.01 has been reported previously and in the present study similar HSQC spectra were observed (Figure 1). The ^{1}H-^{15}N HSQC spectra of Fra a 1.02 was obtained as a properly folded protein. In the presence of myricetin, several peaks were moved, suggesting that binding of some amino acid residues with myricetin occurred. The amino acid residues that interact with the ligand were predicted by comparing the experimental chemical shifts to the predicted chemical shifts for each amino acid. In the presentation, we will show the ^{1}H-^{15}N HSQC spectra in the presence/absence of ligand and the predicted binding site.

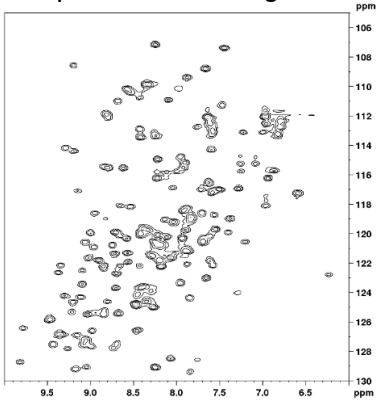

Figure 1: ^{1}H-^{15}N HSQC NMR spectrum of Fra a 1.01

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P.5.06 - STABILIZATION OF CARBON IN THE SOIL VIA INTERACTION OF TANNINS AND MICROORGANISMS UNDER NITROGEN FERTILIZATION

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Tannins are quantitatively significant pools of carbon (C) in foliage, bark and roots of trees. Recent evidence show that tannins may play an important role in plant-soil-microbe interactions with a significant effect on soil C pools. Due to the threat of climate change, we must determine mechanisms behind C stabilization in the soil to mitigate global warming [1].

Forest trees are heavily dependent on symbiosis with mycorrhizal fungi, as the hyphal network significantly improves the uptake of water and nutrients. Recently it was proven that root-tannins stabilize fungal necromass (=dead biomass) via ability of tannins to form complexes with proteins and chitin, thus providing a potentially significant mechanism of C stabilization in the soil [2].

Forest soil C pools are exposed to significant fluctuations due to both natural disturbances and also via forest management. However, how do these practices affect C soil stabilization via interaction with tannins? Here we provide insights into one of the promising forest management aiming to increase C in the soil – nitrogen fertilization in boreal forests [3]. Long-term experiments in Finland with N fertilization provided us explicit conditions to understand impact of N fertilization on soil C stabilization mechanisms, including mechanism governed by root-derived tannins. We analyze soil functioning, soil biome and greenhouse gases exchange to better understand the effect of N fertilization on C stabilization. Though N fertilization improved pool of C in the soil, concomitantly it also decreased tannin production in the roots and concentration of tannins in the soil. Potential changes in root-tannin pool could affect not only soil C pools but also vulnerability to a vast number of disturbances, including insect outbreaks.

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P.5.07 - HETEROLOGOUS PRODUCTION IN *ASPERGILLUS NIGER* AND CHARACTERIZATION OF TWO PLANT LACCASES FROM *ARABIDOPSIS THALIANA*

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Plant laccases catalyze the oxidation of monolignols, a key reaction for lignification in plants. This process reinforces the cell walls of many different cell types in plants, providing mechanical support, nutrient transportation, and pathogen defense. It is still unclear how different plant laccases oxidize monolignols and play specific roles in various physiological processes in plants. There is little information in the literature about the potential biological functions of plant laccases. As an example, only one 3D structure of plant laccase is known to date, corresponding to a laccase from *Zea mays* (ZmLAC3) [1]. Plant laccases are the members of large multigene families. They are highly redundant in plants, and it is believed that not all of them are involved in lignin metabolism. There are 17 laccase genes in *Arabidopsis thaliana* (Arabidopsis), 29 in *Brachypodium distachyon* (Brachypodium) and 53 in *Populus trichocarpa* (poplar). The role of laccases in the lignification of Arabidopsis stems was only recently established. The stems of AtLAC4 and AtLAC17 loss-of-function mutants had moderately reduced lignin levels, whereas the *lac4lac17* double mutant stems had 40% less lignin and an irregular xylem phenotype [2]. This lack of functional and structural studies, combined with family member redundancy, limits our understanding of why such a disproportionately large number of laccase genes evolved during plant evolution. Because gene duplication is a known mechanism for increasing functional diversity, sub- and neofunctionalization events are expected to be found within the laccase gene family of various plant species. As a result of different catalytic affinity of laccases for phenolic compounds, the expansion of laccase gene families in plants is expected to be accompanied by extensive functional diversification, particularly in phenolic metabolism [3].

This work concerns the production and characterization of two plant enzymes, AtLAC4 and AtLAC17 which are strong candidates to be involved in lignin polymerization. The laccase-encoding gene for AtLAC4 a*nd* AtLAC17 were cloned for heterologous expression in *Aspergillus niger* D15#26, to produce protein in the extracellular medium. Purified AtLAC4 and AtLAC17 will be then functionally and structurally characterized using biochemical approaches and protein crystallography. These are the premises to unveil the structural determinants of enzymatic properties in plant laccases.

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P.5.08 - TANNIN AND IRON-REACTIVE PHENOLIC CONTENT OF FOUR GRAPE CULTIVARS THROUGHOUT DEVELOPMENT AND RIPENING

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Polyphenolic compounds, especially condensed tannins and anthocyanins, contribute to overall wine quality. Red wines made from cold-hardy hybrid grape varieties have much lower tannin content than red wines made from *Vitis vinifera* cultivars. This could be attributed to different initial content of tannins in skins and seeds. Although the tannin content of different grape berry tissues has been investigated for *V. vinifera* varieties [1], the berry chemistry of cold-hardy hybrids is poorly understood and studied. Investigating the tannin and phenolics content of cold-hardy hybrids throughout berry development and ripening will help illuminate pathways for optimizing viticultural and winemaking practices to increase red wine quality made from those varieties.

In this study, skins and seeds of three cold-hardy hybrid grape cultivars (Crimson Pearl, Marquette, and Petite Pearl) and *V. vinifera* cv. Pinot noir were separated at five to six phenological time points from one-week post fruit-set to harvest in 2022. After extraction using 70% acetone with trifluoroacetic acid, tannin and iron-reactive phenolic (IRP) contents were quantified using RP-HPLC-DAD and UV-Vis spectrophotometry, respectively.

The content of both tannin and IRP in seeds of all cultivars was the highest one-week post fruit-set and drastically decreased three weeks post fruit-set. The content of seed tannins and IRP slightly increased pre-véraison to decrease again until harvest. The hybrid varieties contained less tannin and IRP than 'Pinot noir' at each phenological time point. After normalization, a greater decrease in tannin content between one-week post fruit-set and later time points for cold-hardy hybrids was observed compared to 'Pinot noir'. First and second order rate equations were investigated to explain the net decrease in phenolics observed from one-week post fruit-set to harvest, but neither model was able to explain the change, which indicated a more complex dynamic during development and ripening. Investigation of tannin and IRP content in skins is ongoing. The cold-hardy hybrid varieties evaluated in this study exhibit a similar trend to *V. vinifera* cv. Pinot noir but have overall lower phenolic content. This could be due to a different regulation of genes involved in the biosynthesis of tannins or to a lower extractability and solubility.

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P.5.09 - GENETIC TRANSFORMATION OF EUCALYPTUS CAMALDULENSIS TO SUPPRESS HYDROLYZABLE TANNIN BIOSYNTHESIS

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Eucalyptus camaldulensis has high resistance to aluminum toxicity, a major limiting factor for plant growth in acidic soils. We previously discovered a novel role for the hydrolyzable tannin, oenothein B, as an aluminum-detoxifying compound in the roots of *E. camaldulensis* [1]. The aim of this study was to generate transgenic *E. camaldulensis* with reduced levels of hydrolyzable tannin biosynthesis in order to better understand the roles of hydrolyzable tannins in aluminum resistance.
Because the genetic transformation of *Eucalyptus* trees remains challenging, we improved the method for *Agrobacterium*-mediated transformation of *E. camaldulensis* by optimizing seed sterilization, explant preparation, and kanamycin selection. We observed that these optimizations led to successful transformation and regeneration of about 3% of hypocotyl explants (Figure 1). We applied the improved transformation method to suppress hydrolyzable tannin biosynthesis in *E. camaldulensis* by antisense or RNAi-mediated knockdown of UGT84A25 and UGT84A26. These genes encode UDP-glycosyltransferases, which catalyze β-glucogallin formation, the second step in hydrolyzable tannin biosynthesis [2]. We thereby generated transgenic lines with reduced UGT84A25 and UGT84A26 expression and lower concentrations of hydrolyzable tannins compared with empty vector control lines. These transgenic lines may be useful to further our understanding of the physiological functions of hydrolyzable tannins in plants, including aluminum resistance.

Wild type Transformant

Figure 1 : Over-expression of GUS reporter gene in *Eucalyptus camaldulensis* transformant

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P.5.10 - TRANSCRIPTOMICS AND METABOLITE PROFING TO ELUCIDATED ANTHOCYANIN BIOSYNTHESIS IN RED CHEMOTYPE OF *SIDERITIS RAESERI*

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Sideritis raeseri Boiss. & Heldr., also known as Greek Mountain Tea, is an endemic plant confined to Greece. The genus Sideritis belongs to the family of Lamiaceae and includes annual or perennial herbaceous plants of mountainous and (sub-) alpine zones of the Euro-Mediterranean area [1]. Furthermore, it constitutes a rich source of natural pharmacologically active compounds. Several *in vitro* and *in vivo* studies have suggested that *S. raeseri* exhibits hypotensive and vasodilatory actions and exerts vasoprotective and gastroprotective effects. Due to its bioactive compounds and well-established use in traditional and modern phytomedicine *S. raeseri* has a great potential for the development of herbal or botanical drug products. However, as a result of the increasing demand from industry and to avoid an overexploitation of natural populations, the cultivation of *S. raeseri* genotypes becomes more and more popular in mountainous areas in Greece. Interestingly, red coloured bracts chemotypes occur frequently within such cultivation, resulting in brownish stems after drying. Such phenotypes are considered of low quality by the consumers and decreasing the market price. To understand the regulation and genetic control of anthocyanin pigmentation in bracts of *S. raeseri* ecotypes, chemical analysis applying LC-MS/MS with MRM quantification and gene expression analysis using RNAseq as well as *de novo* transcriptome assembly was employed to identify expressed structural and regulatory genes of the anthocyanin pathway in different tissues.
Metabolite profiling of methanolic extracts of bracts revealed the presence of various cyanidin derivatives in red tissues while there were absent or below detection level in green tissues. Additionally, the flavone content was in a similar range. Orthologs coding for putative enzymes were retrieved from a *de novo* transcriptome assembly, for the late anthocyanin pathway, (i.e. dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS) and anthocyanin 3-*O*-ß-glucosyltransferase (A3GT)), for enzymes involved in the entire phenylpropanoid and flavonoid pathway, (i.e. phenylalanine ammonia lyase (PAL), chalcone synthase (CHS) and flavonoid 3'-hydroxylase (F3'H)) as well as two well-known transcription factors, (i.e. MYB10 and bHLH), by alignment to characterised sequences as queries. Gene expression analysis was performed in four different tissues of both ecotypes (apical, intermediate, and basal bracts plus leaves) using qPCR. Open reading frames of structural genes were subcloned in yeast expression vectors for functional characterisation of the encoded proteins. The results will lead to a better understanding of the control and regulation of the undesired anthocyanin synthesis in *S. raeseri* ecotypes and enable the development of molecular markers for pre-selection of green ecotypes for cultivation.

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P.5.11 - COMPLEX FLAVONOID ACCUMULATION PROFILES AND REGULATION IN VACCINIUM FRUIT

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Vaccinium berries boast "superfood" status, owing to their impressive content of polyphenols and the human health benefits these provide. This includes flavonoids, such as anthocyanin pigments, proanthocyanidins and flavonols, and phenolics such as chlorogenic acids and stilbenes. Different types of polyphenols are produced at distinct developmental stages and in particular tissue types. For example, proanthocyanidins are produced during early stages of fruit development to deter herbivory prior to seed maturation, while anthocyanins are produced at ripening to attract seed distributers (Figure 1). These differences are primarily controlled at the transcriptional level. Regulatory genes controlling parts of the flavonoid pathway have been identified and characterised in blueberry (*V. corymbosum*) and bilberry (*V. myrtillus*) [1,2]. This includes the *MYBA* (SG6), *MYBPA1*, *MYBPA2* (SG5) genes, which operate within MYB-bHLH-WDR (MBW) complexes. We have characterised the roles for these genes for flavonoid regulation during berry development and have identified new aspects of the MBW regulation networks [3]. This includes hierarchical regulation of *MYBPA1* genes by MYBA and MYBPA2 regulators, which is necessary for coordinated regulation of the flavonoid biosynthetic pathway to produce metabolites at the correct time and in the correct location (Figure 1).

We provide an update on recent advances in flavonoid regulation in *Vaccinium*, making use of new genomic resources and germplasm. This includes examining the mechanisms that contribute to differences in spatial regulation of anthocyanins between blueberries (white-flesh) and bilberries (red-flesh).

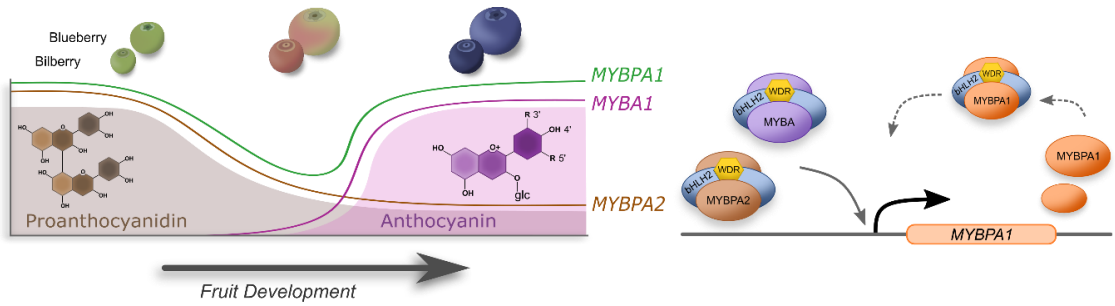

Figure 1: Hierarchical control of *MYBPA1* for proanthocyanidin and anthocyanin biosynthesis

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P.5.12 - THE PATHOGENESIS-RELATED 10 (PR-10) PROTEIN CONTRIBUTES TO FLAVONOID BIOSYNTHESIS IN *MARCHANTIA POLYMORPHA*

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Pathogenesis Related (PR) protein is a general term often given to genes that are strongly induced by stress or pathogen attack but for which the predicted amino acid sequence gives little clue to function. Some PR proteins are of particular interest with respect to biosynthesis of specialized metabolites, including a proposed function of PR10 in the biosynthesis of anthocyanins in flowering plants. However, the function of PR10 has not been studied in non-vascular plants.

We investigated whether a PR10 homologue, *MpPR10*, contributes to flavonoid production in the model liverwort *Marchantia polymorpha* (Marchantia) and examined the possible mode of action. Marchantia produces a variety of phenolics, including bibenzyls and two major types of flavonoids: flavones and auronidin (a red pigment). We found that genetic mutants with loss of function of *Mppr10* had significantly reduced total content of auronidins and flavones but not bibenzyls as compared with wild type. This was reflected in reduced transcript abundance for flavonoid-related biosynthetic and transcription factor genes. Overexpression of *MpPR10* did not affected the expression of flavonoid biosynthetic genes or related transcription factors. We showed that MpPR10 protein binds flavonoids with differential affinities: chalcone and dihydrochalcone had the highest affinity among all tested compounds, and MpPR10 can protect chalcone from self- cyclisation *in vitro*. Our results suggest PR10 has a conserved function in flavonoid biosynthesis across land plants, probably through the binding and protection of intermediates from degradation or untargeted non-enzymatic conversions.

P.5.13 - POLYPHENOL METABOLISMS IN THAWING PERMAFROST MICROBIOMES

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Permafrost is estimated to store 50% of global soil carbon, making it a major player in the carbon economy. As global temperatures increase, massive permafrost carbon stores may be destabilized. Release of stored carbon as methane or carbon dioxide could accelerate global climate warming [1]. In order to predict global warming consequences, we need to understand the potential for microbial processing and release of trapped carbon.

The well-established enzyme latch theory postulates that polyphenols in anoxic systems stabilize carbon by inhibiting the resident microbiome, constraining decomposition and limiting carbon gas emissions [2]. The theory assumes that the oxygen-requiring enzyme phenol oxidase is the primary microbial enzyme for degrading polyphenols. This theory does not account for polyphenol heterogeneity, enzyme diversity or microbial adaptability, and thus may not reflect the reality of natural ecosystems.

By pairing microbiome and geochemical measurements along a permafrost peatland thaw gradient, we found positive relationships between polyphenols and microbial decomposition metrics, indicating that polyphenols do not shut down microbial decomposition pathways. In fact, we found a rich assortment of polyphenol-metabolizing genes encoded and expressed by various microbial lineages under a range of redox conditions. This led us to conclude that polyphenols are under-appreciated substrates in the permafrost carbon cycle. Our data demonstrate that polyphenols should not be assumed to stabilize carbon in anoxic soils, and that the enzyme latch theory has limited applicability to real ecosystems (Figure 1).

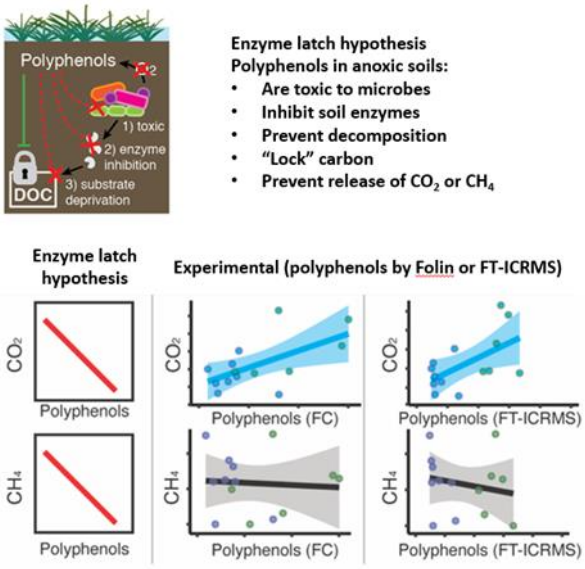

Figure 1: Data from Arctic peatland refutes the enzyme latch hypothesis

[1] Turetsky M.R., Abbott B.W., *et al.*, *Nature Geoscience*, 13, 138-143, 2020.
[2] Freeman, C., Ostle, N. & Kang, H., *Nature*, 409, 149-150, 2001.

P.5.14 - CHANGES IN PHENOLIC COMPOSITION OF *APIUM GRAVEOLENS* L. DUE TO THE INOCULATION OF PLANT GROWTH PROMOTING BACTERIA

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Adverse environmental conditions, such as salinity in soils, are natural processes whose incidence is increasing due to the impact of climate change. The soil salinity can affect various aspects of plant growth and development by imposing osmotic stress, ion toxicity, nutrient deficiency, and oxidative stress, which could lead to an increase in the use of fertilizers and other resources. Plant growth promoting bacteria have been described as microorganisms that cannot only colonize crops rhizosphere but also improve their development through direct and indirect mechanisms [1], so they can be considered as an alternative to chemical fertilizers. Moreover, this type of biofertilizers have been reported to be beneficial for the accumulation of phenolic compounds in different vegetables [2], which could be interesting since vegetables are one of the main sources of phenolic compounds, whose consumption has been related to different beneficial effects on human health.

Thus, in this work, the effect of four different bacterial species (*Pseudomonas alvandae*, *Priestia aryabhattai*, *Rhizobium laguerreae* and *Rhizobium sp.*) on the phenolic composition of celery (*Apium graveolens* L.) has been studied. Plants were cultivated under three different growth scenarios: normal conditions and two different type of saline conditions (100 mM NaCl and 50 mM Na_2SO_4). Plants were inoculated at day 3 of growth and irrigated with the corresponding medium for 64 days of grown. Soil salinity was controlled by conductimetry and, once plants were harvested, they were freeze-dried and grounded to provide a homogenous powder. Phenolic compounds were extracted by using an hydroalcoholic solution (MeOH:H_2O, 80:20) and, after removing the organic solvent, the phenolic composition was determined by means of HPLC-DAD-MS, following a previously developed methodology [2]. The obtained results showed that phenolic composition of celery is affected by both, the soil salinity and the strain employed as biofertilizer. Moreover, it has been observed that the studied biofertilizers are more efficient under saline conditions than under normal conditions and, also, that the type of salinity can determine the effect of each strain on the phenolic accumulation in celery plants.

[1] Kumar B.D. & Jacob J., *Journal of Spices and Aromatic Crops*, 28, 77-95, 2019.
[2] Jiménez-Gómez A., García-Estévez I., Escribano-Bailón M.T., García-Fraile P., Rivas R., *Foods,* 10, 424, https://doi.org/10.3390/foods10020424, 2021.

P.5.15 - CHANGES IN COMPOSITION OF ELLAGITANNINS DURING RIPENING OF POMEGRANATE FRUIT

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The fruits of pomegranate (*Punica granatum* L.) are used as raw materials for various beverages, cosmetics, and supplements. Punicalagin, an ellagitannin monomer, is well known to contain as a main polyphenol in the pericarp. We have reported that the isolation and characterization of ellagitannins including new ellagitannin oligomers with antiglycation property from the arils of pomegranate [1]. In the present study, we investigated the changes in the composition of identified ellagitannins during the ripening process of pomegranate fruit by HPLC analysis.

Extracts with 70% aqueous acetone of pomegranate flowers or fruits collected periodically from May to October 2013 were used as samples for analysis (Figure 1). HPLC analysis was performed on each sample to quantify punicalagin, punicalin, and ellagitannin oligomers identified our previous study, as well as low molecular polyphenols such as ellagic acid, and to evaluate changes in ellagitannin component composition during the ripening process of pomegranate. We also compared the ellagitannins in the pericarps and allyls of pomegranate collected in Okayama with commercial pomegranate produced in the United States.

The total amount of ellagitannins increased with the ripening of pomegranate fruit, but the total amount decreased in October when the pomegranate fruit ripened. The amount of punicalagin showed a similar trend. The content of punicalagin to the total amount of ellagitannins was 80-90% in fruit, but about 40% in May, the flower stage. On the other hand, the content of ellagitannin oligomers was about 10% at the fruit stage, but about 50% at the flower stage. Furthermore, the content of ellagitannin oligomers of the collected pomegranate was higher than that of the commercially available pomegranate.

These results indicate that the content and composition of ellagitannins differ greatly depending on factors such as the time of collection of pomegranates, suggesting that the functionality of pomegranates derived from ellagitannins may also change depending on the time of collection and the place of production.

Figure 1: Growth process of pomegranate fruit

[1] Ito H., *et al.*, *Food Chem.*, **152**, 323-330, 2014.

P.5.16 - STILBENE LIGHT ISOMERIZATION

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Stilbenes, belonging to the large family of polyphenols, are secondary metabolites present in several plants, including grapevine. These molecules, although promising in several fields of application thanks to their multiple properties (antimicrobial, antioxidant, anti-inflammatory ...), present limits for their use (solubility, stability in the environment...). Among these limitations, we can mention the photosensitivity of stilbenes. Indeed, light causes isomerization, cyclization and oxidation reactions of these compounds and consequently modifies their biological activities. From the point of view of using plant extracts enriched in stilbenes to limit the use of chemical pesticides (from synthetic chemistry) to control plant diseases, it is therefore essential to ensure the stability of these compounds in the environment to retain their antimicrobial capacity. Therefore, it is important to focus on these photoisomerization phenomena to better understand the impact of light on stilbenes. Our work aims to study the photodegradation of three major stilbenes found in grapevine cane extracts (resveratrol, ε-viniferin and *r*-viniferin). The transformation/degradation of each compound exposed to light was studied in order to characterize the kinetics of this transformation and to identify the nature of the compounds resulting from the photoisomerization. Solutions of transformed compounds at different times of light exposure were then studied for their biological activities (antimicrobial activities). Finally, different natural strategies to improve the photostability of the three major stilbenes were evaluated.

P.5.17 - ELUCIDATION OF PHLORIDZIN BIOSYNTHESIS IN APPLE - TISSUE-SPECIFIC EXPRESSION PATTERN OF A CANDIDATE GENE PRESUMED TO PLAY A KEY ROLE IN PHLORIDZIN FORMATION

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The dihydrochalcone phloridzin accounts for more than 90% of the soluble phenolic components in apple leaves, and is known to have health benefits for the human when included in their diet. The presence of such high amounts of phloridzin makes apple unique since other species accumulate only very low amounts and many closely related species like pear are not able to form phloretin or its glucosylated relative phloridzin [1]. The biosynthetic formation of phloridzin is based on three steps: (1) the formation of dihydro-*p*-coumaroyl-CoA from *p*-coumaroyl-CoA by an NADPH dependent dehydrogenase, (2) the subsequent formation of phloretin by a chalcone synthase, and finally (3) the glucosylation of phloretin at position 2' to phloridzin. While the last two steps are well studied, the first step is still a matter of debate. It is the crucial step that makes *Malus* spp. unique and gives them their ability to produce large amounts of phloridzin compared with other plants. Previously, we successfully completed a challenging purification process and were able to purify for the first time a candidate enzyme from apple leaves, which exhibits strong enzyme activity with *p*-coumaroyl-CoA to form dihydro-*p*-coumaroyl-CoA. In this study, we analysed the expression of the candidate gene in leaves and flower organs from different apple cultivars 'Gala', 'Braeburn', 'Pinova', and 'Topaz'. The gene was expressed in all tissues examined with varying expression levels, dependent on tissue type, tissue age, and cultivar (Figure 1).

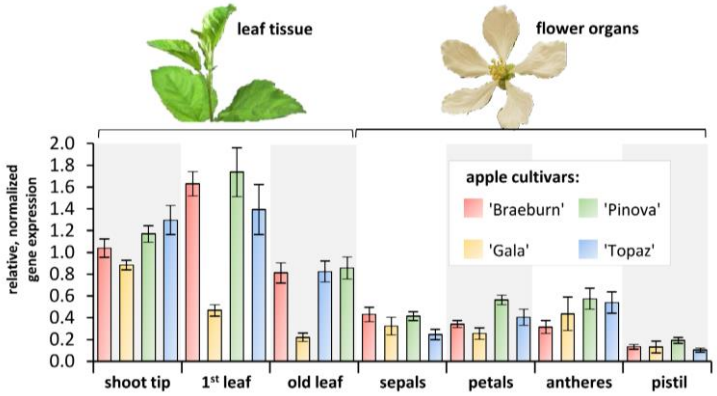

Figure 1: Dehydrogenase candidate gene expression. qPCR was performed with cDNA obtained from leaves and flowers of different apple cultivars.

The results clearly showed heightened expression of the candidate gene in shoots and young leaves of all cultivars, with the expression rates dropping off in the older leaves, with other parts of the plant showing significantly less, and almost none in the pistils. Generally, the cultivar Gala showed the least in almost all plant parts, when compared with the other cultivars.

[1] Gosch C., Halbwirth H. & Stich K., *Phytochemistry* 71, 838-843, 2010.

P.5.18 - THE ROLE OF *PGBHLH94-LIKE* IN THE SYNTHESIS OF HYDROLYSABLE TANNINS AND LIGNINS IN OUTER PEELS OF POMEGRANATE FRUITS

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The outer peels of pomegranate (*Punica granatum*) fruit are highly enriched in metabolites produced from the shikimate pathway and its products, the aromatic amino acids. These include flavonoids, monomers of lignins (monolignols), hydrolysable tannin [HTs; including gallotannins (GTs) and ellagitannins (ETs)]. These metabolites benefit human health and protect the fruit from environmental stresses. To understand the transcriptional control of shikimate pathway-related metabolites in pomegranate, we searched for transcription factors that positively correlated to the levels of gallic acid, the first substrate of the HT biosynthetic pathway. Our previous analysis showed that a bHLH family transcription factor, *PgbHLH94-like,* exhibited a strong correlation with the gallic acid content during three stages of fruit development in two different pomegranate accessions [1]. In this study, we overexpressed *PgbHLH94-like* in pomegranate hairy roots, which were subjected to LC-MS/MS analysis. A total of 166 metabolites related to phenols, and the shikimate pathway were detected. The transgenic hairy roots showed significantly higher levels of shikimate, gallic acid, monolignols, and all the detected metabolites that belong to GTs, relative to the control lines. However, the level of ETs did not significantly change compared to the control hairy roots expressing an empty vector. Notably, there was a significant increase in total lignins in *PgbHLH94-like*-overexpressing hairy roots. RNAseq analysis of the transgenic hairy roots showed that expression of the gene *gallate 1-beta-glucosyltransferase-like* was upregulated, supported by the LC-MS/MS data of high producing 1-galloyl-beta-glucose, the product of gallate 1-beta-glucosyltransferase using gallic acid as substrate. The transcriptome analysis of *PgbHLH94-like*-overexpressing hairy roots also revealed reprogramming of defense response and cell wall-related genes. These results together suggested that *PgbHLH94-like* activates genes in lignin biosynthesis and the GT biosynthetic pathway, but acts as a repressor of genes related to flavonoid biosynthesis. The results also suggest that a high level of GTs does not support ETs formation.

[1] Harel-Beja R., Tian L., Freilich S., Habashi R., Borochov-Neori H., Lahav T., *et al.*, *Tree Genet. Genomes*, 15, 22, https://doi.org/10.1007/s11295-019-1329-6, 2019.

TOPIC 6: BIOMATERIALS, GREEN CHEMISTRY & CIRCULAR BIOECONOMY

PL.6.01 - SUSTAINABLE PRODUCTION AND MODIFICATION OF *P*-HYDROXYCINNAMIC ACIDS: AN ACCESS TO VALUABLE BIO-BASED FUNCTIONAL ADDITIVES AND MATERIALS

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For more than 10 years, URD ABI has been working on the upgrading of biomass through the combination of green chemistry, biotechnologies and downstream process. *p*-Hydroxycinnamic acids (HCA; *e.g.*, coumaric, ferulic, sinapic and caffeic acids) are naturally occurring chemicals that our team has used as building blocks for the design of sustainable functional additives, polymers and materials. This talk will present the work that our team - and (inter)national partners - have conducted to (1) produce these HCA from biomass, and (2) functionalized them through green chemistry and biotechnology to access bisphenol A substitutes, antioxidants, additives for PLA, UV-filters, smart materials…

PL.6.02 - TUNING POLYPHENOLS FOR APPLICATIONS AS THERMOSETTING WOOD ADHESIVES AND PRINTABLE BIO-BASED INKS

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Lignins and condensed tannins are the most abundant natural polyphenols. Despite significant molecular differences, processability and reactivity, both renewable macromers exhibit attributes that make them attractive to design bio-based polymeric systems in thermosetting adhesives and in processable gels for thermoplastics applications. As pioneered in the early 80s by Glasser's group at Virginia Tech, one of the tricks to impart process- ability and to design desirable properties in the final solidified material at once is to tune natural polyphenols in a molecularly controlled manner. In this way, one can engineer their compatibility with other biopolymers as might be needed for flowable polymer blends; one can also engineer their reactivity and network formation kinetics for thermosetting polymer applications. Expanding on the findings and research avenues explored in the early 80s, this overview presents the most significant contributions made in the course of the past decade from our research group at the University of Freiburg. It is thereby demonstrated that flowability, network formation and end-use thermomechanical properties of natural polyphenolic systems can be molecularly designed through controlled molecular derivatization (1). Through fundamental model studies, light is further shed on the underlying thermodynamics of phase development, morphology and physico-mechanical properties (2). For example, hydroxypropylation and bleaching-induced ring cleavage are both powerful to alter the polymer chain branching, stiffness and propensity for intermolecular interactions, thus enabling the fine-tuning of the end properties of polyphenolic polymer systems (3). Such strategy is highly meaningful whether thermosetting or thermoplastic processes are to be employed for the polyphenolic material system. Thus, applications of polyphenolic polymer systems as thermosetting wood adhesives and as processable inks for 3D printing might come one step closer to industrial reality.

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[2] Gleuwitz R., Sivasankarapillai G., Chen Y., Friedrich C. & Laborie M.-P., *Biomacromolecules*, 21, 2276, https://doi.org/10.1021/acs.biomac.0c00220, 2020.
[3] Ebers L., Glasser W. & Laborie M.-P., Compositions comprising modified lignin useful for additive manufacturing, WO2023062239A1, 2023.

O.6.01 - POLYPHENOLS RECOVERY FROM OLIVE MILL WASTE BY USING DEEP EUTECTIC SOLVENTS AND MICROWAVE-ASSISTED EXTRACTION

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Olive oil industry, generate large amounts of waste that may result in important environmental problems, such as soil and water contamination. Therefore, proper management and treatment of waste have become an environmental, economic, and social challenge. These wastes are exceptionally rich in bioactive compounds (e.g. polyphenols) with potential applications in the food, cosmetic, and pharmaceutical industries [1]. Polyphenols are plant metabolites with more than one phenol group in their structure. They are beneficial for human health due to their high antioxidant character, neutralizing the formation of free radicals involved in oxidation processes. Several studies have correlated the intake of these compounds to positive effects on diseases such as cancer, diabetes, and hypertension, among others [2].
The recovery of polyphenols from agri-food waste is an example of circular bioeconomy, which contributes to the valorization of waste while providing solutions to environmental problems. In this context, to search for efficient recovery procedures, unconventional extraction techniques, such as microwave assisted extraction (MAE), have been suggested. On the other hand, natural deep eutectic solvents (NADES) have been proposed as an efficient and green alternative to typical extraction solvents.
In this study, the MAE technique with NADES has been used for the recovery of polyphenols from olive mill waste. An experimental design approach has been applied to evaluate the significance of variables such as solvent, temperature, and extraction time. The extraction efficiency has been evaluated in terms of total polyphenol content by high-performance liquid chromatography with ultraviolet detection (HPLC-UV); the antioxidant capacity has been determined by the ferric reducing antioxidant power (FRAP) assay. In general, 2- to 10-fold higher recovery yields have been obtained with NADES compared to conventional hydro-organic solvents. Additionally, the resulting extracts have also been analyzed by liquid chromatography coupled to mass spectrometry (LC-MS) to identify the main polyphenols of the studied matrix. Because of their remarkable content in phenolic compounds and high antioxidant capacity, olive tree leaves and other olive waste have been identified as an especially suited source for polyphenols recovery.

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O.6.02 - ENYZME-ASSISSTED SUPERCRITICAL FLUID EXTRACTION (EAE-SFE) OF DIHYDROCHALCONES FROM APPLE POMACE

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Snailase is a commercially available complex mixture of more than 20 enzymes, including cellulase, invertase, hemicellulase, pectinase, polygalacturonase, and protease. It is obtained by the extraction of the digestive tracts of the crop of mollusks from the genus *Limax* and is used for pretreatment of cotton-fabrics [1]. We recently showed that snailase is also a powerful tool for the enzymatic hydrolysis of polyphenols and presented a fast, robust and easy assay for the hydrolysis of flavonoid glycosides from plant extracts to simplify flavonoid analysis by HPLC [2]. The enzyme mix showed a broad activity against different flavonoid classes (aurones, chalcones, dihydroflavonols, flavanones, flavones, flavonols, isoflavonoids) in various plant backgrounds (leaves and buds of ornamentals, herbs and trees). In comparison to several other commercial enzymes tested, the highest yields of all aglycones from the different plant extracts were obtained, thus showing the great potential for universal applicability to hydrolyse flavonoid glycosides of different origins.

Figure 1: Enzymatic hydrolysis of flavonoids.

The dihydrochalcone phloretin is a bioactive compound of particular interest as antioxidant, antidiabetic, or natural sweetener. The global market is estimated 6 million $ in 2022 and forecasted to increase up to 10 million $ in 2028, which is driven by both cosmetic and food industry (https://www.businessresearchinsights.com/market-reports/phloretin-market-100618). The main source of phloretin is the glycosylated variant phloridzin, which accounts for more than 70-90% of the soluble phenolic components in apple leaves, roots and fruits. This makes apple production waste products such as apple pomace and green residues from pruning potential sources for mining phloretin. We established a procedure for the direct extraction of phloretin from apple pomace with supercritical CO_2 (sCO_2) after combined enzymatic cell wall degradation and phloridzin hydrolysis (Figure 1). We established a reliable workflow and optimized numerous parameters such as conditions for biomass pretreatment, influence of cultivar, extraction time and temperature, and different sCO_2/organic solvent ratios. This is the first report of enzyme-assisted supercritical fluid extraction of polyphenols with simultaneous processing of the glycosides.

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O.6.03 - LIGNIN-BASED BENZOXAZINES: A VERSATILE PLATFORM OF FUNCTIONAL AND RECYCLABLE POLYPHENOLIC MATERIALS

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Lignin, the second largest source of renewable carbon produced on Earth, epitomized the main source of phenolic compounds for the preparation of bio-derived materials. Characterized by a polyphenolic structure, lignin can be incorporated as mechanical fillers for composites; employed as UV-barrier, antioxidant, or antimicrobial agents; or even used as a matrix in a wide range of value-added materials. Polybenzoxazines (PBZs) emerged as a promising alternative to phenolic and epoxy resins thanks to their competitive features such as superior mechanical properties and thermal stability. The past two decades witnessed the exceptional versatility of their elaboration from renewable resources. However, the heterogeneous and complex structure of lignin hinders the straightforward development of benzoxazine from this biopolymer. We have reported a green and eco-friendly approach to increase the reactivity of lignin toward benzoxazine chemistry [1]. In this study, a soda lignin was esterified in solvent-free condition with phloretic acid, a naturally occurring compound extracted from the leaves of apple trees. This sustainable synthetic route grants technical lignin with ester bonds and an increased number of phenolic reactive sites. Therefore, this enriched platform of ortho-free phenolic rings was employed for the catalyst-free design of a series of lignin-based benzoxazines (LBZs) [2]. The properties of the bio-based resins can be easily tuned depending on the amine used to close the benzoxazine ring. The nature of the amine side group has a strong influence on the mechanical strength, thermal stability, solubility, or surface interaction of the resultant LBZs. Bio-based amines synthons such as long-alkyl chain stearylamine confer hydrophobicity to LBZs coatings, while furfurylamine-based LBZs generate high-T_g materials. Amino-alcohol derivatives grant recyclability to cross-linked lignin-vitrimers thanks to dynamic transesterification exchanges [3,4], joining the recent but growing family of lignin-based vitrimers. This presentation will illustrate the scope of applications of these monocomponent and fully bio-based LBZs (Figure 1).

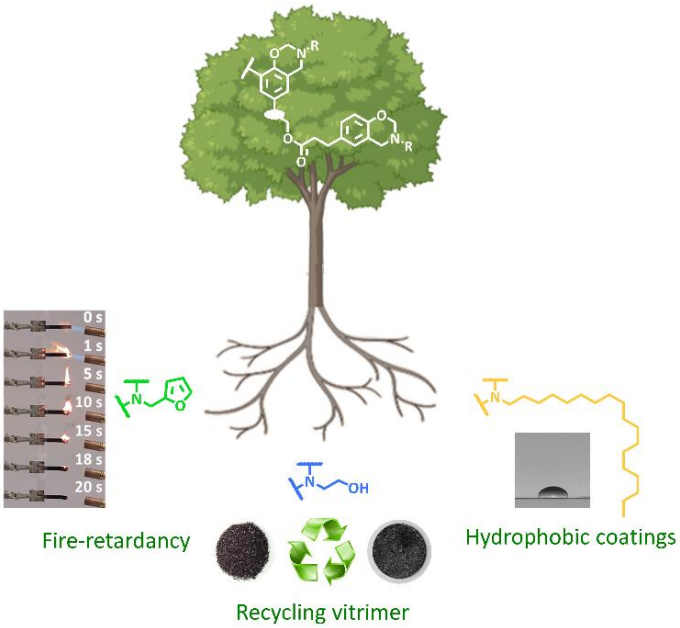

Figure 1: Multi-purpose applications of lignin-based benzoxazines

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O.6.04 - BACK FROM THE PAST: POLYPHENOLS AS ECO-INNOVATIVE MORDANTING SOLUTIONS FOR THE TEXTILE DYEING INDUSTRY

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Before the advent of synthetic colors, natural dyes were widely used to dye natural fibers including wool, linen, cotton and silk. The use of natural products is currently of great interest due to increase awareness concerning environmental and health-related issues. If these natural products are obtained from usually discarded agro-food byproducts/wastes (ABW), symbiosis between circular economy and environmentally responsible practices is achieved.

Most natural dyes (including many polyphenolic compounds) have little to no affinity for the natural fibers requiring the use of metal salts as mordants (e.g., Al^{3+}, Fe^{2+}) to bind to the textile, which can also lead to ecological distress. Previous works on the use of tannin-rich extracts as biomordants have already shown promising results [1]. However, the lack of well-documented research on their potential applications, and their influence on color stability, downplays their role in natural textile dyeing. On another hand, traditional knowledge is a unique source of inspiration and, although it is known that the use of natural dyes may lead to poor to moderate light/color-fastness, the truth is that we still see the brightness and color hue of some of these natural colors in artworks, which have lasted centuries. This resilience is related to the color formulations, i.e. the recipes, developed in the past.

This study is the first approach to the development of new sustainable mordanting methods for the 21st-century textile industry, inspired by the ancient practice of natural dyeing. Eco-friendly biomordants recovered from ABW have been selected, such as oak galls, grape seeds, tartar and pomegranate, to prepare wool and broadcloth samples for dyeing with weld (*Reseda luteola* L.) following 18th-century recipes from French master dyers Antoine Janot and Paul Gout [2-3]. These recipes have been developed for years for the French textile industry of the 18th century, and were used for decades, proving their usefulness and resilience.

Different material/liquor ratios, time, number of repetitions, temperature and dye percentages will be compared. All biomordant results were compared to alum, the most commonly used mordant. The biomordant extracts were analyzed by HPLC-DAD and HPLC-MS. Dyeing assays were characterized by colorimetry (CIELab), molecular fluorescence, and reflectance spectroscopy. Finally, the biomordant-dyeing potential of each formulation used was assessed.

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O.6.05 - INSPIRED BY NATURE: FIBER MATERIALS FUNCTIONALIZED WITH CONDENSED TANNINS OF NORWAY SPRUCE BARK SHOW ANTIMICROBIAL EFFICACY

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The aim of this research was to study the potential of naturally bioactive, tannin-rich extract of Norway spruce bark to create smart functional fiber surfaces. Tannic acid was used as a commercial reference with known antiviral efficacies [1]. Bark extract was obtained from industrial bark side-streams. Test matrices of fiber-networks were prepared to study different approaches in incorporating the functionality into fiber networks and to test their bioactivity (i.e., antibacterial, and antiviral surface properties) aiming at further development of smart lignocellulosic fiber products for e.g., personal protection or other functional use.
We hypothesized that (1) tannic acid and tannin-rich bark extract show broad spectrum antimicrobial activity against both bacteria and viruses; (2) tannic acid and tannin-rich extract as immobilized into fiber samples maintain their antimicrobial activity; (3) tannic acid and tannin-rich extract are evenly localized in fiber matrices, and (4) surface techniques would reveal whether the interaction between bioactive compounds and fiber surface molecules are reversible with non-covalent forces or irreversible via formation via the covalent bonds. Complementary, novel techniques were used, including bioluminescent imaging of bacterial plates, antiviral efficacy tests by enterovirus, morphological analysis by laser microscopy, chemical mapping of bioactive compounds on sample surfaces by ToF-SIMS, and semi-quantitative analysis of potential chemical bonds between impregnated bioactive compounds and the target fiber surfaces by FTIR-ATR spectroscopy, supported by the chemical characterization of the extract by GC-MS and LC-MS, and chemical quantitation of extractives by GC, stilbenes by HPLC and condensed tannins by UHPLC after their thiolytic degradation. According to our results, condensed tannin rich extract obtained from bark side-streams showed potential as functional product which can be used to develop alternatives to substitute e.g., plastic hygienic products, personal protection materials such as face masks, or food packaging materials to prolong the shelf-life of foodstuffs and prevent the spread of infections [2]. In the ongoing study, we are further developing this proof-of-concept to ensure stable chemical bonding in the product prototypes with specific chemistry. The results of the newest findings will be shortly discussed.

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OY.6.01 - POLYPHENOLS DERIVED FROM MANDARIN PROCESSING WASTES : FLAVONOID-NANOPARTICLE-HYDROGEL AS A FUNCTIONAL BIOMATERIAL

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Citrus is the most widely cultivated fruit crop in the World. The most commonly cultivated varieties are oranges, mandarins, grapefruits, lemons, limes, clementines, nectarines and tangerines. These citrus cultivars are industrially processed into juice, thereby generating approx. 25-40% by wt. of biomass in the form of peels and seeds, generally considered as waste. Consequently, a great deal of this nutraceutical-laden biomass is lost, which, if utilized wisely, could revolutionize the functional food industry, since this biomass possesses a plethora of bioactive compounds, mostly within the classes of *polyphenols* and *terpenoids* making them an abundant source of functional bioactives. Mandarin is a potential source of bioflavonoids that possesses a putative anti-oxidative character, and its suitability for developing value added products is evident. In this study, 'kinnow' mandarin (*Citrus nobilis* X *Citrus deliciosa*) biomass was studied for its flavonoid profile. For this, dried and pulverized peels were subjected to supercritical fluid extraction and the extract was observed to contain 47.3±1.06 mg/ml rutin equivalents as total flavonoids. Mass spectral analysis revealed the predominance of polymethoxyflavones (PMFs), chiefly, tangeretin and nobiletin. This conferred upon the biomass a significant anti-oxidant potential which was estimated to be at an IC_{50} of 0.55µg/ml. Flavonoids, however, are known to undergo pre-systemic metabolism which limits their functionality, as was also observed in this study in which nearly 50% flavonoids degraded within the first 2h of gastric exposure, as shown in the Figure 1. To overcome this, nanoencapsulation was envisaged, and flavonoids-laden Poly lactic-co-glycolic acid (PLGA) nanoencapsulates were bioengineered to a particle size between 200-250nm, which exhibited protection of flavonoids in the gastric environment, allowing only 20% to be released in 2h. Further on, the nanoencapsulate was impregnated within alginate hydrogels, which would act as delivery vehicles of the same within the gastrointestinal (GI) system. In doing so, a 100% protection was achieved from pre-systemic release of bioflavonoids. Cryo-SEM (Cryo-scanning electron microscopy) images of the composite corroborate the packing ability of the alginate hydrogel. This work establishes the utility of a waste material towards development of a biomaterial whilst protecting the functionality of the same.

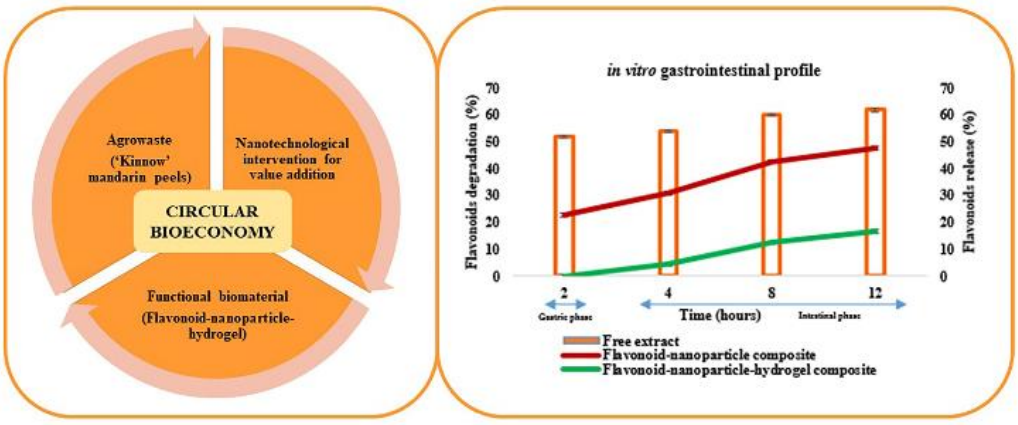

Figure 1: Functional biomaterial exhibiting longer residence in GI environment.

OY.6.02 - FROM GREEN EXTRACTION OF CHESTNUT BY-PRODUCTS TO PHARMACEUTICAL APPLICATION

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Oral mucositis (OM) is an inflammatory condition and a common side effect of oncological treatments, being characterized by painful ulceration, difficulty in swallowing/speaking and an increased risk of infection that can compromise the primary treatment outcome. Natural antioxidants have emerged as an interesting approach to prevent and treat OM by inhibiting the redox imbalance responsible for its development [1]. In this study, chestnut shells, a food by-product, were explored as potential active ingredients against OM [2]. Briefly, Subcritical Water Extraction (SWE), a sustainable methodology, was performed at different temperatures (110-180 °C) aiming to recover antioxidants from chestnut shells. The extraction temperature of 110°C led to the highest phenolic content (xxx), antioxidant/antiradical activities (xxx), and best scavenging efficiency against HOCl (IC_{50} = 4.47 µg/mL) and $ROO^{\bullet}$ (0.73 µmol TE/mg DW). The phenolic profile of the extract obtained at 110 ºC was characterized by high concentrations of phenolic acids (e.g., gallic and protocatechuic acids) and flavanoids (catechin, epicatechin and rutin). Additionally, the antimicrobial activity was demonstrated against a wide range of microorganisms that colonize the oral cavity during OM, including *Staphylococcus aureus*, *Enterococcus faecalis*, and *Escherichia coli.* Also, the extract prepared at 110 °C showed the lowest IC_{50} against HSC3 and TR146 human oral cell lines (1325.03 g/mL and 468.15 g/mL, respectively).
Considering the positive results, the extract obtained at 110 °C was encapsulated and included in an oral film. Eudragit RS30D, a synthetic polymer, was selected for the encapsulation procedure due to its sustained release profile, leading to particles with a mean size of 5.5 µm. The oral films were prepared with Methocel 1000, displaying optimal physicochemical properties in terms of thickness, mass, uniformity, dissolution time in artificial saliva, superficial pH, folding, tensile strength, and elongation. Further studies are needed to evaluate the oral films effects *ex-vivo* and *in-vivo*.

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OY.6.03 - KIWIBERRY LEAVES EXTRACT AS NEW ECO-FRIENDLY COSMETIC INGREDIENT: ASSESSMENT OF *IN-VITRO* AND *IN-VIVO* SAFETY AND INCLUSION IN A TOPICAL FORMULATION DEVELOPMENT

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Wastes and food by-products can be valorised for cosmetic purposes due to their richness in bioactive compounds, particularly antioxidants. Among new cosmeceutical ingredients *Actinidia arguta* (kiwiberry) leaves, one of the main by-product generated during kiwiberry production, arises as an option [1]. Our previously work reported the richness of kiwiberry leaves extracted by a green and eco-friendly technique – Microwave-assisted extraction (MAE) – particularly in phenolic compounds with antioxidant/antiradical potential [2]. The aim of this study was to screen the *in-vitro* safety of the *A. arguta* MAE extract on 3D validated skin and ocular models (namely, EpiSkin™ and SkinEthic™ Human Corneal Epithelial Models, respectively) and the *in-vivo* safety in human volunteers (through a patch test). Briefly, the leaves were extracted according to Silva *et al.* [2]. The viability results of 3D skin and ocular models were, respectively, 55.18% and 101.15%. Regarding the IL-1α released after exposure to the extract, it was 0 pg/mL for the skin model, while for the ocular model was 35.60 pg/mL. These values were significant different from the positive control employed that was sodium lauryl sulfate (522.90 and 55.19 pg/mL, respectively), being the extract classified as non-irritant for both models. Afterwards, a patch test performed in human volunteers (*n*=10) guaranteed the absence of allergic or irritant effects. Considering the positive results, a topical formulation with the extract as active ingredient was developed and stability studies were performed over 180 days at two different storage conditions (25ºC/40% relative humidity (RH) and 40ºC/75% RH). The optimal formulation was composed by extract (38.69%), aqua (16.58%), glycerin (15.00%), Cetiol® V (13.79%), Lanette® N (10%), Tegosoft® AC MB (5.00%), Microcare® PHDG (0.60%), Carbopol® 940 (0.32%), and triethanolamine (drops). The formulation showed stable physical and chemical properties (pH, color, and texture, as well as microbiological) for 180 days. Further *in-vivo* studies, such as hydration and anti-wrinkles effects, should be performed to ensure the skin effects on final consumers.

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OY.6.04 - PHENOLIC COMPOUNDS RECOVERY FROM BREWERY WASTE STREAM: PERFORMANCE ASSESSEMENT OF INTEGRATED ULTRAFILTRATION AND NANOFILTRATION PROCESS

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Brewing industry needs transition towards circular economy model to lower its environmental externalities [1]. A novel and poorly studied according to literature brewery phenolics-rich wastewater was studied in view of functional phenolic compounds (PhC) valorization. This waste stream is representative of phenolics-rich wastewater of different food and beverage industries with recovery and valorization issues. Waste stream PhC was investigated to design intensified recovery process. Membrane filtration processes can recover PhC with mild operating conditions and medium energy requirements.

Integrated membrane process was designed to recover phenolic compounds from studied wastewater, and to reuse process water as second objective. To this purpose, PhC were firstly analyzed by liquid chromatography coupled with antioxidant capacity analysis to gather information such as molecular weight, structures, antioxidant capacity contribution, etc. Then, filtration assays were performed at pilot scale with a 70 L device, at pH 4, 6.5, 10 and 13.5. The water pH influences interactions of compounds between them or with membrane what therefore also plays a role in process productivity and selectivity. Feed water was ultrafiltered in semi-batch mode to clarify wastewater and avoid clogging during concentration. Ultrafiltration was done with a 15 kDa TiO_2-ZrO_2 membrane preselected with a 5 L bench scale device. Clarified wastewater was then nanofiltered in batch mode. Flavonols as (+)-catechin were identified as main contributors of antioxidant capacity. 200 Da nanofiltration membrane was thus chosen for these compounds' retention and concentration. Fouling mechanisms were investigated with zeta potential and size exclusion chromatography analyses.

Productivity performances of membrane process varied with feed water pH. Both for ultrafiltration and nanofiltration, flux dropped rapidly at acid pH but was more stable at alkaline pH. Results of potential zeta and size exclusion chromatography suggested that PhC and carbohydrates aggregates clogged membranes at acid pH. Clarification yielded a 95 % turbidity removal. Intermediate pH of 6.5 was the most advantageous pH condition with high productivity of ultrafiltration with the addition of backpulse device. At nanofiltration stage, PhC and carbohydrates were retained (R_{TPC} and R_{TC}) at 90 and 99 %, respectively. For carbohydrates removal and further purification of PhC, adsorption and desorption processes are currently investigated (adsorbent selection, pH, desorption solvent, etc.).

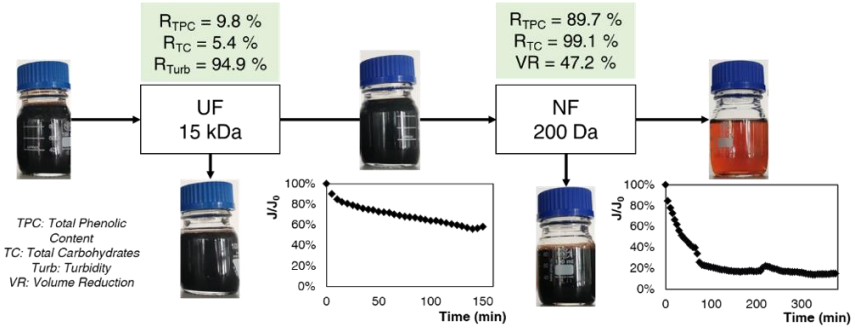

Figure 1: Proposed integrated membrane process for brewery waste stream phenolic recovery

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OY.6.05 - MITIGATION OF AMMONIA LOSS FROM CATTLE MANURE SLURRY BY TANNINS AND TANNIN-BASED POLYMERS

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As required by the NEC directive, Austria has to cut emission of ammonia by 12% until the beginning of 2030 compared to the emissions in 2005. Up to 2018, emissions still increased every year, with the biggest contributions the agricultural sector. To reduce the emission of ammonia from agriculture, especially cow and cattle manure, the addition of tannin and tannin-based polymers was investigated. We monitored the gaseous emissions from manure within the first hours after addition and the nitrogen content for up to one month. In lab trials with ammonia solution as reference, the retention of ammonia in solution after one day of open stirring was 13%, 40% and up to 65% for untreated solution, spruce bark extract and chestnut tannin respectively. The gaseous emission from manure was reduced by 40%, 58% and 66% for chestnut tannin, the tannin polymer, and oak tannin, respectively. For long-term nitrogen loss mitigation, 20% more nitrogen was preserved when spruce or larch bark extract or oak tannin was added to the manure compared to the reference measurements. A combination of the pH lowering effect of tannins shifting the NH_3/NH_4^+ equilibrium more towards NH_4^+ and covalently binding ammonia to flavonoid hydroxy groups provides a viable method for reduction of nitrogen loss in cattle manure (Figure 1). A concentration of 1 wt.% of tannin or tannin-based polymer is sufficient to reach NEC goals.

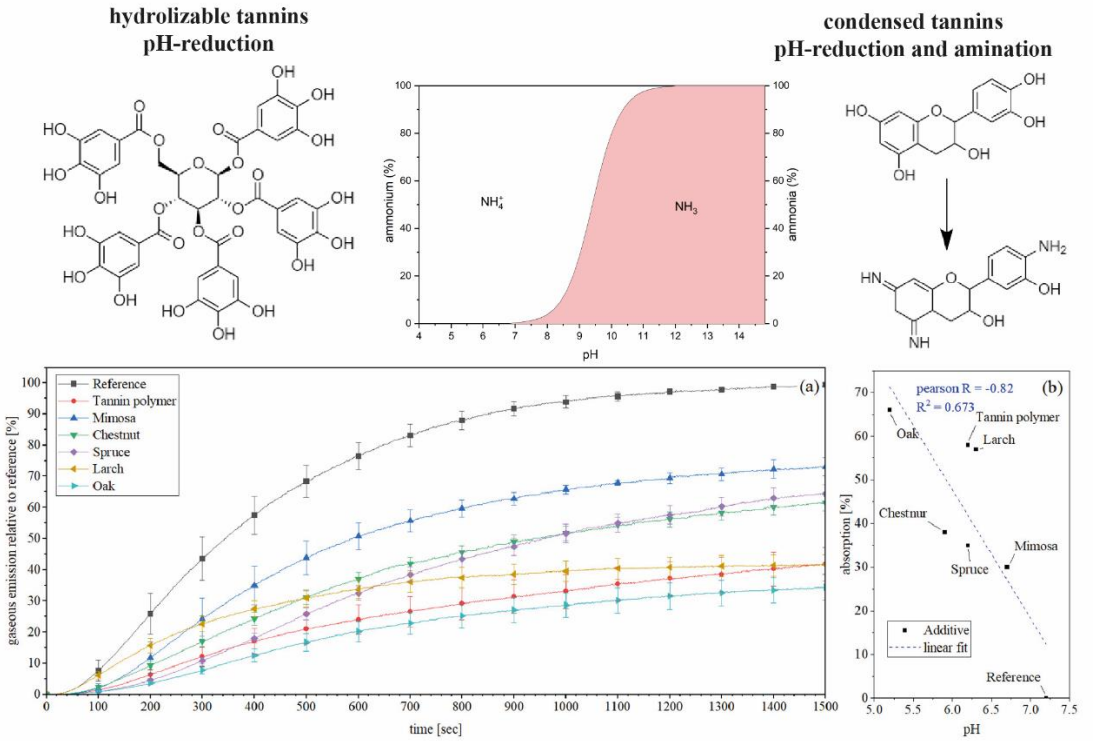

Figure 1: Mechanism of emission reduction by hydrolizable and condensed tannins

OY.6.06 - GREEN EXTRACTION OF POLYPHENOLS FROM RED GRAPE POMACE BY NON-IONIC SURFACTANT MIXTURES

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The use of aqueous solutions of surfactants (especially biocompatible non-ionic surfactants) as substitutes for organic solvents in efficient polyphenol extraction has emerged as cost-effective, easy-to-use and “green’’ technique. However, the application of mixed non-ionic surfactant systems in the extraction of polyphenols from plant matrices e.g., grape pomace, has not yet been investigated.

Aqueous solutions of individual non-ionic surfactants Brij S20 (BS20) and poloxamer 407 (P407) as well as their binary mixtures BS20/P407 (9:1), BS20/P407 (1:1), and BS20/P407 (1:9) in a total concentration of 3% w/v were used for the extraction of polyphenols from lyophilized red grape pomace (*Cabernet Franc* variety). Water was used as control solution. The extraction conditions were set to pH 4, solvent-to-material ratio 100:1 and extraction time 45 minutes. The efficiency of the extraction process was monitored in terms of concentration of individual polyphenols determined by HPLC, DPPH radical scavenging activity and the micelle size measured by DLS.

Good extraction properties of all examined surfactant systems toward polyphenols were observed. The polyphenolic composition of the grape pomace extracts was considerably influenced by the type of surfactant as well as the mass fraction of surfactants in binary mixtures. An increase in solubilization efficiency of BS20/P407 (1:1) and BS20/P407 (9:1) mixed micelles was noticed compared to individual surfactants. The most pronounced increase in solubilization efficiency in mixed micelles was observed for less polar compounds. All investigated aqueous surfactant solutions provided extracts with DPPH radical scavenging activity significantly higher than the aqueous extract. Radical scavenging activities of surfactant-rich extracts were also influenced by the surfactant type and the binary mixture composition. All investigated systems showed an increase in micelle size after extraction, indicating the solubilization of polyphenols into micelles, and the enhancement rate differed depending on the applied surfactant system.

These results show that non-ionic surfactant mixtures can be promising agents for efficient, ”green’’ extraction of polyphenols, which could be useful for further practical application in food and cosmetic industries.

OY.6.07 - OPTIMIZING THE REMOVAL OF PHENOLIC COMPOUNDS FROM SUGARCANE SYRUP WITH ACTIVATED CHARCOAL

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Plant derived syrups are currently supplied to industrial bioreactors to produce value-added biomolecules through fermentation. For instance, sugarcane is processed to extract the sugars contained in this plant and the resulting syrup is used by microorganisms in the biosynthesis of biomolecules, such as bioethanol or β-farnesene. However, this syrup contains phenolic compounds that can impact negatively on the performance of the microorganisms in fermentation [1]. In the literature, purification of phenolic compounds from sugarcane syrup has not yet been described. Therefore, this work aimed to optimize the removal of 59.6 mg/L of phenolic compounds from sugarcane syrup. In this study, activated charcoal was selected due to its high adsorption capacity, while also being low cost and easy to apply in the removal of the desired compounds [2]. The removal of phenolic compounds and the recovery of the syrup after the purification process were optimized through a central composite design with 23 runs, in which three dependent variables were studied: the adsorbent (5 different charcoals), the adsorbent concentration (10, 80 or 150 g of charcoal per L of syrup) and the time of contact (1, 12.5 or 24 h). Results showed that higher charcoal concentration led to better removal of phenolic compounds, but lowered the syrup recovery yield. The condition that was predicted by the model to remove the highest percentage of phenolic content, while keeping the maximum amount of syrup, was charcoal pellets from Proenol at 115 g/L of syrup and 12.5 h of agitation. This optimal condition was validated, resulting in 94.85 % removal of phenolic content and a syrup recovery yield of 43.65 %. This process led to the elimination of many inhibitory compounds, like *p*-coumaric acid (5.53 mg/L), ferulic acid (2.65 mg/L) and 4-hydroxybenzaldehyde (2.15 mg/L). The resulting syrup presented a sugar concentration 5 % higher than the non-purified syrup, as well as some differences in chemical composition (e.g., increase in phosphorous and removal of iron). Therefore, the method for reducing the phenolic content in sugarcane syrup using Proenol pellets at optimal incubation conditions was found suitable to produce a fermentation feedstock with very low phenolic composition.

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OY.6.08 - SYNERGIES BETWEEN FUNGAL ENZYMES.

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Lignin is the third most abundant biopolymer on earth, after cellulose and chitin, and accounts for up to 30% of plant biomass. It could be an inestimable source of aromatics of interest for organic syntheses, and probably the primary one after petrol runs out. However, its heterogeneous, randomly assembled and extremely complex chemical structure (fundamental for its protective role in the plant cell wall) makes its valorisation and exploitation scarce. A solution to this problem can be found in nature. In fact, filamentous fungi stood out as efficient degraders of lignin owing to a synergistic action of redox proteins, secreted by the fungus, that perform an enzymatic combustion. These enzymes are mostly annotated within the “Auxiliary Activities” (AA) class defined in the Carbohydrate Active enZymes (CAZy) database [1]. AA encompass enzymes with different cofactors, such as heme (peroxidases, CAZy family AA2), copper (laccases, AA1) and many others. Attempts to simulate laccase-mediated lignin degradation *in vitro* showed that highly reactive radicals produced by laccase oxidation are prone to repolymerization into higher molecular weight intermediates, that are even more recalcitrant to further enzymatic treatment. During *in vivo* degradation of lignin, fungi can prevent the massive repolymerization observed *in vitro*, leading to the hypothesis that they possess mechanisms to control or redirect the pool of radicals produced during oxidative attack of lignin. A few studies have pointed out the possible role of a few enzymes and their interplay in this event.

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OY.6.09 - TANNIN-FURAN AND LIGNIN-FURAN BASED FOAMS FOR INSULATION AND WASTEWATER TREATMENT

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The continuous increasing demand for energy and the increase in energy prices directly affects families and companies, both benefiting from the energy saving from proper building insulation. The most common insulation materials available as polyurethanes, polystyrene, glass or rock wool present many desirable features, however, nowadays more sustainable and natural alternatives are demanded [1]. Tannin-furanic foams are known to be fire resistant, have low thermal conductivity and high water and chemical stability. Nevertheless, these have continued in constant development of its desirable characteristics with the aim of becoming suitable candidates for the replacement of less sustainable materials. The use of natural renewable feedstocks has increasingly attracted attention, such as the use of vegetal polyphenolic material as tannins or lignin, in which both can be recovered from residues or side products from industries [2]. But as material science is becoming a more interdisciplinary field of knowledge, and modern world presents new challenge and necessities, the need for multipurpose materials rises. Therefore, rigid foams have also been tested as material suitable as adsorbents for wastewater treatment [3]. With the ongoing work, we present tannin and lignin-furanic based foams with several desirable characteristics. These have successfully been tested in the removal of methylene blue from water and new foams were produced by reducing the amount of pentane used, replacing it with sodium bicarbonate as blowing agent and producing more lightweight foams with the addition of controlled amounts of surfactant. Alkali-lignin based foams showed remarkable methylene blue adsorption capacity and the produced tannin-furan foams proved to be good flame retardants.

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OY.6.10 - FORMULATING WITH COLOR: DEVELOPMENT OF SUSTAINABLE COSMECEUTICALS WITH ANTHOCYANINS AND DERIVED PIGMENTS

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Keywords: photoprotection; anthocyanins; UV-filter; color stability; topical formulations;
Exposure to ultraviolet radiation (UV) induces deleterious effects on human skin. Given the skin-heath beneficial properties of several plant-derived molecules, including the visually attractive family of anthocyanins, there has been a growing interest in their application towards skin damage prevention [1]. The aim of this work consisted in exploring blackberries surplus production as a rich source of cyanidin-3-*O*-glucoside, extracted with good purity degree, using relatively simple procedures and mostly water throughout the extractive process. Its derivative, carboxypyranocyanidin-3-*O*-glucoside was further obtained by reaction with pyruvic acid. Effects of both compounds were evaluated on HaCat and HDF skin cells and topical formulations enriched with them were further developed and characterized (Figure 1).

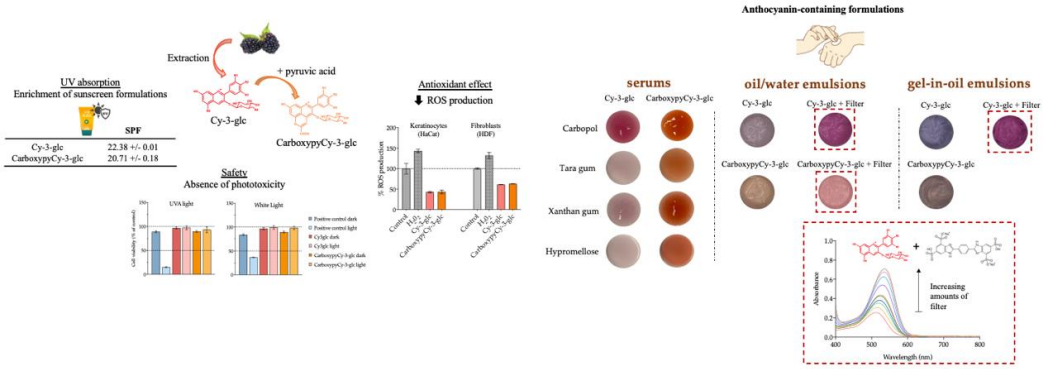

Figure 1: Workflow representation of anthocyanins extraction, *in vitro* evaluation and prototype formulations development.

Interestingly, a strong interaction was detected between the compounds and disodium phenyl dibenzimidazole tetrasulfonate (commercial UV filter), with a notorious color intensification and slight deviation from the original color hue. The mixture did not impair the absorptive capacity of the filter, in fact, a good complementarity between their absorbance spectra was obtained. This new uncovered interaction offers the possibility of natural color diversification and the stability enchancement of these molecules. From the formulations tested, gel-in-oil emulsions (GELTRAP^{TM}) stood out for the highest efficiency in color stabilization both at room temperature and degradation conditions, with the advantage of representing an environmental-friendly process, with the use of a bio-based liquid emulsifier that enables emulsion production by a simple cold process, translating into significant energy and time savings.

Acknowledgments:
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OY.6.11 - SUSTAINABLE EXTRACTION OF BIOPOLIMERS AND BIOACTIVE COMPOUNDS FROM THE LICHEN *EVERNIA PRUNASTRI*

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Traditionally used in Indian, Chinese, and Western medicine, in fragrance or as dyes, lichens may have much more applications in the health, cosmetic or food fields. Indeed, this organism, symbiosis between a fungus and microalgae, synthetizes singular metabolites which are known to have antioxidant, antitumoral, anti-inflammatory, antibacterial and photoprotective properties. It can be for example cited the usnic and evernic acid or the depsides [1]. In the current environmental crisis, it seems important to find alternative sustainable ways to extract bioactive compounds from natural sources. Hydrothermal microwave assisted extraction exhibits the advantages of using only water as solvent and having a faster extraction time, a lower energy consumption and usually a better yield than traditional extractions [2]. In this context, the main objective of this work is to extract and characterize polymers and bioactive soluble compounds from the lichen *Evernia prunastri* using hydrothermal microwave assisted extraction.

The antioxidant properties and the total phenolic compounds of the soluble extracts as well as their polysaccharides content were determined respectively by spectrophotometric techniques and high-pressure liquid chromatography. This work was also focused on a β-glucan polymer precipitated without any additional solvent and its formulation as a gel matrix. The antitumoral, anti-tyrosinase and anti-inflammatory properties of both liquid extracts and polymer were evaluated.

Results indicated that the microwave extraction temperature has a significant influence on the recovered extract yield and properties. Whereas the greatest antioxidant properties (60.2 ± 1.6 g Trolox eq/100 g extracts) and total phenolic compounds (12.5 ± 0.2 g gallic acid/100g of extract) are observed for the soluble extracts obtained at 140°C, the highest yields for the polymer recovery, up to 20%, were obtained at 160 and 180°C. The soluble extracts at 140°C and 200°C and the polymer obtained at 160°C showed anti-tyrosinase properties which could be interesting to regulate the melanin production as the current tyrosinase inhibitors tend to exhibit toxicity or efficiency issues. Lastly, a simple thermal treatment made it possible to form, from the polymer extracted at 180°C, matrices with the rheological properties of a gel. To conclude, it should be highlighted that the extracted soluble bio compounds and polymers showed interesting features for potential application in cosmetics or health.

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P.6.01 - PHENOLS MIXTURES REMOVAL IN WATER AT DIFFERENT TEMPERATURES USING LIGNINOLYTIC YEASTS

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Phenols are aromatic compounds characterized by having one or more hydroxyl groups attached directly to the aromatic ring. The presence of phenols in the environment is a consequence of natural actions and anthropogenic contributions, mainly of an industrial nature. From the point of view of pollution, the presence of these compounds in water and sediments is of great interest, so it is necessary to pay attention to the origin, migration, and distribution of phenols in different types of aqueous effluents [1].
Phenols have been found in various water systems; however, most phenolic compounds can be detected in wastewater of industrial origin [1]. For example, phenol is mainly used in synthetic polymers and plastics including bakelite, pentachlorophenol in bleaching processes, nonylphenol in paper manufacturing, and bisphenol A in the fabrication of durable plastic materials, dental fillings, and coatings inside food and beverage cans. Furthermore, some phenols are employed as pesticides because of their herbicidal, fungicidal, and insecticidal action, which causes their diffusion into the environment through agricultural activities and their entry into the food chain [2,3].
Therefore, the discharge of these pollutants into the environment can cause serious and long-lasting health effects on humans, animals, and other living beings. Consequently, reducing the toxicity of phenolic compounds in water is crucial to avoid risks in ecosystems. Having said that, the proposal is to use yeasts with ligninolytic capacity, isolated from cane bagasse, since they produce enzymes involved in the oxidation of phenolic compounds, thus using an environmentally friendly, efficient, and low-cost process. For this purpose, a combination of *Cryptococcus spp.* and *Candida spp.* yeasts was used to degrade the mixture of Phenol (P), Pentachlorophenol (PCP), Nonylphenol (NP) and Bisphenol A (BPA), in synthetic water at pH 7 with temperature variations of 15°C, 20°C, 25°C, 30°C and 35°C. The combination of yeasts grew in all cases in different time periods, ranging from 36 to 120 hours, depending on the temperature. Achieving removal efficiencies of 70 to 81% of P, 40 to 61% of PCP and 62 to 77% of NP and BPA. According to the results obtained, the combination of the yeasts studied has the capacity to degrade phenols from industrial wastewater and can be used as a remediation method.

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P.6.02 - PREPARATIVE PURIFICATION OF OLEACEIN FROM OLIVE JUICE BY HIGH SPEED COUNTERCURRENT CHROMATOGRAPHY

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Oleacein (hydroxytyrosol decarboxymethyl dialdehyde elenolic acid (3,4-DHPE-DCM-EDA)) is an important bioactive molecule present in recently crushed olive mill by-products, paste, pomace and juice. It is known for its high anti-microbial and anti-inflammatory activities [1]. Nevertheless, as oleacein derives from the transformation of secondary olive metabolites, such as oleuropein, it is present at relatively low amounts, that means that for studying its biological properties, oleacein should be isolated on preparative scale in milligram range. A good technical option was developed by an integrated purification process comprising membrane pressure-driven microfiltration and preparative solid phase extraction [1], followed by high-speed countercurrent chromatography (*HSCCC*) of the oleacein-enriched target-fraction. Relevant process results from the HSCCC purification will be presented, shortly.
Numerous proportions of solvents usually used in all-liquid CCC separations were tested, and the so-called HEMWat biphasic solvent system (1:2:1:2) was selected. The elution process was carried out on a triple coil multilayer countercurrent chromatograph equipped with 850 mL coil column volume. The apparatus was run in the '*head-to-tail*' mode, with rotational speed 800 rpm, and a flow rate of 3.0 mL/min. An amount of 1 g of dried and purified olive juice fraction [1] was dissolved in 20 mL of the solvent system and was submitted to separation. At these conditions the following CCC chromatogram was obtained (Figure 1).

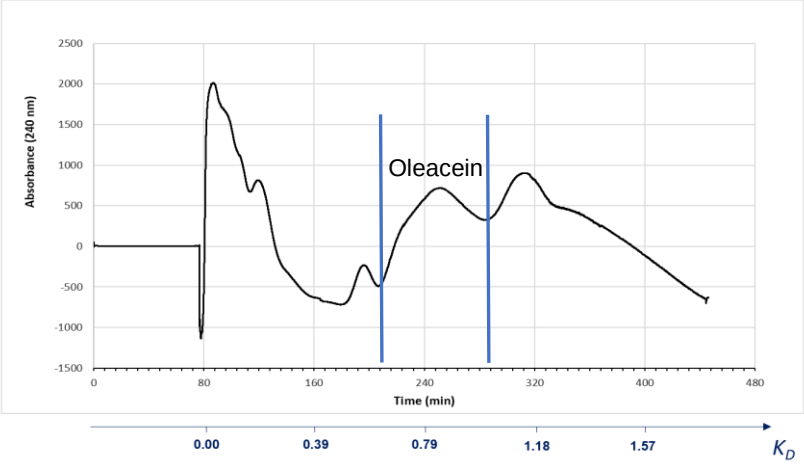

Figure 1: HSCCC UV-chromatogram (λ 240 nm),

Oleacein was separated in a single peak with retention time of 208 to 288 min. An amount of 100 mg of dry matter per batch were obtained. A purity of oleacein of up to 80% was established by HPLC-ESI-MS.

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P.6.03 - POLYPHENOL-IRON-COMPLEX-BASED EMULSIONS FOR HAIR DYEING

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Supramolecular self-assembly and (semi)-infinite-network formation of polyphenols (e.g., tannic acid and gallic acid) and multivalent metal ions (e.g., Fe^{3+}) have recently attracted a great deal of attention in materials science and engieerning, primarily because of the material-independent-coating characteristics of the interface-active polyphenol-metal complex formed in the processes. Although various potential applications have been demonstrated, the uncontrolled formation of unwanted flocs and precipitates, caused by rapidity in the direct complex formation between polyphenols and Fe^{3+}, hampers the commercialization of polyphenol-metal-complex-based formulae [1]. This kinetic issue has been tackled in part by applying the receipt of iron gall ink (IGI) to the fabrication of surfactant-free oil-in-water (o/w) emulsions, derived from tannic acid, gallic acid, and Fe^{2+} [2]. The surfactant-free emulsion technique has been applied to the black hair-dyeing [3].
Inspired by the oxidation reaction of Fe^{2+} to Fe^{3+} in the preparation of the IGI that has been used in Europe since the Middle Ages, we developed a easy-to-apply method for constructing the o/w emulsions, without any surfactants and emulsifiers, based on the air-oxidation of Fe^{2+} to Fe^{3+} in the Fe^{2+}-catecholate complex (Figure 1). Specifically, for the cosmetic applications, the emulsions were formed with a combination of tannic acid, gallic acid, Fe(D-gluconate)$_2$, and natural oil, which are all approved as cosmetic ingredients. The surfactant-free emulsion technology utilizing the hair-dyeing capability of polyphenol-metal complexes could be synergistically combined with the hair-fortifying property of oils and/or active ingredients in the oil phase, suggesting an advanced alternative to the conventional hair-dyeing methods that are considered harmful to humans as well as the environments.

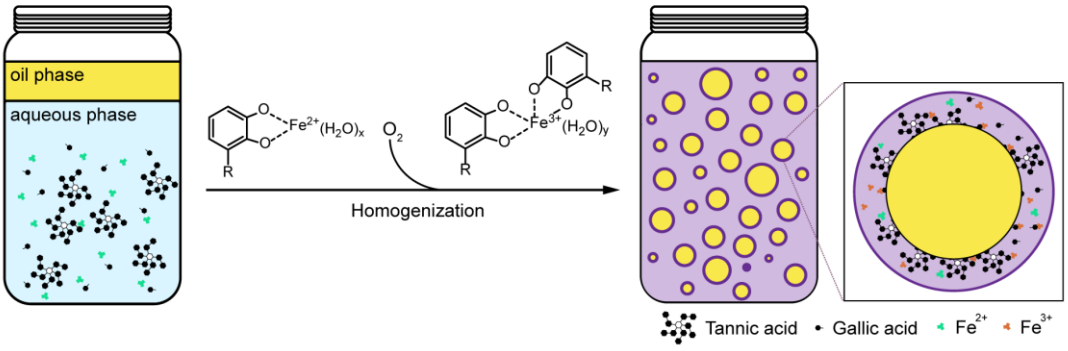

Figure 1: Schematic for surfactant-free emulsion technology based on the IGI-inspired formation of polyphenol-metal complexes.

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P.6.04 - ETHANOL-WASH SOLUTE WASTE FROM INDUSTRIAL SUNFLOWER MEAL PROCESSING IS RICH IN BIOACTIVE COMPOUNDS

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Sunflower meal is a by-product of the oil-producing industry which is mainly used as a protein-rich ingredient in animal feed formulation. Its application in animal nutrition is limited by the presence of some anti-nutrients and poorly digestible fibers. Alternatively, the industrially produced sunflower meal can be used for the preparation of protein isolates for human nutrition. However, the presence of phenols influences negatively protein digestibility, organoleptic and functional properties. To reduce their amount, a pre-treatment of the sunflower meal with an aqueous ethanol solution is recommended.

Sunflower ethanol-wash solute (SEWS) was obtained as waste after pre-treatment of industrial sunflower meal subsequently used for protein isolation. The sunflower meal was washed four times with a 75% aqueous ethanol solution. The ethanol-wash liquids were collected, concentrated, and freeze-dried to prepare a powdery SEWS. The product was found to be rich in carbohydrates (62.14%), lipids (7.73%), and bioactive compounds such as phenols (16.38%) and flavonoids (4.41%). Gas Chromatography-Mass Spectrometry (GC–MS) analyses revealed a prevalence of sucrose (14.01%), linoleic acid (12.10%), and chlorogenic acid (85.41%) based on total ion current (TIC) of polar, nonpolar, and phenolic compounds, respectively. The SEWS was found to be a rich source of microelements iron (259.02 mg/kg), copper (109.36 mg/kg), as well as selenium (0.10 mg/kg). Scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals raised with the increase in SEWS concentrations and reached 52.3% and 69% for 0.05% SEWS when dissolved in water and 70% ethanol, respectively. The antioxidant activity of SEWS in water was not different from that of the sample in ethanol up to 0.04%. This is important for the potential application of the SEWS in the food industry where water is the main solvent used. The highest hydroxyl radical scavenging activity (52.4%) was achieved with 0.1% SEWS. For all studied concentrations, from 0.005% to 0.1%, the SEWS exhibited a higher inhibition capacity than mannitol, which was used as a positive control. The SEWS demonstrated inhibiting capacity against Gram (+) *Curtobacterium flaccumfaciens* PM-YT and *Micrococcus luteus* 2YC-YT established by agar-well diffusion assay. Antifungal activity against *Aspergillus niger* ATCC 1015, *Aspergillus flavus*, and *Fusarium moniliforme* ATCC 38932 was established as well. The lowest minimum inhibitory concentrations (MIC, mg/ml) of the SEWS, 0.313 and 0.625, were estimated for *C. flaccumfaciens* PM-YT and *F. moniliforme* ATCC 38932, respectively.

Although being obtained as waste, the SEWS turned into a value-added novel product containing macro-components, microelements, and bioactive compounds. The biochemical and bioactive properties established outlined the product as a prospective agent for functional food preparation, food biopreservation, or plant bioprotection. Its potential applicability contributes to the better and more efficient use of natural resources which is an emerging issue of recent industrial human life.

Acknowledgment: The research was funded by the Bulgarian National Science Fund, project № КП-06-Н37/21.

P.6.05 - UTILIZATION OF STEAM AND MILLING TREATED BAMBOO LIGNIN AS ANTIOXIDANT POLYPHENOL AND EPOXY RESIN CURING AGENT

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Increased use of lignocellulosic biomass would lower environmental impacts such as the emission of greenhouse gases (e.g. carbon dioxide) and fossil fuel depletion, helping to create a sustainable environment. Advances in technologies such as genetics, biotechnology, process chemistry, and engineering are leading to the concept of biorefinery. In order to develop a method for the total biorefinery processing of lignocellulosic biomass, this study focused on the efficient separation and utilization of a woody structural component, i.e., lignin, from moso bamboo by using steam and milling pretreatments followed by water and acetone extractions (Figure 1). We studied the chemical characteristics of lignin on the steam treatment followed by the milling treatment of moso bamboo. The steam treatment and milling treatment followed by water and acetone extractions was an effective method to obtain low-molecular-weight lignin, i.e. water soluble lignin and acetone soluble lignin, as an antioxidant polyphenol and an epoxy resin curing agent, respectively. The maximum antioxidant activity, i.e. EC50 = 0.127 mg/mL, and the maximum flavonoid content, i.e. 3.94 mg-quercetin equiv./g-dry weight bamboo, of water extract, was obtained at a treatment pressure of 30 atm for a steaming time of 5 min. The acetone soluble lignin that can be used as a curing agent had the following chemical properties: Number average molecular weight 698, Weight average molecular weight 2895, Hydroxyl equivalent 119 g-lignin/eq. Compared with commercial-cured epoxy resins, the lignin-cured epoxy resin obtained in this work have good thermal and mechanical characteristics and can be used in various electrical substrate industry field. This work clarified that the lignin extracted from steam-treated followed by milling-treated lignocellulosic biomass could be used as an antioxidant polyphenol and a curing agent for the synthesis of cured epoxy resin.

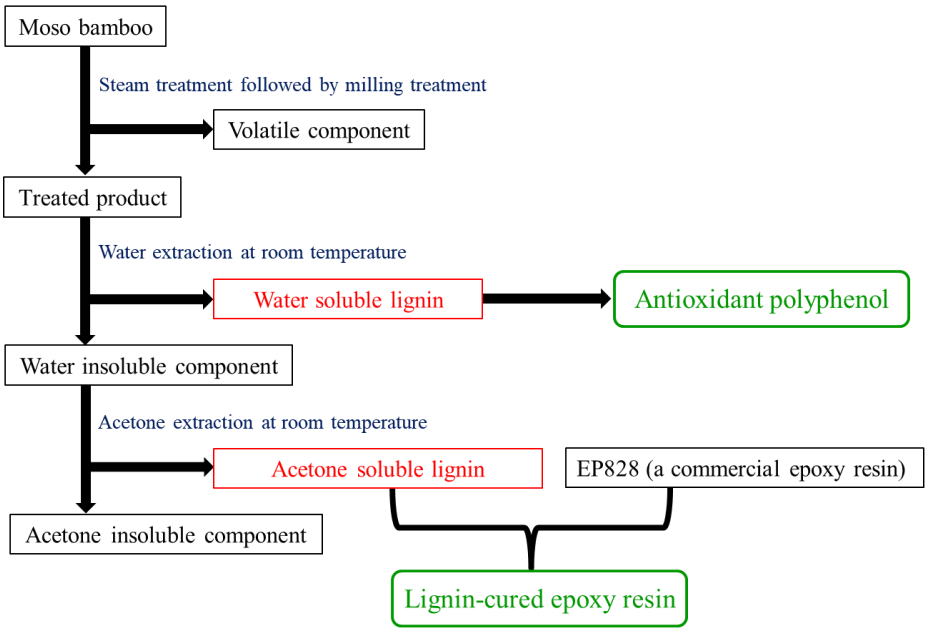

Figure 1: Utilization system of steam and milling treated bamboo lignin

P.6.06 - SURFACE MODIFICATION OF TANNIN-FURANIC FOAMS BY SILYLATION

Sepperer T.*[a], Petutschnigg A.[a], Koopmann A.[b], Torres-Rodriguez J.[b], Sket P.[c], Bedolla D.[d,e], Hüsing N.[b], Elsässer M.[b]

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Tannin-furanic foams are a promising and biogenic alternative to oil-based porous materials. Their hydrophilic character, typically indicated by a contact angle to water of 70°, limits some potential applications. To overcome this, a post-synthetic surface modification step with different organosilanes at 50 °C was investigated with a focus on the final, hydrophobic performance. On the one side, methyltrimethoxysilane and 3-(chloropropyl)trimethoxysilane, which can react with the hydroxy groups of the tannin polymer, were applied (Figure 1). A modified surface structure and a 25 to 50% weight increase, depending on the molecular weight of the silylation agent, were observed. Contrary, a mono-functional silane, trimethylchlorosilan, shows only a slight increase in weight, yet also condenses onto the polymer surface without forming a protective surface coating layer. Contact angle measurements using water show an increase from 70 ° (unmodified) up to 145 ° for a silane-modified foam. Nuclear magnetic resonance and infrared spectroscopy show the formation of covalent bonds between the silane and the biogenic polymer matrix. The obtained material is less prone to absorb water from a humid atmosphere (reduction of 75%) and is highly efficient for the removal of non-polar contaminants from water

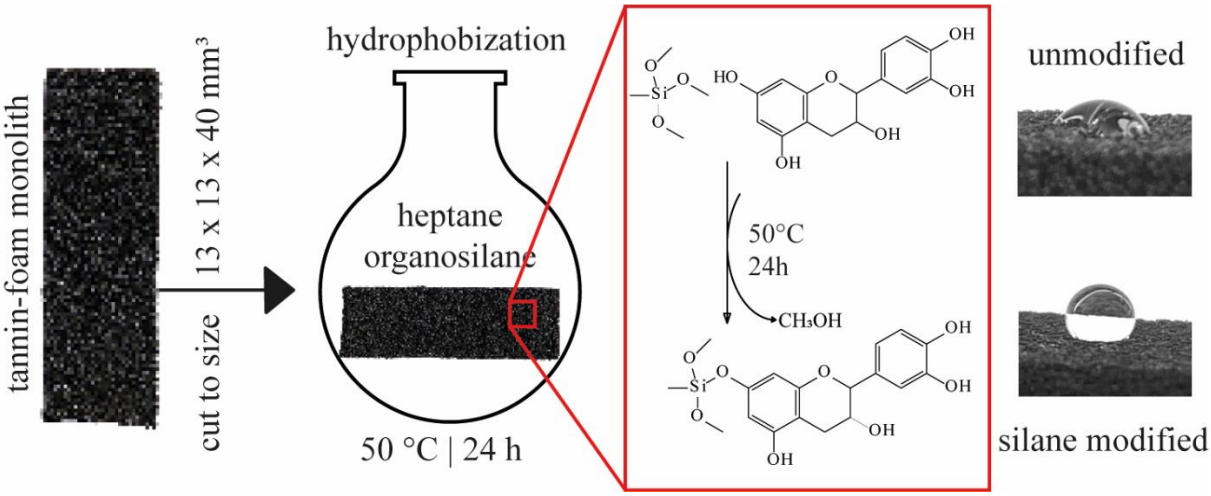

Figure 1: Schematic representation of the surface modification procedure

P.6.07 - CHEMICAL PROFILING AND ANTIFUNGAL ACTIVITY OF SUSTAINABLE PHENOLIC-RICH PLANT EXTRACTS

Lombardi A.*[a], Campo M.[b], Vignolini P.[b], Papalini M.[c], Pizzetti M.[c], Bernini R.[a]

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Fungal infections pose a major challenge to the academic community and industry operating in biomedical and agri-food fields [1]. In this context, the antimicrobial properties of phenolic compounds and their relevant presence as secondary metabolites in plants and related by-products provides concrete opportunities for the development of new alternative strategies to replace synthetic products as biocidal and food preservatives, in line with the sustainability principles [2].
In this communication, the antifungal activities of phenolic-rich extracts obtained from various plant matrices against pathogens of food interest and dermatophytes were evaluated (Figure 1).
In detail, tests were carried out on extracts obtained from pomegranate (*Punica granatum* L.*)*, chestnut (*Castanea sativa* Miller), vine (*Vitis vinifera* L.), olive (*Olea europaea* L.) and green tea (*Camellia sinensis* Kuntze) matrices, wastes and by-products using sustainable procedures [3]. An HPLC/DAD/MS analysis was performed to define the qualitative and quantitative phenolic profile of extracts. The antifungal activities were carried out through an *in vitro* diffusion method against filamentous fungi *Rhizopus stolonifer*, *Alternaria* sp., *Aspergillus brasiliensis* and *Trichophyton interdigitale*. The emerging scenario revealed a wide range of specific inhibitory activities, showing, for some extracts, promising uses in the control of fungal infections and food contamination.

Figure 1: Antifungal *in vitro* diffusion test against *Trichophyton interdigitale*

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P.6.08 - ISOLATION OF PROANTHOCYANIDINS FROM WESTERN RED CEDAR BARK: ADVANCING BIO-BASED POLYPHENOLIC MATERIALS

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Although it has been known for a long time that aqueous extracts of tree barks are a substantial source of proanthocyanidins [1], the latter are yet to be used in creating value-added materials. This could be explained, in part, by the natural distribution of tannins in the plant kingdom: it is rare, if ever, for two plant species to share the same tannin pool [2]. This makes research and development in the area of bark proanthocyanidins extremely diverse. Hence, there is a need for detailed investigations on proanthocyanidin extracts and their uses.
Western Red Cedar (*Thuja plicata* Donn) is a commercially important tree in British Columbia, Canada. While its wood has a vibrant market, the bark has remained underutilized and is commonly regarded as waste in the forest industry. In this study, western red cedar bark is found to contain proanthocyanidins (1.65%, oven-dried basis), with the majority being water-soluble at room temperature, RT (~60%) and the remaining extractable only at high temperature, HT (~40%). Based on mass spectrometry analyses, the RT water fraction is rich in branched structures with a low degree of polymerization (DP). In contrast, the HT fraction is rich in linear structures with a high DP. These structural variations in cedar bark proanthocyandins are predicted to affect their physicochemical properties and open new opportunities for designing polyphenolic materials for a variety of applications, especially in the health sector, including biomedicine, pharmaceuticals, and functional foods.

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P.6.09 - NOVEL APPLICATIONS OF POLYPHENOL-RICH EXTRACTS FROM AGROINDUSTRIAL WASTES WITHIN CIRCULAR ECONOMY

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This communication is dedicated to Prof. Annalisa Romani (University of Florence, Italy), who passed away in January 2022

Phenolic compounds are natural secondary metabolites found in plants exhibiting antioxidant, anti-inflammatory, antidiabetic, antimicrobial, cardioprotective, and anticancer activities. In view of these beneficial health effects, they are used as nutraceuticals, cosmeceuticals and active ingredients for food, feed, and new materials. According to the *circular economy* strategy (Figure 1), these bioactive compounds can be recovered from agro-industrial wastes generated in the agriculture and food sectors, promoting *green processes*.
This communication will describe the most recent results obtained in our laboratories using olive leaves, olive pomace, pomegranate seeds and peel as raw materials to obtain polyphenol-rich extracts with sustainable procedures [1]. Their chemical characterization, biological activities and novel applications will be reported [2,3].

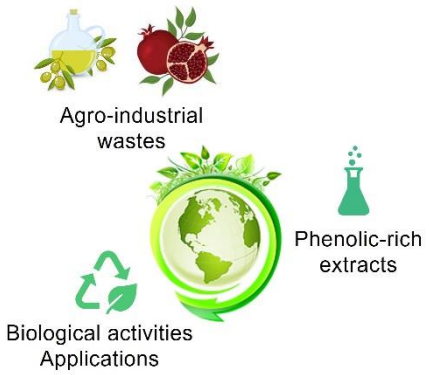

Figure 1: The circular economy model.

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P.6.10 – CURCUMIN LOADED ZEIN MICROPARTICLES FOR ACTIVE WOUND HEALING

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Treating wounds, regardless of the type, is a challenge that doctors, and health care workers face in their everyday life. The normal healing process encompasses numerous molecular and cellular mechanisms in all its phases, from inflammation to regeneration of the damaged tissues [1]. Every single step requires a precise sequence of biochemical events and the active participation of specific kinds of cells and molecules. Yet, what happens in chronic wounds is slightly different: the inflammation process does not evolve in standard times; instead, the cascade of the subsequent events that lead to the healing is delayed. This condition increases the chances for the wound to be infected by dangerous pathogens. Therefore, an ideal dressing should support the wound during its healing process, following the dynamics of tissue repair, while, at the same time, preventing the initial inflammatory phase from lasting too long. One strategy can be the release of anti-inflammatory and anti-bacterial compounds to protect the lesion from a likely bacterial contamination.
Within this frame, zein proteins represent a promising material [2]. Their large availability alongside with their slow degradation rate in water makes them particularly suitable for this kind of application, especially when combined with naturally derived anti-inflammatory drugs.
In this study, we fabricated zein microparticles via Spray Drying (Figure 1), blank and loaded with molecules presenting anti-inflammatory and antioxidant properties. Spray-drying, consisting in the atomization of a solution, the evaporation of the solvent and eventually the separation of the powder from the drying gas, guarantees a high reproducibility and is therefore a widely used process to transform a solution, suspension, or emulsion into a powder.
The so obtained formulations were chemically and physically characterized, in terms of morphology, size, zeta potential and atomic composition. Tests about the degradation rate of the microparticles showed a slow and controlled degradation mechanism. Therefore, also the release behavior of the system is studied considering different drugs, with a particular attention towards a derivative of curcumin, a phenolic compound that has shown important antioxidant activity [3]. Therefore, antioxidant and antibacterial properties of the loaded zein microparticles have been evaluated, alongside their biocompatibility on keratinocytes.

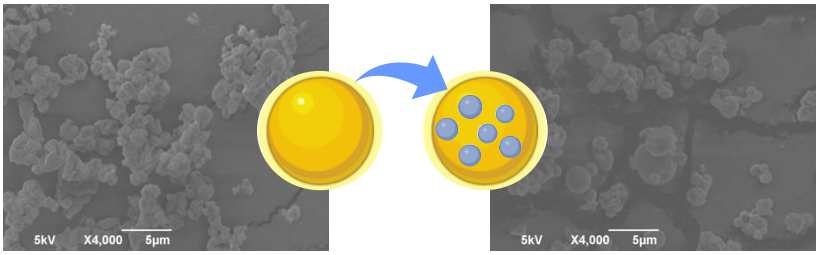

Figure 1: Schematic of the proposed system, with SEM images of the zein.

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P.6.11 - EFFICIENCY STUDY OF MACROPOROUS RESINS FOR THE RECOVERY OF SINAPIC ACID FROM MUSTARD BRAN HYDROLYSATE

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Sinapic acid, the most major phenolic compound in mustard bran hydrolysate, has drawn much attention according to its potential antioxidant, anti-inflammatory, and anticancer activities. However, due to its low concentration and selectivity in the crude extracts, it is necessary to purify it to meet the requirements of food, pharmaceutical and cosmetic markets. In this study, the purification of sinapic acid from mustard bran biocatalytic production was carried out by macroporous resins. Firstly, the efficiency parameters for the adsorption and desorption of sinapic acid were assessed on four Amberlite series resins (XAD-16, FPX-66, XAD-7, and XAD-1180). Generally, these four resins all showed a strong affinity for sinapic acid. More specifically, the adsorption capacities of XAD16 and FPX66 are significantly higher than XAD1180 and XAD7 ($p<0.05$); no significant difference ($p>0.05$) is noticed for the recovery rate and the desorption ratio for all tested resins. Next, the adsorption kinetics, adsorption isotherms, as well as the corresponding models were investigated to elucidate the adsorption mechanism and performance. The results showed that adsorption kinetics and isotherms of sinapic acid follow the pseudo-second-order model and the Langmuir model for the four resins. It is worth mentioning that XAD16 reached the adsorption equilibrium at a lower concentration, so the desorption optimization was studied only on this resin. It has been shown that the 90% ethanol fraction is the best elution concentration for sinapic acid desorption. The results provided helpful information for the performance of macroporous resins on the sinapic acid adsorption from mustard bran aqueous solution while providing the basis for studying the adsorption in dynamic mode.

P.6.12 - ANTHOCYANINS COLOR STABILIZATION BY MAYA BLUE METHOD

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Anthocyanins are secondary metabolites present in fruits, flowers and, the products derived from them. However, anthocyanin use, as an industrial colorant, is limited due to its instability towards environmental factors. The pH and hydration are the main factors in its degradation. Recent research focuses on stabilizing these pigments and making them useful in color applications. This is possible through structural stabilization or medium control; which can be approached by copigmentation, acylation, or encapsulation, among others [1].
Hibiscus flower is an agricultural product with high content of anthocyanins and phenolic acids. The principal use of hibiscus in Mexico is as a beverage, although its dyes are valuable for the color industry [2]. In turn, Maya blue is a mineral-organic pigment developed by the ancient Maya culture. The pigment, made from palygorskite and indigo extract, is stable to chemicals and time [3]. This work aims to stabilize hibiscus flower anthocyanins color with sepiolite, employing the Maya Blue-like method.
A Hibiscus infusion was made from the grounded flower in acidified water (1% HCl v/v) at 100 °C for 30 min. After that, the interaction was carried out with 0.1 g hibiscus anthocyanins/1 g sepiolite at 100 °C for 1 hour. An Anthocyanins degradation test was made, on the composite liquid phase and the control, weekly for 60 days. In parallel, color measurements of the samples and the control were carried out. Structural characterization was performed by scanning electron microscopy and by X-ray diffraction (XRD). The Rietveld method was employed to analyze XRD patterns. The composite liquid phase shows less degradation (60%) over time than the control hibiscus infusion (72%) (Figure 1). Color measurements indicate differences between the solid and liquid phases. Besides, significant gaps in samples over time were found. XRD analysis shows differences between sepiolite and composite sample structures.

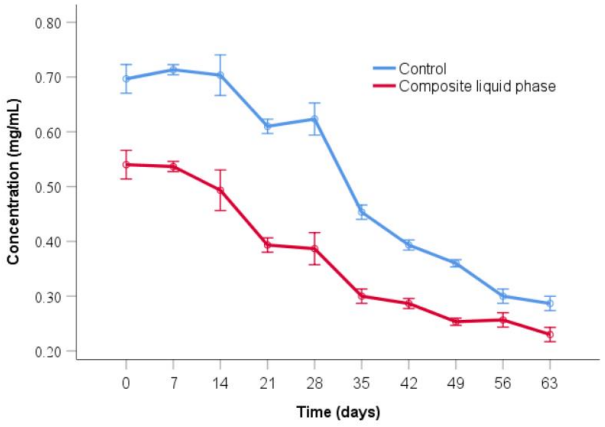

Figure 1: Anthocyanins content in Hibiscus infusion heated (Control) and Composite liquid phase.

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P.6.13 - POLYPHENOL COMPOSITION OF CHESTNUT WOOD AND BARK EXTRACTS

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Industrial-scale polyphenol extracts are produced from well-selected plant species and plant parts, the final selection depending on the target use of the industrial product. For example, industrial chestnut extract is made of both chestnut wood and bark and the extract can be used in many applications such as leather tanning processing. Although the chemical composition of the chestnut extract is quite well known, the further development of the product would benefit from better knowledge of the separate polyphenol composition of the wood and bark, i.e. their similarities and differences. So far, this kind of work has not been conducted in detail.

The purpose of this study was to do a proper qualitative and quantitative examination of different types of chestnut extracts. The analyses were done by ultrahigh performance liquid chromatography coupled to a diode array detector and two types of mass spectrometers. First, Waters Xevo triple quadrupole mass spectrometer was used to record the group-specific polyphenol fingerprints of especially the oligomeric and polymeric polyphenols. Second, Thermo QExactive Orbitrap high-resolution mass spectrometer was used to accurately characterize the smaller weight polyphenols. In the poster, we show the differences of the bark and wood extracts and the major compounds found from the extracts (in mg/g dry weight).

P.6.14 - A SUSTAINABLE VALORIZATION OF CORK WASTE THROUGH AN EFFICIENT MICROWAVE-ASSISTED EXTRACTION AND ULTRASOUND- ASSISTED EXTRACTION OF BIOACTIVE PHENOLIC COMPOUNDS

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Cork industry generate a large amount of cork powder rich in bioactive compounds resulting from cork processing industries. There is an urgent need to search for new solutions to make extraction processes more sustainable to obtain these bioactive compounds in a circular economy approach. Under this context, searching for novel methods by means of extraction efficiency and yield, time and solvents while reducing the environment impact are the main challenges of nowadays [1].
This study aimed to optimize the extraction conditions for UAE and MAE techniques:
UAE: time (3- 26 minutes) and % ethanol in solvent (22- 90%) (with fixed: 20 °C, 70% amplitude, 500 W, 20 kHz). MAE: time, % ethanol in solvent, and temperature; (5- 25 minutes), % ethanol in solvent (10-90%), and temperature (50-150 °C)
With the intent to maximize the extraction yield (EY) and the total phenolic content (TPC) of cork extracts through RSM analysis. The factorial design was applied considering temperature, %ethanol and temperature (in case of MAE) ranges described previously.
Extraction yield (EY) and total phenolic content (TPC) were the response variables in the factorial regression. EY was gravimetrically determined and TPC was calculated as the sum of all phenolic compounds individually identified by HPLC-DAD and LC-MS/MS.
The experimental data from the cork sample fit well to second-order polynomial models and were thus used to determine the optimal UAE conditions (20 min and 75% ethanol concentration) that resulted in the highest EY (23.74 ± 3.71 mg extract/g dry weight) and TPC (0.69 ± 0.03 mg/g dw). Finally, different UAE cycles were applied to the cork sample obtained at the optimal extraction conditions in order to increase the extraction EY and TPC even further. In light of this, the use of two UAE cycles significantly improved ($p<0.05$) EY (32.24 ± 0.82 mg extract/ g dw) and TPC (0.98 ± 0.015 mg/g dw) of cork industry waste. These results were comparable to those obtained using the conventional maceration method (35 °C, 7 days).
The optimal conditions obtained for MAE were 25 min, 150 °C, and 90% ethanol, and it was possible to obtain an EY of 127 ± 7 mg/g DW and a TPC of 1.85 ± 0.3 mg/g DW. These results were compared to the traditional and MAE optimal conditions (25 minutes) allowed to obtain around 40% more TPC and a two-fold EY than the one obtained in a traditional maceration. Even when compare to UAE technique, MAE doubled the quantities of TPC and EY in half of the time. Overall, MAE resulted a valuable green technology suitable to obtain bioactive compounds from cork industry wastes to tackle grow prosperity while reducing the environmental impact in a circular economy approach.

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P.6.15 - METABOLOMIC ANALYSIS FROM POTATO BY-PRODUCTS DISCLOSES INTERESTING AND VALUABLE FLAVONOIDS

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While edible potato tubers grow under the soil, potato fields are covered with a dense green biomass of leaves, flowers, and berries. By-products like flowers and berries are usually unused by the farmers and stay in the fields with no further applicability. However, these potato residuals contain highly valuable metabolites that might be used in pharmaceutical industry and for the development of plant-based pesticides. Apart from the well-known glycoalkaloids, flavonoids are among the major secondary metabolites present in potatoes, which carry advantageous biological activities, such as antioxidant, anti-inflammatory, and anticancer properties [1]. In order to enhance the circular bioeconomy in potato fields by giving a destination to the unused and rich biomass, this research aims at the extraction and quantification of major secondary metabolites from potato by-products, as well as their bioactivity assessment against plant pathogens.

For this, an initial non-targeted analysis via RP-UPLC-ESI-Orbitrap MS was performed on samples from flowers and berries from different genotypes and different sites (field and greenhouse). Later on, an in-depth analysis of this data was conducted in order to identify metabolites present in these samples mainly based on their bioactivity and novelty for potato tissues. From the non-targeted analysis, 19% of the 551 detected and annotated metabolites were secondary metabolites. Besides the intensively described chlorogenic acid, solanine and chaconine, the data also indicates secondary metabolites from the flavonoid chemical class, of which many have not yet been described in *Solanum tuberosum*. These flavonoids showed distinct accumulation among the tissues and genotypes, with some of them being exclusively detected in flowers, and others in berries.

In parallel, methanolic extraction and HPLC-MS/MS quantification of major glycoalkaloids (GAs), a class of nitrogen-containing steroidal glycosides well-known in potato, were also performed in order to check their levels in relation to genotype, tissue, developmental stage, and growth condition. The analysis of the cultivar Quarta showed that the content of GAs decreases along the phenological development of the potato plant, with flower tissue containing 4 to 7 times more GAs than berries in advanced development stage.

From the preliminary results, we conclude that potato residuals contain a highly diverse metabolome, with interesting candidates that can serve as increment for pharmaceutical and crop protection products. Next steps of this research to be discussed involve the extraction and quantification of flavonoids from commercial genotypes in different developmental stages, and the bioactivity assessment of these compounds against phytopathogens.

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P.6.16 - EXTRACTING CAFFEOYLQUINIC ACIDS FROM COFFEE SILVERSKIN USING MICROWAVE-ASSISTED EXTRACTION

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Silverskin, a by-product of the coffee roasting industry, is rich in several bioactive compounds, including caffeoylquinic acids (CQA). Conventional solid-liquid extraction (SLE) can be used to obtain CQA, but this method requires a lot of time and energy, and, in some cases, the employment of organic solvents. Thus, to achieve cost-effective extraction of bioactive compounds from natural matrices, rapid, efficient and clean alternative methods (*e.g.* microwave-assisted extraction (MAE)), are essential [1,2].
In this work, the possibility of using MAE as a fast method to produce aqueous coffee silverskin extracts rich in CQA was studied, regarding the influence of temperature (40 - 150 °C) and extraction time (2 - 90 min). For comparison, an optimized hydroethanolic (1:1) SLE was performed. The CQA profile was analyzed by RP-HPLC-DAD at 320 nm [1].
When the temperature increased from 40 to 80 °C, the extraction efficiency of the predominant compound, 5-CQA, also increased significantly. However, temperatures higher than 80 °C resulted in a lower extraction yield for this compound and higher for 3- and 4-CQA, which may be due to the isomerization of 5-CQA when subjected to temperatures of 100 - 200 °C [2]. In order to avoid the loss of bioactive compounds and minimize adverse effects of processing, 80 °C was set as the optimal temperature of extraction. Regarding extraction time, the highest value for 5-CQA was found at 5 min (0.67 mg/g dry sample). This is a short extraction time with a significantly higher concentration compared to SLE (0.57 mg/g dry sample).
In conclusion, MAE proved to be a more effective and efficient extraction method than SLE. These silverskin extracts rich in CGA can be interesting for the development of functional foods, simultaneously contributing to a circular bioeconomy.

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P.6.17 - OLIVE OIL WASTE AS A GREAT SOURCE OF POLYPHENOLS: EXTRACT CHARACTERIZATION AND COMPOUND IDENTIFICATION

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Waste from the olive industry is a noticeable source of antioxidant compounds that can be extracted and reused for the production of raw materials related to the chemical, cosmetic, and pharmaceutical sectors. Some remarkable components are flavonoids, such as luteolin and its numerous glycoside derivatives. Another large group of phenolic compounds is related to dihydroxyphenyl ethanol and its esters with elenoic acid, giving rise to renowned compounds, such as oleuropein, highly appreciated for their beneficial properties for health. The presence of some hydroxybenzoic and hydroxycinnamic acids is also highlighted. Despite the abundant literature available on this topic in the scientific forums, some issues on structural elucidation, characterization, and quantification of these compounds are still pending.

This work aims at studying the phenolic composition of olive oil waste samples using liquid chromatography with ultraviolet detection coupled to mass spectrometry (LC-UV-MS) to identify and quantify the main compounds. Olive leaf residue samples have been crushed, homogenized, and subjected to a solid-liquid extraction treatment with mechanical agitation at 80ºC for 2h using a hydroorganic solvent. The polyphenols identification in the resulting extracts has been carried out by high-resolution mass spectrometry (HRMS) using data-dependent acquisition mode using an Orbitrap HRMS instrument. More than 50 different phenolic compounds have been annotated tentatively, of which about 20 have been confirmed from the corresponding standards. Some of the most quantitatively noticeable compounds are oleuropein and its aglycone, luteolin-7-glucoside, 3-hydroxytyrosol, and verbascoside. Finally, many of these secondary metabolites present in olive leaf residues also occur in other matrices related to the olive oil production process, such as pomace pastes. We can conclude that these matrices may offer excellent opportunities as sources of antioxidant compounds and that their recovery can contribute to the recycling and revaluation of these residues. Some issues to be solved, such as the fractionation of extracts and purification of compounds, will be addressed in further studies.

P.6.18 - POLYPHENOLIC BIOACTIVE POTENTIAL OF SOFTWOOD FOREST WASTE BIOMASS (BARK AND NEEDELS)

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The interest in extraction of bioactive compounds from biomass, especially polyphenols, recently increased, due to their valuable biological potential as natural source of antioxidants [1]. The phenolic compounds are commonly produced by plants as secondary metabolites, as a measure of protection and adaptation to different stress conditions and which could be used in a wide range of applications, from chemicals and pharmaceuticals to green polymers and bio-based materials [2].
The present research aimed to characterize the phytochemical composition of forest biomass (bark and needles) belonging to different conifer species, such as spruce (Picea abies Mill.) and fir (Abies) in order to identify the main bioactive compounds specific to each type of biomass. For this, the bark and needle samples were subjected to extraction in hydroalcoholic solution under the action of a microwave field (MAE) that followed an extraction protocol: pressure 40 bar, ramp 10 min, temperature 50°C, time 5 min., and the resulted extracts were characterized in term of total polyphenols, total flavonoids and antioxidant activity by UV-Vis spectrophotometric methods and by UHPLC-MS/MS for the identification and quantification of bioactive compounds from the class of polyphenols.
The obtained results indicated that the bioactive composition of the coniferous forest biomass depends on the species and different parts of the plant. 3,4 dihydroxybenzoic, 4 hydroxybenzoic and ferulic acids, rutin, isorhamnetin and apigenin are the main phytochemical components of spruce biomass, while syringic acid, catechin, myricetin, quercetin, galangin, t-resveratrol characterize the fir biomass (needles and bark). The results indicate that coniferous forest biomass, with low economic value, has the potential to be used as an alternative source of bioactive compounds with antioxidant potential that can be used for the development of new food supplements, medical devices, personal care products (creams, gels). In this context, developing industrial upscaling strategies are imperative for the long-term success of biorefineries, as part of a circular bio-economy.

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P.6.19 - ANTIOXIDANT, ANTIMICROBIAL COMPOSITE FILMS BASED ON SODIUM ALGINATE AND GALLNUT EXTRACT

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Among a variety of natural extracts, gallnut extract (*Quercus infectoria*) containing the highest naturally occurring levels of tannin (gallotannin, 50-75%) with a mixture of small molecules including gallic acid and ellagic acid, has been reported to display excellent antioxidant capacity and high antimicrobial potential against common pathogens [1]. Sodium alginate obtained from brown seaweeds has shown great potential as film forming material owing to its excellent film forming properties, non-toxicity, and unique colloidal properties [2].

In this study, sodium alginate composite films were produced by solvent casting in water, and the effect of gallnut extract addition at different weight ratios on the functional and physical properties of the developed films were evaluated in terms of mechanical properties, water vapor permeability, thermal stability, microstructure, optical properties, antioxidant capacity, and antimicrobial properties [3]. The antimicrobial activity of the produced films was evaluated against two common pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli*, and two pathogenic fungi, *Aspergillus niger* and *Penicillium digitatum*. Increasing gallnut extract content from 0 to 25 wt% (based on sodium alginate weight) increased tensile strength and elongation at break of sodium alginate films in the range of 48-103% and 135-185%, respectively, without affecting their thermal stability. In addition, increasing gallnut extract from 2.5 to 50 wt% reduced water vapor permeability of composite films in the range of 28.5-50.1% compared to the neat sodium alginate films and increased light barrier properties. As revealed by scanning electron microscopy and Fourier-transform infrared analysis, such improvements in functional and physical properties of sodium alginate films can be attributed to the good compatibility between the polymeric matrix and the incorporated gallnut extract along with the increase in interactions between sodium alginate molecules and gallnut extract polyphenolic compounds. Moreover, the incorporation of gallnut extract significantly improved the antioxidant capacity of composite films most likely due to the high total phenolic content of the incorporated gallnut extract. Furthermore, the resulting films showed good antibacterial activity against Gram-positive and Gram-negative pathogenic bacteria, suggesting their significant contribution, as antibacterial packaging materials, towards shelf-life extension and food safety preservation.

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P.6.20 - GREEN SYNTHESIS OF ORTHOGONAL PHENOLIC MONOMERS AND METAL NANOPARTICLES

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In recent years, green synthesis is generating great interest as a sustainable and environmentally friendly strategy to prepare a wide range of materials and nanomaterials. For this strategy, it is key to reduce the negative effects associated with traditional material preparation methods commonly used in laboratories and industry. On the other hand, the interest in bio-based materials, like polyphenols, has increased in recent decades due to both ecological and economical concerns [1].
We herein explored the sustainable synthesis of phenolic monomers with orthogonal functionalities (Figure 1). These monomers are high-value precursors for the synthesis of multifunctional dendritic polymers. As main tool, we have employed thia-Michael addition reactions on α,β-unsaturated esters generated *in situ*, using Knoevenagel decarboxylation reactions [2]. This is a "one-pot" method, sustainable, scalable and highly versatile, which provides phenolic AB_xC type monomers.

Furthermore, these orthogonal biomonomers were employed for the green synthesis of silver and gold nanoparticles (AgNPs, AuNPs), overcoming the main limitations in the chemical synthesis of metal NPs: the health and environmental issue from the use of organic solvents [3]. The new phenolic compounds rapidly converted the metal salts into metal nanoparticles, and in parallel formed *in situ* natural coatings that provided stability and biocompatibility.

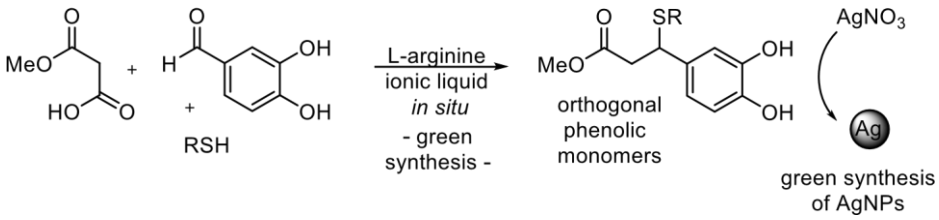

Figure 1: Green synthesis of orthogonal phenolic monomers and AgNPs.

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P.6.22 - GENOME-WIDE TRANSCRIPTIONAL ANALYSES REVEAL PATHWAYS FOR THE METABOLISM OF LIGNIN-RELATED COMPOUNDS IN *XANTHOMONAS CITRI* SUBSP. *CITRI*

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Lignin is the second biopolymer most abundant on Earth, and a carbon-rich agro- industrial residue, which makes it an attractive renewable feedstock for chemicals production. However, the structural heterogeneity of the compounds derived from the lignin depolymerization imposes technical challenges for reaching high purity products with high yields. In this context, the study of bacteria capable of funneling complex mixtures of lignin-derived molecules into specific target products, and whose molecular strategies are still little explored, such as those from the genus *Xanthomonas*, has the potential to reveal new metabolic routes for the bioconversion of this abundant agro- industrial residue. Therefore, the purpose of this study is to define which lignin model compounds are metabolized by *Xanthomonas citri subsp. citri* strain 306 (*Xac* 306) and identify which metabolic pathways *Xac* 306 uses to bioconvert these aromatics. For this purpose, we used a multidisciplinary approach combining genome data mining, growth studies, HPLC analyses and RNA-seq studies. The growth curves and HPLC analyses showed that *Xac 306* can metabolize a variety of lignin-related compounds, such as coniferyl alcohol, vanillin, 4-hydroxybenzaldehyde, 4-hydroxybenzoate, syringaldehyde among others. The transcriptional profile of *Xac* 306 in response to the aforementioned aromatic compounds supported the initials predictions based on a genome mining approach for the syringate funneling pathway and for the catabolism of H and G-type lignin monomers via protocatechuate ortho-cleavage. Moreover, transcriptome analyses showed the up-regulation of two gene clusters (XAC0353-54) and (XAC0881-82-83), which encode enzymes with activities compatible with those required for the conversion of sinapyl alcohol to syringate. The RNA-seq data also revealed unprecedented candidate genes encoding for putative NAD(P)-dependent oxidoreductases possibly involved in peripheral pathways for the metabolism of lignin-derived aryl aldehydes. Biochemical validation of some of the hypotheses raised above are in progress and support a role for XAC0353-54 in the bioconversion of sinapyl alcohol up to sinapate, revealing an enzymatic pathway not yet reported for microorganisms in the literature. In summary, the present study exhibits some evidence for the presence of a complete pathway for coniferyl alcohol and sinapyl alcohol catabolism in *Xac* 306 and expands the current knowledge on the bioconversion of S-type lignin-derived compounds, which might be instrumental for the development of microbial platforms for the valorization of S-rich lignins.

P.6.23 - HESPERIDIN-LOADED NANOPARTICLES AS A PROMISING SUSTAINABLE RELEASE SYSTEM FOR ENVIRONMENTAL APPLICATIONS

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During their evolution, plants developed the capacity to produce a wide diversity of secondary metabolites, which they use in their responses and adaptation mechanisms to biotic and abiotic factors. Botanical pesticides have gained attention as potential natural pesticides and suitable alternatives to traditional pesticides because they decompose rapidly in nature owing to their contact with air, moisture, high temperatures, and sunlight. Flavonoids, a subclass of polyphenols, have been extensively studied for this purpose because of their involvement in plant defence responses against insects and pathogens. One of these interesting molecules is hesperidin, whose bactericidal and insecticidal activities have been studied either alone or in coordination complexes [1,2]. However, botanical pesticides may exhibit performance variation under different conditions, have low efficiency, are slow to kill, and have a limited shelf life compared to conventional pesticides. Their encapsulation in biodegradable polymers such as polysaccharides may help overcome this problem by providing a sustainable release system. The present study aimed to immobilize hesperidin in pectin nanoparticles (HES-PecNPs). Different hesperidin concentrations (0.1, 0.3, and 0.5 mg/mL) were tested to evaluate the most advantageous particle for environmental applications. The hydrodynamic size and zeta potential of the particles were characterized using dynamic light scattering (DLS), and their encapsulation efficiencies were determined. The most stable HES-PecNP was further characterized by determining its water absorption capacity and performing release and stability studies.

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P.6.24 - HESPERIDIN AND PECTIN SEQUENTIAL EXTRACTION FOR ORANGE WASTE VALORIZATION

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Orange is the most consumed citrus worldwide, representing more than 55% of world's citrus consumption. From the whole fruit, only 50% is consumed while the remaining is discarded as organic waste. Such wastes are economically and environmentally problematic, contributing to pollution problems and the development of pathogenic organisms. However, these wastes, composed mainly by peels, leftover pulp and seeds, when properly exploited, can be a source of value-added compounds, since they are rich in different bioactive compounds, such as pectin and flavonoids (namely hesperidin), phenolic acids, essential oils, terpenoids, and others.

Hesperidin, a glycosylated flavanone, is one of the most abundant flavonoids found in citrus fruits. This phenolic compound has recently been investigated for biomedical applications, such as an anti-inflammatory agent but its insolubility in water makes its extraction difficult. Furthermore, the majority of the reported methods are non-selective for this flavonoid, which contributes to the presence of impurities and the need of additional purification methodologies.

Pectin is a heteropolysaccharide, most commonly found in the cell wall of fruits, with defined functional properties determined by the degree of esterification, which can be classified as low methoxyl or high methoxyl pectin. Pectin have been most commonly researched for their gelling properties, and they are frequently used as a gelling, thickening, and stabilizing agent in the food industry for the production of jams and jellies. However, it is also been under investigation as a promising anti-cancer agent.

The present work focused on the development and optimization of a green approach to a sequential extraction of pectin and hesperidin, while simultaneously maximizing the reuse of orange waste and solvents used. The extractions were based in green solvents with pH changes. The obtained yields averaged 18.7 ± 1.18% for pectin, and 1.07 ± 0.08% for hesperidin. Both compounds were obtained with high purity. Pectin showed AUA above 65% (66.2 ± 1.25%) and DE above 50% (59.4 ± 0.74%), while hesperidin purity, 84.01%, was assessed by HPLC-PDA. The solvents used throughout the work were recovered and reintroduced in the sequential extraction system.

P.6.25 - DEEP EUTECTIC SOLVENT-BASED RECOVERY OF LIGNINS WITH POTENT ANTIOXIDANT PROPERTIES FROM AGRI-FOOD BY-PRODUCTS

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Lignins are antioxidant phenolic polymers which are finding increasing applications in a variety of fields. Consequently, there is a growing need for easily available and sustainable sources as well as for green extraction methodologies of these compounds. Within this context, we developed efficient strategies for the recovery of lignins of good purity endowed with potent antioxidant properties from different agri-food by-products based on the use of deep eutectic solvents (DES), which represent a valuable and green alternative to conventional organic solvents for fractionation and processing of lignocellulosic biomasses [1].
In particular, a tunable DES-based processing for valorization of chestnut wood fiber, a clean and largely available solid waste of the tannin industry, as a source of lignin was first developed [2]. This involved a first extraction of the material with 2:1 mol/mol choline chloride/tartaric acid at 50 °C for 90 min, followed by treatment of the solid residue with 1: 2 mol/mol choline chloride/lactic acid at 120 °C for 8 h. The first treatment afforded a sample containing lignin and ellagic acid as the main low molecular weight phenolic compound, whereas the second one allowed to obtain an ellagic acid-free sample containing mainly a structurally homogeneous guaiacyl-syringyl lignin, as demonstrated by electron paramagnetic resonance (EPR) and chemical degradation analysis. Both samples exhibiting very potent antioxidant properties in different chemical assays, and, in particular, the one from the mild treatment was also able to provide a controlled release of ellagic acid in phosphate buffer at pH 7.4. This result could be of interest for a possible exploitation of this sample in the biomedical sector, for the sustained release of this water-insoluble and bioactive compound under physiologically relevant conditions.
The DES-based treatment at 120 °C, combined with ball milling, was adopted also for the recovery of lignins from the shells of edible nuts [3]. Extensive spectroscopic and chromatographic analysis confirmed that the main phenolic constituents present in the shell extracts were lignins, which again exhibited very promising antioxidant properties, particularly in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (EC_{50} values ranging from 0.03 to 0.19 mg/mL). Notably, a significant improvement of the antioxidant properties was observed for lignins from specific shells further to nanoparticlization or incorporation into TiO_2-based hybrid materials. These latter, in particular, showed also promising photoprotective properties against DNA damage in skin explants.

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P.6.26 - SUSTAINABLE VALUE CHAIN FOR COLOUR – POLYPHENOLS AS COLOURANTS AND AIDING CHEMICALS

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Bio-based materials and new technologies gain growing interest in different applications, as companies actively want to enhance products sustainability and remove environmental and hazardous pollutants during the manufacturing process. Degradable and recyclable textile and package innovations, for example, are increasing. However, currently synthetic colourants are used, and synthetic dyes and pigments are designed to be stable, which poses contradiction with biodegradability. There is increasing interest to advance the usage of environmentally sound colourants from fungal and plant sources in different applications.
Majority of the currently used natural colourants are obtained from plants in which phenolic compounds have substantial role. Polyphenols include flavonoids, tannic acid and ellagitannin, compunds which show high potential as dyes, mordants and other aiding chemicals for example in textile colouration processes.

In addition to our research we announce here the Biocolours 2024 event [1] welcoming researchers from all over the world to Helsinki, Finland, to share findings of natural and bio-based colourants within the theme of 'Sustainable Value Chain for Colour'. Biodegradable materials are used to increasing extent, and we need alternatives to the current synthetically produced, oil-based colourants. Biocolours 2024 wants to look at bio-based colourants from multidisciplinary viewpoints combining research with businesses. We want to open the floor for presentations highlighting the entire value chain from the production of colourants, their applications and design to marketing and even consumer aspects.

Colour is an integral part of the built and designed environment, but the average consumer seldom thinks about the material basis of colour, and the environmental and safety issues related to colourants. For the Biocolours2024 we want to call for papers developing new methods of biocolourants' large-scale production, characterization, and applications. We are eager to hear presentations widely from different fields about research aiming towards fundamental understanding of biocolourants, and also interpretations about the societal acceptance and consumers needs for successful implementation.

We believe that natural colourants are not only history and small-scale craft practise, but also have huge potential, and they are already part of the more sustainable colour futures.

[1] Biocolours 2024 https://www.helsinki.fi/en/conferences/biocolours2024.
[2] BioColour – Bio-based dyes and pigments for colour palette, https://biocolour.fi.

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P.6.27 - ENVIRONMENTALLY FRIENDLY PACKAGING THAT IS EDIBLE OR BIODEGRADABLE

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Films and coatings are the two categories of edible/biodegradable packaging. However, coatings can only be edible. The terms coating and films are frequently used in literature data when referring to edible or biodegradable packaging. Biopolymers are typically used for both edible and biodegradable packaging types. Even though edible and biodegradable packaging has been around for a while, it can be thought of as the next level of packaging. These package designs connect the three qualities: utility, preservation, and packaging. The unique benefit of edible/biodegradable packaging is that it may contain bioactive substances that might enhance the nutritional profile of wrapped/packed foods or lengthen their shelf life. The packaging gains antibacterial and antioxidant capabilities from the bioactive ingredients. However, since these packaging are biodegradable, as opposed to the widely used plastic polymers, the main advantage of edible/biodegradable packaging is its ecological element. This is the primary cause of the increased acceptance of certain kinds of packaging. The ability to be made from food industry leftovers and so lessen waste accumulation is another specific benefit of edible or biodegradable packaging. The creation of edible/biodegradable packaging involves the utilization of polysaccharides, proteins, lipids, and a variety of other ingredients. These types of packaging are used as a delivery strategy for bioactive substances due to their functional characteristics. The main objective of the lecture is to highlight the key production procedures and subsequent experimental steps while also providing an overview of the current difficulties pertaining to biodegradable packaging.

P.6.28 - POLYPHENOL CONTENT IN LEAVES OF LETTUCE (*LACTUCA SATIVA*) GROWN WITH BIOMASS ASH FERTILIZATION

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Lettuce is a common vegetable appreciated for its green and tasty leaves. Three different types have a wide, most of all culinary applications, apart from other specific uses. Not only sensory properties, but as well the presence of bioactive compounds decide on the value of this vegetable. Lettuce bioactive compounds are chlorophyll, carotenoids and polyphenols. The last play an important role, as the antioxidants most of all, in health benefits of the vegetable. The concentration and availability of nutritionally important compounds in lettuce depends on many factors, among them fertilization. Lettuce requires quite intensive fertilization. The alternative for common mineral fertilisers could be ash resulting from burning biomass as a fuel. Presented work compares polyphenol concentrations in lettuces 'Lento' cultivar fertilised with commercially available fertiliser and ash from plant biomass. The plants grown in polyethylene pots (Kick-Brauchmann system), with 9 kg of subsoil, with basic fertilization 5 g nitrogen per pot with addition of various doses of commercially available "biochar" – product of biodegradable fraction of urban garbage combustion, and ash from wood chips combustion - "ash". Doses of 20 and 40 g of biochar per pot and 50 and 100 g of ash per pot were applied. The results showed that addition of 50 g of ash with 20 g of biochar resulted in the increase both dihydroxyphenols (as caffeic acid) and flavonoids – 33% comparing to control assay. Ash used without biochar resulted in the increase of phenolics as well, 43-48%, depending on dose. Taking into account the increase in the content of polyphenols, the use of ash from the combustion of natural fuel - plant biomass seems to be an interesting alternative.

P.6.29 - BIOACTIVE COMPOUNDS IN APPLE POMACE RESIDUE AND EXTRACT STABILISATION FOR POTENTIAL PRACTICAL APPLICATIONS

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Apple pomace is an insufficiently utilized waste from the apple industry which shows a high potential to be processed for its nutritional and pharmaceutical potential [1], but also through fermentation processes to obtain biobased products to meet societal needs, in the circular bioeconomy system [2].
In this study, we propose superior valorization of apple pomace through the solvent microwave-assisted extraction of polyphenolic and triterpenic compounds and, further, the embedding of resulted bioactive extract into mesoporous MCM-41-type silica matrix in order to reduce the extract sensitivity and enhance its stability for practical applications. Response surface methodology by Box–Behnken design was used to optimized the extraction parameters in order to maximize the polyphenols yield. The apple pomace extract was characterized for total polyphenols, total flavonoids and antioxidant activity by UV-Vis methods and by UHPLC-MS/MS and UHPLC-DAD for profiling the bioactive compounds, while the embedding of the polyphenolic compounds into MCM-41-type silica matrix was confirmed by FTIR and UV-Vis spectroscopies. The biological activity was determined by evaluating the antimicrobial activity (*S. aureus, E. coli, S. flexneri, S. typhimurium vs enterica*), prebiotic effect (*L. acidophilus, L. paracasei, L. plantarum*) and *in vitro* biocompatibility.
The results showed that the optimal extraction conditions were as follows: microwave power 480 W, extraction time 105 s, ethanol concentration 50 % and ratio of solvent to raw material 30:1. The lyophilized extract obtained from apple pomace contained high polyphenolic (136 mg GAE/g, dw) and flavonoids (34 mg QE/g, dw) contents and had a good antioxidant activity (1800 µmoli Trolox equivalent/g, dw). UHPLC-MS/MS characterization of the apple pomace extract indicated important amounts of antioxidant bioactive compounds such as quercetin, myricetin, epi-catechin and p-hydroxybenzoic, 3,4-dihydroxybenzoic, cinnamic, ferulic, p-coumaric and chlorogenic acids, but also the presence of other specific bioactive compounds such as procyanidins, dihydrochalcones and flavonol derivatives, proving the importance of apple pomace as valuable source of bioactive compounds. The apple polyphenolic extract was successfully encapsulated into MCM-41-type silica matrix and demonstrate antimicrobial activity for the pathogenic strains, especially against S. *flexneri* strain, a microorganism involved in dysentery, but also prebiotic effect, especially on the L. *paracasei* strain at non-cytotoxic concentrations on L929 murine fibroblasts cell line (lower than 1.2 mg/mL). These results support further potential utilization of MCM41 silica matrix impregnated with polyphenolic extract for developing new food preparation and functional health products.

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Acknowledgments: This work was funded by Romanian Ministry of Education and Research, under NUCLEU Program-Financing Contract no. 20N/2023, Project PN 23 15 04 02 and Program 1-Development of the national research and development system, Subprogram 1.1. Institutional performance-Projects to finance excellence in RDI, Contract No. 19PFE/30.12.2021

P.6.30 - XYLOSOURCED POLYPHENOLIC DERIVATIVES WITH SELF-ASSEMBLING PROPERTIES FOR ENHANCING THEIR PHARMACEUTICAL ACTIVITIES

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Industrialists using petrochemical-based compounds are more and more concerned in the safety and diversification of their supply sources which has led to a growing interest in recent years to seek alternative bio-solutions. In this context and taking into account the concept of circular economy, research in wood chemistry is intended to obtain molecules that can become new materials, biotechnological tools or be used in industry, and thus be valorized, with emphasis on the use of wood industry by-products, which would also add significant value to these wastes.

Thus, wood extractives are a type of compound that can be used in response to the global public health problems. One type of particular interest is polyphenols, the major secondary metabolites of plants. They show many important properties, such as antioxidant, cardio-protective, anticancer, anti-aging, anti-inflammatory, antimicrobial.

For example, regarding their antibacterial properties, a large number of authors agree that polyphenols cause either alteration of cellular proteins or inhibition of membrane enzymes and disruption of the bacterial membrane which origins physiological and morphological changes, producing leakage of intracellular compounds. They also exhibit synergistic effect with traditional antibiotics which can enhance their efficacy, decrease their dose, and thus reduce their adverse effects. Some compounds are found in significant amounts in wood extracts and have outstanding antibacterial activity.

Despite all the benefits that polyphenols present, many studies have shown that their bioavailability is low, which is a challenge to overcome. One strategy to improve their bioavailability is chemical modification of their basic structure to obtain a form with favorable kinetics in the organism.

The overall aim of this study is to selectively modify these polyphenols to obtain poly-functional compounds with self-assembling properties (Figure 1) and thus improve their biological properties, by acting on the target with different modes of action, while conferring them transporter properties within organisms.

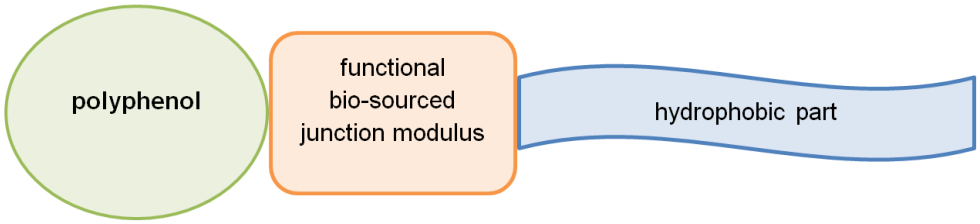

Figure 1: Model structure of a poly-functional self-assembling compound.

P.6.31 - NANOENCAPSULATION OF RED CABBAGE ANTHOCYANINS BY COACERVATION OF WHEY PROTEINS AND HIGH METHOXYL PECTIN

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Anthocyanins (ACNs) are phenolic molecules known for their antioxidant prowess, health benefits, and strong coloring capacity. An important source of ACNs is represented by red cabbage (RC - *Brassica oleracea* L. var. capitata f. rubra), whose discarded leaves may be valorized from a circular economy perspective. Nevertheless, ACNs, due to their susceptibility to degradation, need to be protected from environmental stresses [1]. In this work, we encapsulated ACNs extracted from RC leaves and stems, exploiting a coacervation process based on the ionic interaction between two colloids deriving from food industry by-products, i.e., whey proteins (WP) and apple high methoxylated pectin (HMP). Four different combinations were tested and compared. The solution containing 1% w/v HMP, and 2.5% w/v pre-heated (60°C x 40 min) WP solution gave the best results in terms of lower particle dimensions, narrower size distribution (polydispersity index, PDI < 0.24) and higher surface charge (ζ-potential). Increasing the concentration of ACNs significantly affected mean diameter and ζ-potential (fig. 1). The encapsulation efficiency (EE%) of the process was 29.6% ± 2.5, in agreement with previous results on other ACNs sources [2]. Fourier-transform (FT) IR analysis confirmed the presence of phenolic compounds entrapped in the biopolymeric structure. Owing to its simplicity, this coacervation approach may represent a valid, sustainable, and easily scalable strategy to encapsulate RC anthocyanins.

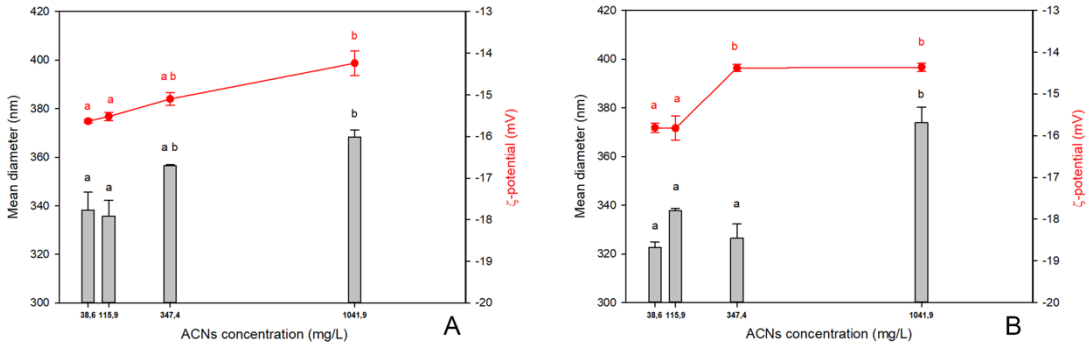

Figure 1: Effect of ACNs concentration on the mean diameter and ζ-potential of coacervates on day 0 (A) and day 14 (B). Different lowercase letters indicate significative differences (p<0.05) between samples. Each data is expressed as mean of triplicates ± standard error.

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P.6.32 - PHYTOCHEMICAL SCREENING, CHARACTERIZATION OF CHEMICAL COMPOUNDS, EVALUATION OF ANTIOXIDANT AND ANTIFUNGAL ACTIVITIES OF OF *LETESTUA DURISSIMA* (NKONG AFANE) WOOD EXTRACTS FROM GABON

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The sustainable management of the Gabonese forest is an essential point of the strategic plan "Gabon Emerging Markets", as part of its "Green Gabon" pillar since 2009. Gabon has indeed an ambitious vision in the forest-wood sector by 2025, which is to make Gabon a world leader in certified tropical woods, driven by an innovative industry, promoting a sustainably managed forest, carbon sink and a sanctuary of biodiversity. A path of innovation could be the valorization of extractives present in by-products of high natural durability wood species exploited in Gabon or of species used in traditional medicine such as *Letestua durissima* for the search for new bio-active molecules. Development of new markets based on the valorization of wood extractives constitutes therefore an attractive way of diversification of the wood industry. In this context, the characterization of the chemical composition and the evaluation of the antioxidant and antifungal activities of the extractives present in the different compartments (bark, sapwood and heartwood) of *Letestua durissima* wood, also called kong afan in Gabon, have been investigated. The overall extract contents of the different parts of the wood studied vary between 37.3 and 7.69% with high total contents of polyphenols (87.24% acetone extract from the bark), condensed tannins (119.3% toluene-ethanol extract from the bark) and flavonoids (20.26% toluene-ethanol extract from the bark). The presence of condensed tannins was confirmed by chromatographic analysis. Phytochemical screening revealed also, in addition to phenolic compounds mentioned above, the presence of alkaloids, sterols, triterpenes and saponins supporting the use of *Letestua durissima* in traditional medicine. The extracts of *Letestua durissima* show fungistatic effect against white rot and brown rot fungi (acetone, toluene-ethanol and aqueous extracts of bark, sapwood and heartwood). Evaluation of antioxidant properties, using DPPH method, of extracts obtained with different solvents show strong radical inhibition capacity. The different families of polyphenols identified possess numerous biological properties among which antioxidant activities that could be valuable in different areas as in cosmetics, for example, to limit the effects of aging due to radical species.

P.6.33 - BIOBASED BORONIC ESTER VITRIMER RESIN FROM EPOXIDIZED LINSEED OIL FOR RECYCLABLE CARBON FIBER COMPOSITES

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In the last decades, fibers reinforced polymer composites (FRPC) have experienced a continuously rising demand, since they are widely employed in various applications. Most commonly used matrices are the epoxy thermosets, since they have excellent thermomechanical properties and they are lightweight [1]. However, those materials cannot be reprocessed or recycled at the end of their lifetime, which causes a rapid consumption of fossil-based feedstocks and environmental concerns. Moreover, the permanent cross-linking of the matrix makes very difficult the recovery of the valuable reinforcements inside the composite [2,3].
To overcome these drawbacks, we propose a new bio-based, vitrimer resin for carbon fiber reinforced composites that can be thermally reprocessed multiple times, keeping unaltered its physicochemical properties thanks to the dynamic boronic ester exchange. The new composite materials can also be easily recycled by vitrimer hydrolysis under mild conditions, making possible the full recovery of the carbon fibers contributing to “circular economy”. The developed material is synthetized by the reaction between the epoxidized linseed oil and a diboronic ester dithiol dynamic cross- linker, through a thiol-epoxy “click” reaction [4-6]. Both the syntheses of the cross-linker and the vitrimer have been developed following green chemistry principles. In particular, the synthesis of the new vitrimer takes advantages of a catalyst and is a solvent free process.

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P.6.34 - TRANSFORMED ROOT CULTURE IN *POPULUS* FOR THE PRODUCTION OF SPECIALIZED METABOLITES

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During the last decade, a boom of interest for plant-based product as a food, pharmaceutical or cosmetics both in developing and industrialised nations has been observed. The international trade of plant-based products is becoming a key asset in worldwide economy. To cope up with the ever-increasing demand, the companies are looking for alternative ways for sustainable production of natural and plant-derived products in a safe and environment friendly way. Keeping this in view, poplar (*Populus*, *Salicaceae*), local grown tree species of Centre-Val de Loire region (France) has been studied for the sustainable production of specialized metabolites from hairy root culture during the present study. Hairy roots, also known as transformed roots were obtained by co-cultivation of *in vitro* leaves of hybrid poplar (*P. tremula*♀ x *P. alba* ♂) with *Agrobacterium rhizogenes* strain 15834 on Murashige and Skoog (MS) (1962) medium supplemented with acetosyringone. Fast growing root lines were selected and grown individually on MS medium supplemented with polyvinylpyrrolidone. Hairy roots so obtained showed the typical characteristics including number of root hairs, fast growth on plant growth regulator free medium and negative geotropism. Transgenic nature of transformed roots was confirmed by amplification of *rol* genes using PCR. Hairy roots were cultivated both in agar-gelled and liquid media. UPHLC-MS analyses of hairy roots showed the presence of twelve major compounds including; catechin, nigracin, salireposide, trichocarpin and tremulacin based on literature studies. Root extracts exhibited antioxidant activity as observed by DPPH and FRAP assays. This is the first report of successful induction of hairy roots and their sustainable growth in hybrid poplar. Hairy roots produced the natural compounds, which have potential to be used in various medicines and cosmetics. Further studies for large- scale production of hairy roots of hybrid poplar using RITA@ system are underway.

P.6.35 - WOOD KNOT POLYPHENOLS: FROM WOOD DURABILITY TO ANTI-AGEING APPLICATION

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The work presented here is in conjunction with "ResiNoeud", a sustainable development and circular economy project. "ResiNoeud" aims to enhance valorization of wood industry co-products, especially knots from softwood species. The growing demand of consumers for greener and more natural cosmetics is pushing industries to study the various properties found in plants for human applications. This is why the use of wood extracts rich in polyphenols can be beneficial for the cosmetic industry.
Indeed, the knots can afford bio-sourced molecules with high added value, which can then be used as active ingredients in anti-ageing and anti-pollution cosmetics.
The knot is defined as the part of the branch that is gradually incorporated into the trunk. It is formed by the extension of the rings of the trunk into the rings of the branch. Knots have a role of mechanical support [1] but also of conduction of water and nutrients to the leaves, as well as a role of storage of carbon reserves. They also play a role of protection and defense against the entry of pathogens into the trunk thanks to their high concentration of extractives. Indeed, the protective function in plants, and in trunk wood in particular, is mainly provided by compounds called extractives. These extractives belong to different chemical families among which polyphenols. Knot wood contains extractives in high concentration as shown by Willför et al. [2]. These knots, considered as waste in the paper or wood industries, could be valorized towards high added value markets. Indeed, the high and varied extractives content of knots can confer them various biological activities, such as antioxidant or antibacterial activities, for example, and could therefore be valorized in the field of cosmetics.
Skin ageing (dermis and epidermis) are due to oxidative stress, which involves reactive oxygen species (ROS) that damage DNA or cell membrane components. However, these are not harmful compounds, as ROS are need them for cellular communication. The main problem is that these ROS are produced in excess during oxidative stress and our organism can no longer maintain the balance. Exogenous antioxidants are then precious to help organism to fight against free radicals.
This study focuses on the valorization of softwood knots provided by primary wood processing industries through phytochemical screening and extraction and purification of molecules of interest. The composition of the extracts is studied by liquid chromatography, high-resolution mass and through molecular networks. Finally, to observe their potential antioxidant or anti-ageing activities, the extracts are submitted to enzymatic and colorimetric tests such as xanthine oxidase inhibition, or such as the DPPH antiradical test.

[1] Eder M., Jungnikl K. & Burgert, I., *Trees*, 23, 79-84, 2009.
[2] Willför S., Nisula L., Hemming J., Reunanen M. & Holmbom, B., *Holzforschung*, 58, 335-344, 2005.

P.6.36 - COMPARISON OF DIFFERENT PROCESSES FOR THE EXTRACTION OF SPECIALIZED METABOLITES FROM HAIRY ROOTS IN *POPULUS*

Bruat M.*[a], Heng S.[a], Laffon M.[a], Malik S.[a], Colas C.[b], Destandau É.[b], Héricourt F.[a], Depierreux C.[a], Chefdor F.[a], Carpin S.[a], Lamblin F.[a]

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The choice of solvent and extraction processes from plants are the key steps for isolation and identification of compounds possessing biological properties to be used as active ingredients for cosmetic and pharmaceutical industries. Optimization of extraction processes depends on the various factors such as solvent selection, extraction duration, mixing ratio related to the nature of phyto-constituents. The present study aims to standardize the extraction method to obtain specialized metabolites from hairy root cultures of hybrid poplar (*P. tremula*♀ x *P .alba* ♂) exhibiting biological properties. Ethanol (70% and 90%) or ethyl acetate were employed using different mass:solvent ratios (1:10-1:40). Samples were subjected to ultasonication for 15, 30 or 60 minutes. Total flavonoids and phenolics compounds and also antioxidant activities were measured using colorimetric tests. Results showed that ethanol (70%) was efficient in extracting total phenolics and flavonoids and showed higher anti-oxidant activities compared to ethyl acetate. Mass: solvent ratio 1:40 with ultrasonication for 1 hr gave the optimum response irrespective of the solvent used. No difference was observed between the chemical profile of extracts obtained by UHPLC-MS analysis from 70% and 90% ethanol extracts and confirmed the presence of catechin, isolariciresinol 9'-O-beta-D-glucoside, salicortin, tremulacin, gentisic acid 5-O-beta-glucoside, tremuloidin, isograndidentatin A. However, catechin, gentisic acid 5-O-beta-glucoside, tremuloidin were found only in ethanol extract, while ethyl acetate showed the presence of isograndidentatin A. Our study allows us to conclude that the choice of the solvent will determine the nature of the isolated molecules.

P.6.37 - POST-MODIFICATION OF POLYPHENOL POLYPHOSPHAZENE COLLOIDS AS A NEW WAY TOWARDS ADVANCED BIO-BASED FLAME RETARDANT COATINGS

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The design of new bio-based and environmentally-friendly flame retardants (FR) is of great importance, especially in the construction, electrical, and textile industries. Due to updates in safety and environmental regulations, syntheses of FR materials continuously meet new challenges. Therefore, the current interest is the development of new, halogen-free FR to reduce the environmental and health impact [1]. Polyphosphazenes or here in particular phosphornitrilic chloride trimer (HCCP) can be used for the production of bio-based FR due to their high phosphorus and nitrogen content and their adaptability. This was combined with Lignin, a waste product extracted from wood in the paper industry, due to its high number of hydroxy groups and aromatic amount.

The successful synthesis of Lignin/HCCP FR has already been confirmed. Such halogen-free cyclomatrix polyphosphazene materials showed suitable properties in thermal analyses, which allow for application on textiles or cardboard.

For further optimization and to ensure a complete crosslinking additional post-modifications were carried out in different variants with the bio-based FR. In this work, additional smaller polyphenols like gallic acid [2] were added to the synthesis to ensure complete cross-linking of the colloids and especially to increase the thermal stability as well as surface adhesion. Mild reaction conditions, simple purification, and scalability are favorable to improve sustainability even further [3]. This new FR material promises high thermal stability, low degradation, and high charring conditions.

The influence of the chemical composition and coating amount of the bio-based polyphenol-polyphosphazenes colloids on the thermal stability and performance of FR was systematically investigated in bulk and on coated cardboards on textiles. Various analytical methods like thermogravimetric analysis, vertical flame tests, and Limiting Oxidation Index (LOI) measurements were used.

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[3] Deniz A., *et al.*, *ACS Applied Polymer Materials*, *2*, 5345-5351, https://doi.org/10.1021/acsapm.0c00574, 2020.

P.6.38 - CHESTNUT SHELLS LIGNIN FOR SUSTAINABLE GENERATION OF SILVER NANOPARTICLES AND IMPLEMENTATION OF POLYLACTIC ACID ELECTROSPUN FIBERS WITH HIGH ANTIOXIDANT AND TARGETED ANTIBACTERIAL ACTIVITIES

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Among natural phenolic polymers attracting increasing interest as versatile, robust and multifunctional compounds, of particular relevance is lignin, whose potent antioxidant properties have prompted its applications in a variety of fields such as food packaging, wound healing, drug delivery, and tissue regeneration.[1] On this basis, and in a green chemistry perspective, several studies have been directed to the exploitation of sustainable and easily available sources of this polymer, such as by-products and wastes from the agri-food industry. In this context, we recently reported a combined mechanochemical/deep eutectic solvent (DES)-based protocol for the recovery of lignin from the shells of edible nuts.[2] Among these, chestnut shell lignin exhibited remarkable antioxidant properties in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays.
Herein, we report the use of chestnut shell lignin as a reducing agent for a solvent-free mechanochemical synthesis of silver nanoparticles (AgNPs). The optimized protocol, involving ball milling of lignin/$AgNO_3$ at a 85:15 w/w ratio for 180 min at 50 osc/s led to a 8 $\pm$ 1 % Ag(0) amount (max theoretical 9.5%) as determined by X-ray diffraction analysis. The lignin/AgNPs were incorporated in varying amounts in poly(D,L-lactic acid) (PDLLA)-based electrospun fibers containing also chitosan or cellulose nanocrystals (Figure, panel a). The antioxidant properties of the materials were evaluated by the DPPH and FRAP assays, showing good results particularly in the case of the fibers containing PDLLA (96.9% w/w), lignin/AgNPs (2.1% w/w), and chitosan (1% w/w). All the lignin/AgNPs containing electrospun fibers showed excellent cytocompatibility toward mesenchymal stem cells (hMSC). The presence of AgNPs conferred antibacterial properties to the fibers where the surface colonization from the pathogens *Escherichia coli* and *Staphylococcus epidermidis* was reduced of about 1.5 logarithms in comparison to the bulk PDLLA; moreover, when hMSC where cultivated onto materials' surface, the presence of AgNPS protected cells from infection preserving their viability thus offering a targeted protection from infection (Figure, panel b-c).

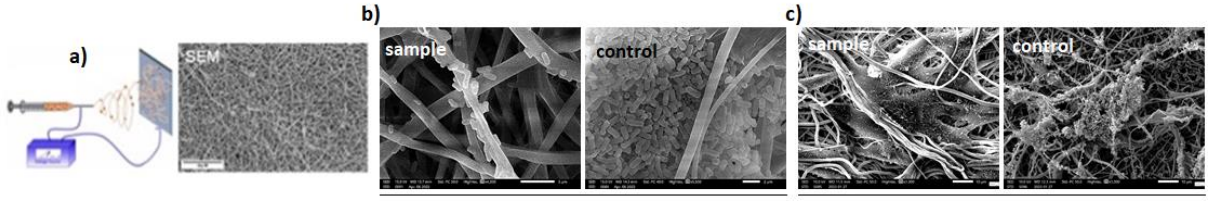

Figure: a) fabrication of PDLLA electrospun fibers containing lignin/AgNPs and chitosan; b) antibacterial activity on *E. coli* ; c) targeted activity on hMSC-*E. coli* co-culture.

[1] Panzella L. & Napolitano A., *Antioxidants*, 6, 30, https://doi.org/10.3390/antiox6020030, 2017.
[2] Argenziano R., Moccia F., Esposito R., D'Errico G., Panzella L. & Napolitano A. *Antioxidants,* 11, 1860, https://doi.org/10.3390/antiox11101860, 2022.

P.6.39 - SYNTHESIS, CHARCTERIZATION AND DEGRADATION OF SUPRAMACROMOLECULAR MICROGELS

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Microgels are colloidal polymer networks that combine properties of rigid particles and flexible macromolecules. Their unique stimuli-responsiveness in combination with their colloidal phase behaviour make them useful for many applications ranging from engineering to biomedicine. Microgels are mostly crosslinked with covalent bonds by the use of *N,N'*-methylenebisacrylamide [1]. Recently, the use of tannic acid as crosslinker for microgels as well as the investigation of properties of such novel supramacromolecular microgels came into focus [2]. In this research, we aim to use polyphenols as natural supramolecular crosslinker to make microgels pH-sensitive and degradable. These are important properties for functional drug delivery systems. These microgels are characterized regarding their chemical structure by Raman spectroscopy. Also, the crosslinker content within the microgels is determined quantitatively using calibration curves. Furthermore, their size and size distribution are investigated *via* dynamic light scattering. The hydrogen bonds used to crosslink the microgel structure introduce a pH-sensitivity, which is not only investigated by dynamic light scattering, but also by microscopy.

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P.6.40 - MICROWAVE-ASSISTED EXTRACTION FOR THE RECOVERY OF ANTIOXIDANT COMPOUNDS FROM *ROBINIA PSEUDOACACIA* WOOD

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Nowadays, there is a growing demand for healthier products. Within this framework, the food industry is investing in the innovation of functional products enriched with bioactive compounds (e.g., phenolic compounds, peptides or fatty acids) that provide health benefits to the consumer. The main natural source of these value-added compounds is vegetal biomass, like *Robinia pseudoacacia*. This angiosperm is recognized as an invasive species in many countries due to its rapid growth and high regeneration potential, among other features. Its control and/or elimination from ecosystems where it is not native could be beneficial from an environmental, economic and social point of view.

Microwave-assisted extraction is an energy-efficient technology that allows uniform heating of the sample and shorter treatment times, reducing the degradation of polyphenolic compounds and increasing their recovery respect to conventional treatments. The main objective of this study was to evaluate the suitability of hydrothermal microwave treatment to produce liquors rich in phenolic compounds with potential antioxidant activity from *Robinia pseudoacacia* wood.

The bioactive compounds of extracts obtained after microwave treatment were tested for total phenolic and flavonoid content, as well as for antioxidant activity by ABTS and FRAP methods. Additionally, the phenolic constituents were determined by HPLC-ESI-MS. The results obtained revealed a high content of phenolic compounds (TPC and TFC), of which 17 were identified (9 phenolic acids, 6 flavonoids and 2 stilbenes), demonstrating great antioxidant activity (ABTS and FRAP) [1]. The conclusions obtained in this work revealed that the assistance of the extraction by microwaves constitutes a suitable end eco-friendly methodology for the valorization of *Robinia pseudoacacia* wood.

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Acknowledgements: Authors are grateful to the MINECO (Spain) for the financial support of this work in the framework of the projects “Cutting-edge strategies for a sustainable biorefinery based on valorization of invasive species” with reference PID2019-110031RB-I00 and to Consellería de Cultura, Educación e Ordenación Universitaria (Xunta de Galicia) through the contract ED431C 2017/62-GRC to Competitive Reference Group BV1, programs partially funded by FEDER. Alba Pérez-Pérez would like to express her gratitude to the Spanish Ministry of Science and Innovation for her FPU doctoral grant (Reference FPU21/02446). Beatriz Rodríguez-Martínez would like to express her gratitude to the University of Vigo for her predoctoral grant (Reference PREUVIGO-22). Beatriz Gullón and Pedro Ferreira-Santos would like to express their gratitude to the Spanish Ministry of Economy and Competitiveness for their postdoctoral grant (References RYC2018-026177-I and FJC2021-046978-I, respectively). Pablo G. del Río would like to express gratitude to the Consellería de Cultura, Educación, Formación Profesional e Universidades (Xunta de Galicia) for his postdoctoral grant (Reference ED481B-2022-020).

P.6.41 - SYNERGIC EFFECT OF EUTECTIC SOLVENTS AND MICROWAVE TECHNOLOGY IN THE EXTRACTION OF PHENOLIC COMPOUNDS FROM *FUCUS VESICULOSUS*

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The employment of third generation aquatic biomass, such as algae, has gained popularity as an alternative to non-renewable sources. Specifically, *Fucus vesiculosus* (FV) is an interesting resource for its application in the food and health industry due to its high potential for obtaining high added value compounds such as phenols, proteins, carotenoids and polysaccharides [1].
Conventional extraction is the most used method for phenolics and antioxidants isolation. This process employs organic compounds as solvents which carry some inconveniences along such as high volatility, low yields, high costs, high toxicity, environmental issues, etc. Due to these drawbacks, new chemicals known as deep eutectic solvents (DES) have been developed, which are considered more sustainable and environmentally friendly. These solvents present interest properties such as their low melting point, low volatility, low vapor pressure, low toxicity, high biodegradability, and low cost [2]. In addition, due to their internal nature they can extract a wide variety of compounds and therefore the use of DES in the extraction of bioactive compounds from different types of biomasses has expanded in recent years. On the other hand, it is increasingly necessary to process biomass more effectively and economically. Accordingly, the application of microwave (MW) heating as an intensifier has become a method with high potential to reduce energy expenditure. MW also has a number of advantages such as faster and more efficient heating or higher selectivity with the biomass and the solvent [3].
In the present work, the optimization of the extraction process of phenolic compounds (measured by total phenolic and flavonoid content-TPC and TFC respectively) with antioxidant capacity (measured by DPPH, ABTS, FRAP and TAC) from FV was carried out. For this purpose, the percentage of water in the DES (10-50%), the extraction temperature (100-150 ºC) and the extraction time (0-40 min) were evaluated and optimized by means of a multiple response surface method (RSM), in which the accuracy of the design was verified. The most important phenolic compounds were identified and quantified by means of HPLC-ESI. In this way, the use of innovative solvents, such as DES, and a more sustainable type of heating, such as microwaves, could be an interesting alternative for the recovery of this brown algae's bioactive compounds.

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The authors acknowledge the financial support received from "Xunta de Galicia" (GRC ED431C 2021/46), partially funded by the FEDER Program of the European Union ("Unha maneira de facer Europa") and for the project PID2019-110031RB-I00. Beatriz Rodríguez-Martínez is grateful to the University of Vigo for her predoctoral support (PREUVIGO-22). Also, Alba Pérez-Pérez (FPU21/02446), Beatriz Gullón (RYC2018-026177-I) and Pedro Ferreira-Santos (FJC2021-046978-I) would like to express gratitude to the Ministry of Universities for their financial support. Pablo G. del Río would like to express gratitude to Consellería de Cultura, Educación, Formación Profesional e Universidades of Xunta de Galicia for financial support (ED481B-2022-020).

P.6.42 - PRESSURIZED LIQUID EXTRACTION FOR THE DETERMINATION OF POLYPHENOLS

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Pressurized liquid extraction (PLE), is considered an advanced extraction technique because it can save time and reduce solvents compared to traditional extraction methods. It is a solid-liquid extraction performed at high temperature (50-200 °C) and high pressures (10-15 MPa) [1]. This technique is also called accelerated solvent extraction (ASE), pressurized fluid extraction (PFE), pressurized hot solvent extraction (PHSE), high-pressure solvent extraction (HPSE), high-pressure high-temperature solvent extraction (HPHTSE), and subcritical solvent extraction (SSE) [2]. The aim of the study was to critically review recent studies using PLE for the determination of polyphenols. The present study examined recently published studies between January 2013 to January 2023. We focused first on effects of the different PLE parameters such as temperature, solvent composition and sample pretreatment including the possibility of using PLE on-line coupled with solid-phase extraction (SPE). Further, we examined extraction efficiency in comparison to the other extraction methods for polyphenols determination. Different studies demonstrated that PLE could provide better or comparable results than the traditional methods with significant reduction of solvent consumption and reduction of time with better reproducibility. Although phenolic compounds are regularly regarded as heat-labile compounds the highest extraction yields of total phenolic compounds described in these studies were achieved at temperatures above 100 °C. This could be due to the possible braking of lignin-phenolic acid bonds or the breaking of lignin giving rise to more phenolic acids at higher temperatures [3]. Therefore, PLE could be a promising method for the preparation of highly concentrated phenolic compounds extracts not just for analytical proposes, but also in several industrial applications. Furthermore, by changing the extraction parameters such as temperature, phenolic compounds can be extracted with high selectively. While it is known that thermal degradation of different phenolic compounds depends on temperature, treatment time, pH and solvent type, pre-treatment, extraction environment and source of the material [3], it is very important to optimize PLE for each specific samples in order to achieve high yield or selectivity.

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P.6.43 - NOVEL EXTRACTION OF ANTIOXIDANT POLYPHENOLS FROM GRAPE POMACE RESIDUE USING DEEP EUTECTIC SOLVENTS

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Grape Pomace (GP) resulting from wine industries can cause serious environmental and economic impacts. However, these by-products are often undervalued but constitute a potential source of bioactive and functional compounds (mainly polyphenols) that can be applied in several food and pharmacological industries. The growing demand for sustainable extraction processes requires the development of new and effective solvents to replace toxic organic solvents. Deep eutectic solvents (DESs) are a smart and green alternative due to their non-toxicity and biocompatibility. This work aimed to study the effectiveness of DESs to extract antioxidant phenolic compounds from GP. Tree different DES were synthesized: DES 1 - sodium acetate:Lactic acid (molar ratio 1:5) with 30% water; DES 2 - Chlorine chloride:Acetic acid:Water (1:1:10); DES 3 - Chlorine chloride:Citric acid (2:1) with 30% water. Ethanol 50% was used a control solvent. The extraction efficiency was evaluated by measuring the total phenolic (TPC) and flavonoid (TFC) content and antioxidant activity (ABTS, FRAP and total antioxidant capacity (TAC)). The best extraction results were obtained with choline chloride:citric acid (TPC: 110.62 ± 2.3 mg GAE/g GP and TFC: 162.06 ± 6.6 mg RE/g GP), representing values around 40% higher than hydroethanolic extraction. The extracts obtained by DES 3 showed high antioxidant activity in FRAP (156.5 ± 7.7 mg TE/g GP) and TAC (183.3 ± 9.8 mg TE/g GP) methods and DES 2 in ABTS assay (183.7 ± 6.7 mg TE/g GP). However, all DESs tested show similar and higher extraction efficiency than hydroethanol. Additionally, the phenolic profile was determined by HPLC-MS/MS, and revealed the presence of phenolic acids, flavonoids and stilbene in the extracts.
Overall, coupled with the non-toxic, biodegradable, low-cost, and environmentally friendly characteristics of DESs, our results provided robust evidence that DESs represent an effective alternative to volatile organic solvents for the recovery of bioactive compounds from agri-food by-products.

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P.6.44 - VALORISATION OF POLYPHENOLS FROM INDUSTRIAL BLACK TEA WASTE

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Global black tea production has been predicted to rise at an annual growth rate of 2.2% over the next five years in order to satisfy the rising demand of customers. It is also predicted that black tea production could reach 4.4 million tons in 2027, which will generate very large quantities of spent black tea waste [1]. However, currently only a very small portion of this spent biomass is recycled (e.g., for caffeine extraction, enrichment of soil, animal feed, etc.). The majority of spent black tea residue is discarded as waste, which not only results in huge biomass loss, but also causes numerous environmental issues [1,2]. Therefore, research into innovative and sustainable solutions to better valorize black tea waste is still highly needed [1].

One of valuable approaches for tea waste valorization is to extract tea polyphenols, present in the waste, then valorize them as bioactive molecules. The objective of our study was to evaluate the efficiency of ultrasound-assisted maceration (UAM) on tea polyphenol recovery from industrial spent black tea waste. Extraction efficacy between the UAM and non-UAM methods (control) was assessed on the total phenolic content (Figure 1) and antioxidant capacities of extracts. The results showed that UAM could improve both quantity and antioxidant potency of polyphenolic extract from the biomass waste. The optimal conditions for polyphenol recovery in this study were ethanol/water 7:3 (v/v) as extraction solvents, UAM setting at 20 kHz / 60% amplitude / cycles 20s-on and 10s-off over 30 mins, then followed by 30 mins of classical maceration (total extraction time = 1h). The polyphenolic composition is currently being analyzed by HPLC. Furthermore, the inhibitory activities of the obtained extracts against tyrosinase, a key enzyme involved in human skin pigmentation and in enzymatic browning of vegetables or fruits, will be investigated. The final results of this work would give more information on polyphenols issued from industrial black tea waste, which could be useful for developing their potential valorization in the agricultural, cosmetic and pharmaceutical fields.

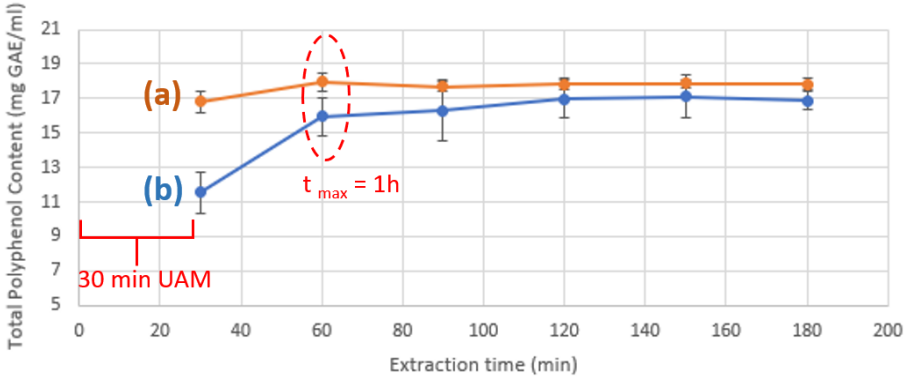

Figure 1: Polyphenol extraction monitoring curves. (a): ultrasound-assisted maceration (UAM) setting at 20 kHz / 60% amplitude / cycles 20s-on and 10s-off over 30 mins, followed by 30 mins of classical maceration; (b): non-UAM method (control). Extraction solvents were ethanol/water 7:3 (v/v).

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P.6.45 - OPTIMIZATION OF EPICATECHIN- AND EPICATECHIN GALLATE-NANOLIPOSOME PREPARATION APPROACHES

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Objective: Epicatechins, as the most commonly-encountered monomeric flavan-3-ols in many plants, are reported to have various biological activities. However, the unexpected sensitivity, instability and low bioavailability limited their therapeutic potential. Liposomes are widely explored and desirable vehicles to overcome the challenges due to their amphiphilic and biocompatible characteristics. The purpose of this study was to establish the optimized approaches for preparation of EC- and ECG-nanoliposome.
Methods: Five different liposome preparation techniques including thin film dispersion, ethanol injection, reverse-phase evaporation, thin film dispersion-calcium acetate gradient, and ethanol injection-calcium acetate gradient (EtOH-CAG) were used to prepare epicatechin liposome (EC-Lip) and epicatechin gallate liposome (ECG-Lip). The encapsulation efficiencies (EE%) and drug loading (DL%) were assessed by ultrafiltration centrifugation and HPLC methods. Quantitative descriptive analysis (QDA) was used as the main criterion to select the optimum preparation approach.
Results: The EE% for both of the EC-Lip and ECG-Lip prepared by EtOH-CAG were higher than that of the other four approaches. Moreover, the dispersion obtained was visually observed to be uniformly distributed and clear, also with the light blue opalescence characteristic of nanoliposomes. QDA also exhibited the above identical results that the EtOH-CAG was the optimum preparation method, by five factor evaluations of EE%, DL%, appearance, morphology and stability. The EtOH-CAG preparation process was as follows: soybean lecithin: cholesterol ratio 4:1, and lipid volume: water volume ratio 1:8. The EE% and DL% of the EC-Lip prepared under the conditions were up to 93.75% and 11.03%, respectively, and those of the ECG-Lip attained to 92.52% and 10.885%, respectively.
Conclusion: Our study provided the optimal method for the preparation of EC- and ECG-nanoliposome system, which could avoid adverse effects of flavan-3-ols and expand the multiple applications of these bioactive phenolic compounds.

P.6.46 - FUNCTIONALIZED DIALYSIS MEMBRANES WITH ANTIOXIDANT ACTIVITY

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Hemodialysis treatment is required in patients with end-stage chronic kidney disease. However, this treatment causes oxidative stress, increasing morbidity and mortality. To solve this problem, it is intended to develop bioactive membranes able of reducing the production of free radicals during the hemodialysis process.

Hydroxytyrosol, 2-[(3',4'-dihydroxy)phenyl]ethanol, is an antioxidant extracted from the olive tree and has a high anti- radical activity. However, due to its high hydrophilicity, it is not possible to effectively trap this antioxidant in polysulfone membranes. Thus, more lipophilic esters of hydroxytyrosol were synthesized by esterification with butyric (HyTy-C4), caprylic (HyTy-C8) and palmitic (HyTyC16) fatty acids [1]. The identity and purity of the synthesized compounds was confirmed by HPLC and NMR. Subsequently, polysulfone membranes containing □-tocopherol (control +) and the synthetized antioxidants at concentration of 6, 12 and 24 mM were prepared. It was found that the compounds were fully incorporated into the membrane, with no leakage when in contact with aqueous solutions.

The membrane antioxidant capacity was determined using the DPPH radical assay. All compounds were found to have high and similar antiradical activity to α-tocopherol.

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P.6.47 - TECHNO-ECONOMIC ASSESSMENT OF EXTRACTIVE BIOREFINERIES TO PROCURE HIGH-VALUE POLYPHENOLIC BIOACTIVES FROM ONION WASTE

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In recent years, there has been increasing attention to polyphenols and their potential applications in the pharmaceutical, nutraceutical and cosmeceutical industries [1]. On the other hand, there is a growing demand to explore the feasibility of utilising agri-food residues for the procurement of bioactives, as part of the principles of a circular bio-economy. In response, H2020 CBE JU project PHENOLEXA aims to unlock the potential of polyphenols-rich agri-side streams (olive, grape shoots and leaves, chicory and onion residues) to obtain high-value natural polyphenols via the development of cascade smart extractive biorefinery. As part of this project, we focused on the evaluation of the techno-economic feasibility of Solid-Liquid Extraction (SLE) and Subcritical Water Extraction (SWE) biorefineries to procure polyphenols from red onion skins.

In order to achieve the main aim of the current project, a few tasks have been accomplished: design of SLE and SWE technological sequences with a plant capacity of 1,600 tonnes w.w. of onion skins/year including chromatography as the main purification stage; calculation of material and energy balances based on lab-scale experimental data provided by Celabor, Belgium; CAPEX, OPEX and profitability analyses using corresponding theoretical methods; sensitivity analysis on the effects of the key economic variables on the economic performance. As it can be seen in Figure 1, despite somewhat superior technical performance of the SWE process (greater polyphenols and extraction yields), the economic performance of the two studied biorefineries is somewhat similar.

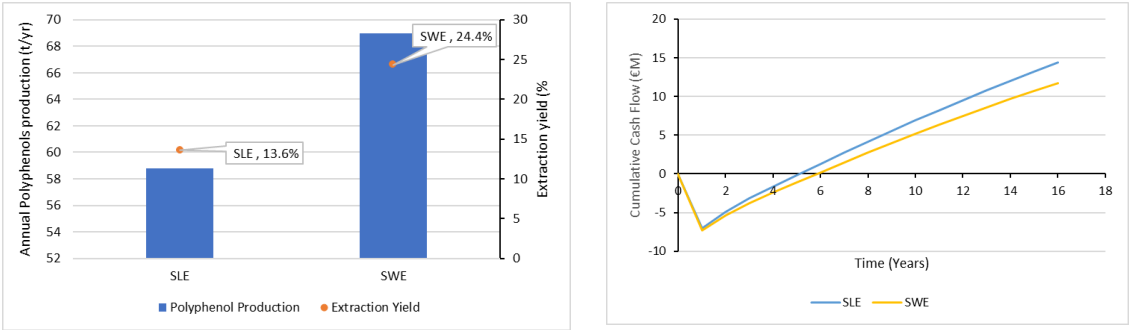

Figure 1: Technical performance (left) and cumulative cash flow (right) comparison for SLE and SWE processes.

The total capital expenditure amounts to €1.19M and €1.23M for SLE and SWE accordingly, with the chromatographic purification being the most expensive stage taking up to 35-37 % of the total capital costs. SWE has a larger operational expenditure, on average spending €2.8M per year which is 55% more than SLE. It is worth noting that SWE requires over 66% more energy for operation due to high temperature and pressure at the extraction stage. SWE and SLE biorefineries break even at 5.8 and 5.1 years accordingly and the polyphenols price has the most significant effect on the biorefinery economic performance. Overall, it was demonstrated that an extractive biorefinery to procure natural polyphenols from red onion skins is a commercially attractive bio-economy process.

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