BOOK OF ABSTRACTS

AUTUMN SCHOOL IN FOOD CHEMISTRY

2nd edition Italian School in Food Chemistry for PhD student

Pavia, September 20th-22nd 2023

Analytical Methods in Food Science

Edited by Adele Papetti and Jean Daniel Coisson

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PROGRAM

Wednesday - September 20th 2023

h 15:15	**Opening ceremony** Prof. Adele Papetti, University of Pavia Prof. Jean Daniel Coisson, University of Piemonte Orientale Prof. Hellas Cena, Pro-Rector for Third Mission University of Pavia
h 15:30	**Prof. Chiara Dall'Asta, University of Parma** L1: Functional and Novel Foods
h 16:30	**PhD student 3rd year presentation** C1: Dr. Ilaria Frosi, University of Pavia *"Gaining health from Lombard agri-food waste"*
h 17:00	**Short Communications** C2: Dr. Melissa Fanzaga, University of Milan *"In vitro safety assessment of phenylketonuria (PKU) dietary supplements exploiting Caco-2 intestinal cells"* C3: Dr. Agostina Colacicco, University of Milan *"Combining biocatalysis and flow facilities for the preparation of valuable aglycones from natural citrus glycosides"*

Thursday - September 21st 2023

h 9:00	**Prof. Gianni Sagratini, University of Camerino** L2: Liquid Chromatography-Mass Spectrometry in Food Analysis and Characterization
h 10:00	**Short Communications** C4: Dr. Federica Vento, University of Messina "E*valuation of volatile profile of chili pepper by using headspace solid-phase microextraction coupled with gas chromatography analysis*" C5: Dr. Andrea Caratti, University of Turin "*A New Workflow for Rationalizing Raw Data Exploration in GC×GC: Computer Vision to Unravel Diagnostic Signatures*"

	C6: Dr. Samanta Corsetti, University of Camerino *"Calabrian chili pepper flavouring extracts: preliminary characterization and valorisation of this variety towards the PGI mark"* C7: Dr. Roberto Laganà Vinci, University of Messina "*New functional foods: characterisation of products derived from Cannabis sativa L. by advanced chromatographic techniques"*
h 11:15	**Prof. Chiara Di Lorenzo, University of Milan** L3: Immunochemical approaches in food analysis
h 13:30	**Poster Session**
h 14:30	**Prof. Paola Dugo, University of Messina** L4: Lipidomic analysis in foods
h 15:30	**Short Communications** C8: Dr. Federica Grasso, University of Genoa *"Green extraction and characterization of gelatin coming from unsorted dehydrated canned tuna side streams"* C9: Dr. Luciano Mangiapelo, University of Perugia *"Optimization of the extraction of bioactive compounds from grape pomace"* C10: Dr. Francesco Limongelli, University of Bari *"Green approach to extract polyphenols from S. europea L."* C11: Dr. Nicola Pinna, University of Perugia "*Chromatographic and spectrophotometric characterization of pumpkin waste"*
h 16:45	**Dr. Matteo Perini, Edmund Mach Foundation** L5: Isotopical analysis in food chemistry

Friday - September 22nd 2023

h 9:30	**Prof. Davide Bertelli, University of Modena and Reggio Emilia** L6: Nuclear Magnetic Resonance spectroscopy in food characterization and authentication
h 10:30	**Prof. Giosuè Costa, University of Catanzaro** L7: In Silico Approaches in Food Chemistry
h 12:00	**Short Communications** C12: Dr. Lorenza D'Adduzio, University of Milan *"Innovative plant derived extract with health promoting activity"* C13: Dr. Riccardo Zecchi, University of Florence *"Rice nutraceuticals and their localization inside the seed before and after cooking: an overview through mass spectrometry imaging analysis"* C14: Dr. Agnese Santanatoglia, University of Camerino *"Acrylamide content and antioxidant activity among 8 different filter coffee extraction methods"* C15: Dr. Emanuela Marchese, University of Catanzaro *"Integrating structure- and ligand-based approaches for virtual screening of natural products."*
h 12:45	Remarks and perspectives (Adele Papetti & Jean Daniel Coisson)

LECTURES

L1: FUNCTIONAL AND NOVEL FOODS

Chiara Dall'Asta

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Functional and Novel foods are pivotal concepts in food science, especially in the light of the global challenges affecting market trends and consumer's choices. This lecture will providing a nuanced understanding of their significance in food science, the rigorous scientific methodologies underpinning their claims, as well as the regulatory environment in which they operate.

Different categories of functional foods will be described through real-market case studies, and a critical evaluation of the scientific evidence supporting the health claims associated with functional foods, as well as the role of regulatory bodies in ensuring consumer safety and accurate product labelling will be explored.

In parallel, the concept of novel foods is examined in depth, including sustainability considerations that are paramount in the contemporary food landscape. The safety assessment process required for bringing a novel food candidate to the market will be described through case studies such as edible insects, innovative plant ingredients, (micro)algae and microorganisms, especially in relation to the EU process for novel food approval.

The crucial role of food chemistry in the design and development of a new ingredient – from the valorization of under-exploited materials to the molecular characterization and the comprehensive safety assessment – will be critically discussed.

L2: LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY IN FOOD ANALYSIS AND CHARACTERIZATION

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Since several years ago, researchers, food manufacturers as well as the public in general, have become very interested in the quality of food products, which are very complex mixtures consisting of naturally occurring compounds (lipids, carbohydrates, proteins, vitamins, organic acids, and volatile organic compounds-VOCs) and other substances generally coming from technological processes, agrochemical treatments, or packaging materials [1].
Combined liquid chromatography–mass spectrometry (LC–MS) can be considered as being one of the most important techniques of the last decade of twentieth century applied to various fields of research including food science. The extreme speed of development and its acceptance and high spread, especially considering its price tag, is astonishing. LC–MS has become the method-of-choice for analytical support [2]. LC-MS interfacing and its application have been frequently reviewed with emphasis on the development of interfaces for atmospheric-pressure ionization (API). In this lesson, we will describe the basic principles of chromatography, in particular of high pressure liquid chromatography (HPLC), by underlining the differences with the ultra-HPLC (UHPLC) technique. The main ionization sources will be described (ESI, APCI, APPI) as well as the main mass spectrometers (MS, MS/MS) systems, by showing some applications in the field of food science. Finally, the differences between low resolution (LR) and high resolution (HR) mass spectrometry (MS) will be addressed.

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L3: IMMUNOCHEMICAL APPROACHES IN FOOD ANALYSIS

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Immunochemical assays are analytical techniques based on the recognition between an antigen and an antibody. These techniques are specific and sensitive and allow the detection and/or the quantification of analytes in a complex matrix as foods, including allergens. Food allergy is an immunological hypersensitivity to foods, affecting approximately 2.5% of the general population. The prevalence of food allergy in adults has been estimated to be 2-3%, while in infancy has been reported to be 4.7%. Foods mostly involved in allergic reactions are cow's milk, egg, wheat, soy, peanut, tree nuts, fish, and shellfish. Analytical testing systems based on immunochemistry are widely used: in the food industry, to verify whether allergens are present in their raw materials and in the finished products; in the market surveillance; in academia to stimulate research into food allergy and allergen detection. Depending on the different purposes, different immunochemical assays can be used. Enzyme linked Immunosorbent Assay (ELISA) and lateral flow analysis are widely applied in food manufacturing industries for the rapid detection of allergens. Immunosensors are becoming also popular to detect traces of allergenic adulterants (e.g., hazelnut proteins in olive oil), that could have serious health implication other than the economic damage. Immunochemistry is widely used for research purposes not only to detect allergens in foods, but also to study the interactions between antigens and the human immune system to identify the proteins involved in allergic reactions. The different aspects of the immunochemical approaches will be explained and discussed, providing examples and case studies.

L4: LIPIDOMIC ANALYSIS IN FOOD

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In the last decades, lipidomics has emerged as a cutting-edge approach among omics- techniques, since lipids revealed to be essential molecules in the regulation of metabolic pathways. To this regard, the content of essential fatty acids (EFAs), as well as nutritional indices such as the levels of omega-3 and omega-6 FAs and their ratio are essential parameter to evaluate the beneficial properties of food products. In addition, the investigation of complex lipids in their native forms is proved to be crucial to obtain additional information about lipids role and on FA arrangement into each species.

For this reason, the present talk is aimed to a detailed elucidation of intact lipids in different functional foods, including the profiling of vegetable oils, hemp products and the wastes of the fish industry, according to current trends in food science, such as the valorization of unconventional and sustainable food products and the minimization of food wastes and the promotion of recycle strategies (circular economy models).

From an analytical point of view, two parallel and complementary approaches are commonly used in the lipidomic field: gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography-mass spectrometry (HPLC-MS). The former is essential for the detailed characterization of the total FA composition, being able to reliably identify the position of the double bonds along the carbon chain (omega nomenclature) and/or the configuration of the double bond (cis/trans), while the latter is employed for the elucidation of intact lipids (e.g., triglycerides, diglycerides, phospholipids, free FAs) as they are originally present in the sample, thus adding important information about storage conditions and how they will be metabolized. In both cases, particular emphasis is paid on the miniaturization and automation of the entire analytical workflow by using robotic workstations able to perform the sample preparation in a fully automated manner and online with the chromatographic system. Also, mass spectral library with embedded retention data (linear retention index, LRI) were built in both GC and HPLC methods to achieve a fast, reliable and automatic identification of lipid species through the development of a dedicated software, which applies a dual-filter identification strategy by exploiting the complementarity between MS and LRI data

Keywords: *lipidomic analysis; functional foods; chromatographic techniques; mass spectral database; linear retention indices*

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L5: STABLE ISOTOPE ANALYSIS FOR THE AUTHENTICITY AND TRACEABILITY OF FOOD PRODUCTS, SUPPLEMENTS AND DRUGS

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Stable isotope ratio analysis (SIRA) is now increasingly used for the traceability and authenticity not only of food products (e.g. milk, cheese or vegetable oils), but also of drugs (such as curcumin) and food supplements with pharmacological properties (such as monacolin K, produced by the fermentation of red yeast rice). The numerous official methods are flanked by various scientific works that demonstrate the power of this technique and its applicability in official and unofficial controls. By coupling isotope ratio mass spectrometry (IRMS) with separative techniques, such as liquid chromatography and gas chromatography, it is now possible to identify frauds ranging from the replacement of natural molecules with their synthetic form, to the identification of added adulterants, even at low concentrations.

L6: NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IN FOOD CHARACTERIZATION AND AUTHENTICATION

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Nuclear magnetic resonance (NMR) spectroscopy is a spectroscopic technique capable of observing local magnetic fields around atomic nuclei. This spectroscopy technique is based on the absorption of electromagnetic radiation by nuclei of the atoms in the radiofrequency region from 4 to 900 MHz. Over the past fifty years, NMR has become more and more important in many research fields.
NMR is a versatile technique more and more used for quality control in many different scientific fields. As an example, NMR spectroscopy is routinely used to study the chemical structure of new natural or synthetic compounds and determine the concentration and purity of a substance in pharmaceutical and organic chemistries.

NMR can also be employed to quantitatively analyze mixtures containing known compounds (qNMR). In food science, NMR can be exploited for targeted and untargeted characterization, authentication, and quality control of food. Additionally, NMR is used to study the physicochemical transformations occurring during food processing and storage. Recently, NMR has been proposed as an alternative method to x-ray crystallography for the determination of protein structure and the study of protein-ligand interaction.
The examination of NMR spectra of isolated compounds is an easy task. Conversely, the interpretation of spectra obtained from complex samples, such as foods, requires the employment of advanced spectral statistical analyses.
In this lesson, different NMR applications will be shown to demonstrate the versatility and potentiality of this technique in food chemistry. The application of targeted and untargeted approaches on spectral data will be examined by taking different kinds of food as an example: honeybee products, oenological products, herbs and derivatives, chocolate, fruit juices etc.

L7: IN SILICO APPROACHES IN FOOD CHEMISTRY

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In contemporary scientific research, computational methodologies have played a pivotal role in the quest for bioactive agents. Nowadays, academic institutions and pharmaceutical/biotech companies across the globe routinely harness the power of computational design tools. Computer-aided drug design encompasses a spectrum of computational methods and resources that expedite the discovery of novel bioactive chemical entities. This includes not only synthetic compounds but also natural substances and food components with potential nutraceutical properties.
The significance of computational tools in this field was underscored in 2003 when the Nobel Prize in Chemistry was awarded to Martin Karplus, Michael Levitt, and Arieh Warshel for their pioneering work on "the development of multiscale models for complex chemical systems." This recognition underscores the fusion of experimental science with theoretical chemistry, as it addresses the fundamental question of how to design bioactive agents that precisely complement their target molecules.
One of the chief advantages of these computational approaches lies in their potential to significantly reduce time and resource investments in the drug development process. Computational methods, such as Virtual Screening (VS) techniques, offer a means to streamline the identification phase, resulting in cost and time savings [1-2]. Over recent years, our research team has devoted considerable efforts to the realm of natural products and food chemistry [3-11]. Our goal has been to discover novel bioactive compounds present in common foods capable of binding to and modulating the activity of critical targets involved in complex diseases.
Among the array of computational tools at our disposal, the Structure-based Virtual Screening (VS) approach stands out as a potent method for identifying potentially bioactive components within functional foods. Beginning with the 3D structure of the target of interest, readily accessible through the Protein Data Bank (PDB), we have conducted in silico analyses to select the most promising compounds based on their theoretical binding affinity. In this presentation, we provide an overview of the capabilities of in silico methods, as well as share successful applications in this exciting field.

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COMMUNICATIONS

C1: GAINING HEALTH FROM LOMBARD AGRI-FOOD WASTE

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Introduction

Nowadays, it is clear how innovation must begin from recycle. According to FAO data on food lost and waste every year [1], there has been an increasing interest in the reutilization of organic by-products, as they boast a high presence of bioactive secondary metabolites, dietary fiber, essential oils and pigments that can be recovered to produce new raw materials. Considering this contest, we focused our attention on the valorisation of different Lombard agri-food wastes as possible sources of polyphenols, the main active components in Botanicals. Several epidemiological studies have shown the correlation between advanced glycation end products (AGEs) and the development of different chronical disorders [2]. Due to the current increasing interest in the anti-AGEs agents of natural origin [3], the object of our work, supported by Cariplo Foundation, was the screening of various polyphenolic extracts obtained from cereals and vegetables agri-food wastes for their antiglycative capacities and the following formulation of the most promising extract obtained as an added-value ingredient for food supplements. Rice husk, corn cob, wheat by-product, melon and pumpkin peel and asparagus bottom part were evaluated as new raw materials.

Materials and Methods

Polyphenols extraction methods

Polyphenols from agri-food wastes were extracted using a conventional approach, consisting in the use of a thermostatic bath (CSE) and hydro-alcoholic solvents, and innovative extraction methods, involving the use of a microwave reactor (MAE) with both hydroalcoholic mixtures and natural deep eutectic solvents (NaDES). Parameters that generally affect the extraction, such as temperature, time, solid to solvent ratio and percentage of ethanol or water, were optimize according to a design of experiment approach. Afterwards, the extracts were filtered and suitably treated for HPLC analysis. The extract with the highest recovery of phenolic compounds was selected for antiglycative studies.

Evaluation of antiglycative and antioxidant capacity

Different *in vitro* BSA-based systems were set-up to evaluate the antiglycative capacity of the different agro-food waste extracts at different stages of the glycation reaction. The inhibition of Amadori products and of advanced glycation end products (AGEs) formation was evaluated by NBT assay and BSA-methylglyoxal, BSA-glucose system (4,5), respectively. The trapping capacity of the extract against methylglyoxal and glyoxal, well known AGEs precursor, was evaluated using the RP-HPLC-DAD method described by Mesias et al. (2013), with slight modifications (5). Moreover, DPPH and ABTS assays were performed to determine its antioxidant ability (6).

RP-HPLC-ESI/MS^n analysis

The polyphenolic profile of the most promising extract obtained was identified through RP-HPLC-ESI/MS^n analysis. A Thermo Finnigan Surveyor plus HPLC apparatus connected to a LCQ Advantage Max ion trap spectrometry through ESI source was used (7).

Bioaccessibility studies

The bioaccessibility of the most promising extract obtained was assessed using the standardised INFOGEST protocol 2.0 (8,9). Different mixture of electrolytes and enzymes were set-up to simulate the oral, gastric and intestinal phase. The colon phase was performed according to the protocol described by Zhang et al. (2011)

(10). After each step, the phases were stopped at 90°C for 5 min, cooled at room temperature and centrifuged at 4°C-5000 rpm-30 min. The supernatants were collected and freeze-dried until analysis.

Preparation of pectin/zein hydrogel beads

The Pectin/zein hydrogel beads were prepared following the method proposed by Liu et al. (11). Briefly, a pectin solution was injected into a 75% ethanol solution containing zein and calcium chloride under constant stirring at ambient temperature. The concentrations of zein in the ethanol solution studied were 1, 5.5, 10 w/v, while the concentrations of calcium chloride were 0.5%, 2.25, 4 w/v and the concentrations of pectin were 0.5, 1.75 and 3 w/v. The droplets were allowed to cure in the reception phase for 15 min followed by washing with ethanol and water, then dried in oven for 12 hours.

RP-HPLC-DAD quantitative analysis of p-coumaric acid

An HPLC Agilent 1200 system (Waldbronn, Germany) equipped with a mobile-phase online degasser, a quaternary pump, autosampler, column heater and a diode array detector (DAD) was used.
Chromatograms were recorded at 320 nm. The chromatographic method was validated according to ICH guidelines on bioanalytical method validation (12), using *p*-coumaric acid as external standard.

Results and Discussion

Microwave assisted extraction with hydroalcoholic solvents resulted to be the best extraction method for the polyphenolic recovery from all the agri-food wastes tested, except melon peel, that gives higher yields within 30 minutes of conventional extraction. The NaDES mixtures were unable to recover high quantities of polyphenols, perhaps due to problems related to the post-extraction isolation of the actives from the solvents that needs more investigations. The best extraction conditions obtained by DOE, reported for rice husk extract (RHE), corn cob extract (CBE), wheat by-product extract (WBE), asparagus bottom part extract (ABE), pumpkin peel extract (PPE) and melon peel extract (MPE), are summarised in Table 1

Table 1. Best extraction conditions set up for the different agri-food waste screened.

	MAE					***CSE***
	RHE	*CBE*	*WBE*	*ABE*	*PPE*	*MPE*
Ethanol (%)	80	62.4	23,8	10	0	21.3
Temperature (°C)	90	88	40	40	60	52.8
Time (min)	5	5	5	5	5	30
Solvent to solid ratio (mL/g)	35	35	24.5	50	50	51.5

RHE, CBE, MPE, PPE, WBE, and ABE extracts were then tested for antiglycative and antioxidant activities. RHE give the most promising results, with an ability to inhibit 70-90% of AGEs generated in the used *in vitro* systems, in addition to a good capacity to directly trap glyoxal and methylglyoxal. In parallel, RHE showed good antioxidant activity, as demonstrated by the scavenger capacity against ABTS cation and DPPH stable radicals. For these reasons, this extract was selected for further investigations.
RHE LC-MS/MS polyphenolic profile registered in negative ionization mode highlighted the presence of 15 compounds, including phenolic acids and flavones, among which coumaric acid and tricin were the most abundant compounds. Thus, they were selected as marker compounds for bioaccessibility studies, to assess the suitability of the value-added ingredient for food supplements. INFOGEST protocol 2.0 was used to evaluate the RHE *in vitro* bioaccessibility. The bioaccessibility index (BI) of both marker compounds was calculated considering the peak area of the analyte before and after each digestion step and compered with the peak area expected following the gradual dilution occurring during the in vitro digestion protocol. Results showed that both polyphenols are affected by digestion enzymes and pH. As expected, a formulation is needed to stabilize the extract during digestion. Ten different formulations of pectin/zein hydrogel beads

were studied to encapsulate and preserve the extract until the intestinal district with a design of experiment approach. The best formulation obtained effectively showed an improved stability of RHE during digestion.

Conclusions

Microwave-assisted extraction showed to be an effective method for the isolation of polyphenols from various agri-food wastes. RHE was the extract with highest antiglycative capacities in the incubation system consisting of a protein and sugar or MGO tested; moreover, it could directly trap MGO, avoiding the AGEs formation. However, the in vitro bioaccessibility studies indicated the limited stability of the extract's polyphenols. To address this concern, encapsulating RHE within pectin/zein beads emerged as a possible strategy, offering improved stability during simulated digestion and targeted release within the intestinal environment.

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SHORT COMMUNICATIONS

C2: IN VITRO SAFETY ASSESSMENT OF PHENYLKETONURIA (PKU) DIETARY SUPPLEMENTS EXPLOITING CACO-2 INTESTINAL CELLS

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Introduction

Phenylketonuria (PKU) is a rare genetic metabolic disorder that consists in the inability of patients to convert the aminoacid phenylalanine to tyrosine, caused by mutations in the phenylalanine hydroxylase (PAH) gene [1]. Currently, dietary restriction of phenylalanine intake starting from the neonatal period remains the main PKU treatment [2][3]. Glycomacropeptide (GMP) is a whey-based natural protein low in Phe, being modified to provide an alternative protein source for PKU patients, showing nutraceutical properties as well [4]. In this context, we evaluated the safety of two dietary supplements for PKU patients, namely Phe-free protein substitute (L-AAs), GMP and the mixture of the two (1:1), exploiting Caco-2 cells, a well-known immortalized cell line, widely used as a model of the intestinal epithelial barrier [5].

Materials and Methods

Human intestinal Caco-2 cells, obtained from INSERM (Paris, France), where routinely cultured in complete DMEM, supplemented with 10% heat-inactivated fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT, USA). For differentiation, they were seeded on polycarbonate filters (Transwell, Corning Inc., Lowell, MA, US) and allowed to differentiate for 15 days with regular medium changes three times weekly. The MTT experiments, useful to evaluate cellular vitality, were conducted on human intestinal Caco-2 cells treated with L-AAs, GMP, the mixture of the two (1:1), or vehicle, applying methods already published [6]. The trans-epithelial electrical resistance (TEER) of differentiated Caco-2 cells to evaluate the integrity of treated Caco-2 cells monolayer was measured at 37 °C using the voltmeter apparatus Millicell (Millipore Co., Billerica, MA, USA). Antioxidant activity of dietary supplements has been assessed trough fluorometric Intracellular ROS Assay and Lipid peroxidation (MDA) assay, performed on Caco-2 cells, applying methods already published [7]. Anti-inflammatory activity has been assessed evaluating nitric oxide level and performing Western Blot analysis on Caco-2 Cells, according to already published procedures [7]. The expression levels of target proteins, on milk or BSA blocked membrane, were detected by primary antibodies as follows: anti-iNos and anti-β-actin. Secondary antibodies conjugated with HRP, and a chemiluminescent reagent were used to visualize target proteins and their signal was quantified using the Image Lab Software (Biorad). The internal control β-actin was used to normalize loading variations.

Results and Discussion

Results suggested that free L-AAs sample, alone and in the mixture, was safe for intestinal cells from 0.1 to 10 mg/mL, whereas GMP was safe at all the concentrations tested in the range 0.1 – 50 mg/mL. Results of TEER measurement show a significant decrease of TEER value after 120 minutes of treatment in cells treated with L-AAs versus the control cells. Results demonstrated that in Caco-2 cells pre-treated with L-AAs, the ROS production got even worse versus the H_2O_2 stimulated cells (up to 833.5 ± 141.4%). On the contrary GMP restored the ROS basal levels both alone and in the 1:1 mixture. Results of Lipid peroxidation (MDA) assay are coherent with the results obtained evaluating ROS modulation in Caco-2 cells. LPS-induced NO and iNOS overproduction in Caco-2 cells were restored to the basal level by the GMP and 1:1 mixture treatment (10mg/mL), differently from the L-AAs treatment (10mg/mL), which worsened the induced inflammatory status of Caco-2 cells. Results concerning the pro- and anti-inflammatory activity of L-AAs, GMP, and the 1:1

mixture, respectively were confirmed by the evaluation of pro-inflammatory (IL-1β, IL-6, IFN-γ, and TNF- α) and anti-inflammatory (IL10) cytokines measured in LPS-stimulated Caco-2 cell culture supernatants.

Conclusions

The disruption of pro-oxidant/antioxidant balance in phenylketonuric patients is strongly related to their dietary treatment [3]. In this context, our findings clearly indicate that the consumption of free L-AAs got dramatically worse the intestinal oxidative and inflammatory status and that the GMP as well as the 1:1 free L-AA/GMP mixture positively restore the physiological oxidative and inflammatory status in Caco-2 cells. Moreover, MTT experiments, useful to evaluate cellular viability, clearly demonstrate that GMP is a safer product than free L-AAs.

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C3: COMBINING BIOCATALYSIS AND FLOW FACILITIES FOR THE PREPARATION OF VALUABLE AGLYCONES FROM NATURAL CITRUS GLYCOSIDES

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Introduction

Agri-food waste is a significant environmental and economic problem as tons of residues (seeds, peels, leaves, branches, trunks, and roots) which are typically unexploited, need to be managed and disposed. Starting from these residues, green technologies, such as biocatalysis, can be used to obtain natural valuable compounds with attractive biological properties.

In this work we focused on hesperidin (HES) and rutin (RT), glycosides typically found in citrus species especially in fruits and their by-products (*e.g.,* peels and leaves) [1]. By employing a commercially available rhamnosidase (RN) and a home-prepared β-halo-thermophilic gycosidase (HOR) from *Halothermothrix orenii* the corresponding aglycones, hesperetin (HP) and quercetin (Q), which are characterized by better bioavailability have been obtained [2]. After and in-depth study in batch mode for the determination of the best reaction conditions, an in continuous process was developed through a combination of enzyme immobilization techniques and flow reactors, thus increasing the sustainability and the overall reaction yields.

To further reduce the process-related costs a co-immobilization of both the enzymes on the same matrix (*i.e.,* glyoxyl-agarose) has been developed to prepare a high-performing multi-active biocatalyst [3-4].

To further increase the solubility of the starting material a green solvent 2,2,5,5-tetramethyloxolane (TMO) has been employed in a biphasic system (50:50 TMO-buffer) [5].

Due to the recovery and reuse of all the materials involved in the flow biotransformation this strategy can be considered a zero-waste process.

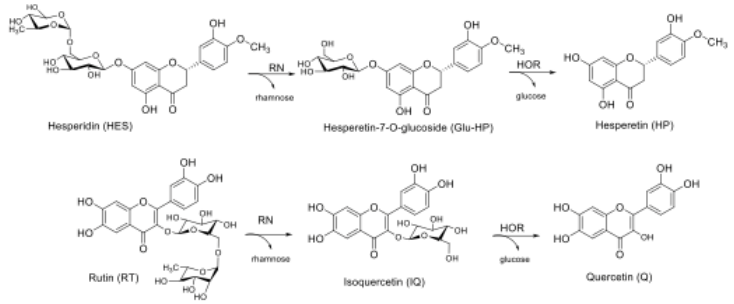

Figure 1. Obtainment of Quercetin (Q) and Hesperetin (HP) as aglycones from the corresponding natural rutinosides.

Materials and Methods

Starting from the natural rutinosides HES and RT a highly-productive and rapid one-pot, 2-step flow biotransformation was developed.

The first step was investigating free enzyme-mediated batch biotransformations in a biphasic system (*i.e.,* 50:50 water/TMO, 1 mg/mL catalyst concentration, 5 g/L substrate loading). Once the operation conditions were set, the next step was to enhance the catalyst stability. So, the two single proteins (imm-RN and imm-

HOR) were immobilized through a covalent bond with the matrix, agarose previously functionalized with aldehyde groups (*i.e.,* glioxyl-agarose). Subsequently the two enzymes were co-immobilized on the same support with the final aim of running enzymatic cascades into one-pot reaction systems.
Imaging analysis was carried out *via* scanning electron microscopy (SEM) to characterize the carrier before and after immobilization, and *via* confocal microscopy to analyze spatial distribution of the enzymes.
Flow bioprocesses have been developed, to accelerate biotransformations and enhance the productivity. As with batch reaction single steps were firstly optimized.
Finally, to directly obtain the desired aglycones HP and Q from the corresponding rutinosides co-immobilized RN-HOR preparation was employed.

Results and Discussion

With free-enzymes-mediated batch biotransformations Glu-HP and IQ were obtained with 15% and 30% m.c. in 14 h and 3 h, while HP and Q with 18% and 32% m.c. in 2 and 0.5 h, respectively.
During immobilization processed different concentrations of the two enzymes (RN and HOR) have been tested (1, 2, 5 mg/g_{resin}); the highest recovered activity (35%, 53% respectively) was obtained employing 1 mg/g_{resin} of pure proteins.
Regarding imaging studies, particles bound to the enzymes showed no changes in their surface and no surface aggregation phenomena, furthermore in the single-enzyme preparations the biocatalysts were localized across the porous surface of glyoxyl-agarose beads. While in the combined formulation, a spatial organization with HOR more internally localized surrounded by RN was observed.
Lastly the reactions were tested in continuous systems with the co-immobilized enzymes, which gave complete conversion in just 5 min of residence time.
Since all the material involved in the developed procedure can be recovered and reused, the designed strategy can be considered an ultra-efficient, zero-waste process.

Conclusions

Starting from natural glycosides hesperidin and rutin, recoverable from citrus residues, and using enzymes it was possible to obtain aglycones that can be commercialized as natural according to FDA and EMA regulation. To increase enzymes stability two strategies were applied, the use of extremozymes and immobilization techniques. This last approach allows to set up in continuous flow systems. Biocatalysis and flow facilities allowed for rapid reaction times and higher yields (>99% m.c., 5 min) with respect to batch reactions (60-70%, 0.5-1 h). To avoid drawbacks typical of step-by-step cascade reactions enzyme co-immobilization was deemed the best strategy. The enhanced catalytic performance of the two step flow bioprocess was allowed by the compartmentalized microenvironment, the special organization and the right density of the co-immobilized enzymes. The recovery and the potential reuse of all the materials involved in the biotransformations, including solvents, make this process appealing for its overall sustainability.

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C4: EVALUATION OF VOLATILE PROFILE OF CHILI PEPPER BY USING HEADSPACE SOLID-PHASE MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY ANALYSIS

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Introduction

Chili peppers are widely used as food or spices in many countries and as flavouring ingredient by food industry to produce various food products. In this study the volatile profiles of 17 chili pepper varieties was investigated by SPME followed by gas chromatographic analysis coupled to mass spectrometry and FID detectors. The results allowed evaluating the quality of the cultivars analysed, and to determine marker compounds responsible for their aromatic characteristics. Most of the studies in literature are limited to the identification of volatile compounds without any sensorial information, and therefore, the real contribution of individual compounds to the overall aroma has not been defined. In this respect, the sensory profile of chili peppers was also investigated in order to find a correlation with volatile components and to evaluate which notes contribute mainly to the perceived aroma. Furthermore, the volatile profile of three commercial extra virgin olive oils (EVOOs) flavoured with chili pepper was examined and their profile was compared with that of conventional unflavoured extra virgin oils to evaluate the impact of chili addition on the oils composition.

Materials and Methods

- *Standard Compounds*
  A C7–C40 n-alkanes (1000 µg/mL) standard mixture in hexane supplied by Merck Life Science was utilized for ALKANEs linear retention indices (LRIs) calculation. Forty-seven standard compounds supplied by Merck Life Science were used for the training of the panellist for sensory analysis.
- *Samples*
  Seventeen fresh chili pepper samples belonging to the genus Capsicum were analysed. In addition were analysed three chili pepper extra virgin olive oils. From the information reported on the label, one of them was flavoured with Merkén pepper, the second was flavored with a mix of Capsicum chinense peppers, and the third was flavored with the addition of a mix of different chili peppers.
- *SPME Extraction Conditions*
  The method used for extracting volatile analytes in chili peppers and flavoured extra virgin olive oils was the following: (DVB/CAR/PDMS) 50/30 µm fiber, sample conditioning of 5 minute at 50°C and an extraction of 50 minute at the same temperature with a stirring rate of 300 rpm. After extraction, the analytes were manually injected in splitless mode and are thermally desorbed for 1 minute at 260°C in the GC injector port.
- *GC–MS and GC-FID Analysis*
  GC-MS and GC-FID analysis were carried out for qualitative and quantitative purposes, respectively. GCMS analyses were carried out on a GC-QP2020 system. For the separation, an SLB-5 ms fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm df) was used. Helium was used as carrier gas at a constant

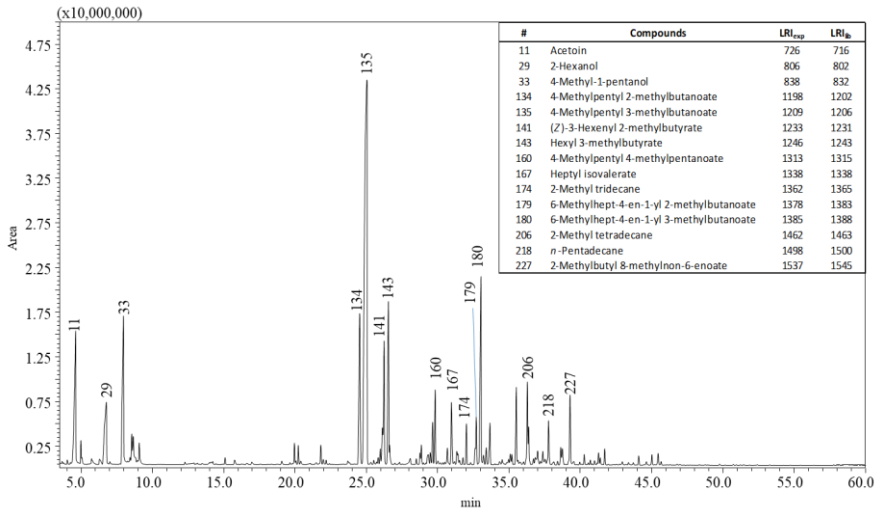
linear velocity of 30.0 cm/s. The temperature program was from 40°C, held for 1 min, to 350°C at 3°C/min, held for 5 min. The interface and ion source temperatures were 250°C and 200°C, respectively. The acquisition was made in scan mode in the mass range of 40–500 m/z, with a scanning rate interval of 0.2s. Data handling was supported by GCMS solution ver. 4.30 software. For the characterization were used W11N17 and FFNSC 4.0 databases. GC-FID analyses were carried out on a GC2010 system. Column, gas carrier and flow mode, oven temperature program and injection parameters were the same as for MS applications. The FID temperature was set at 280°C (sampling rate 200 ms), and hydrogen and air flows were 40 mL/min and 400 mL/min, respectively. Data were collected by LabSolution software ver. 5.92.

- *Sensorial Evaluation Procedure*

  Sensory Analysis was carried out by a panel of 7 analysts trained to distinguish and describe the aroma characteristics of 47 pure standards. The first step was to carry out a screening of all the chili pepper samples to identify the descriptors. For the sensorial analysis, the chili peppers were chopped one at a time with an immersion blender to reduce them into pieces of 2/3 mm and the mixture was then placed on a sheet of absorbent paper to drain the moisture. Each panelist smelled the preparation for about 30 minutes in order to identify the flavour notes.

Results and Discussion

The analyzed chili pepper samples showed different volatile profiles. An example of the GC-MS analysis with main components indicated is shown in Figure 1. More than two hundred and fifty compounds have been identified in total, representing between 87% and 91% of the total composition of each chili pepper.



#	Compounds	LRI_{exp}	LRI_{lit}
11	Acetoin	726	716
29	2-Hexanol	806	802
33	4-Methyl-1-pentanol	838	832
134	4-Methylpentyl 2-methylbutanoate	1198	1202
135	4-Methylpentyl 3-methylbutanoate	1209	1206
141	(*Z*)-3-Hexenyl 2-methylbutyrate	1233	1231
143	Hexyl 3-methylbutyrate	1246	1243
160	4-Methylpentyl 4-methylpentanoate	1313	1315
167	Heptyl isovalerate	1338	1338
174	2-Methyl tridecane	1362	1365
179	6-Methylhept-4-en-1-yl 2-methylbutanoate	1378	1383
180	6-Methylhept-4-en-1-yl 3-methylbutanoate	1385	1388
206	2-Methyl tetradecane	1462	1463
218	*n*-Pentadecane	1498	1500
227	2-Methylbutyl 8-methylnon-6-enoate	1537	1545

Figure 1. GC-MS volatile profile for sample 1 (C. Chinense-Naga Morich).

An example of sensory analysis is shown in Figure 2. Sensory testing has revealed a wide range of odors in the 17 varieties of chili peppers examined.

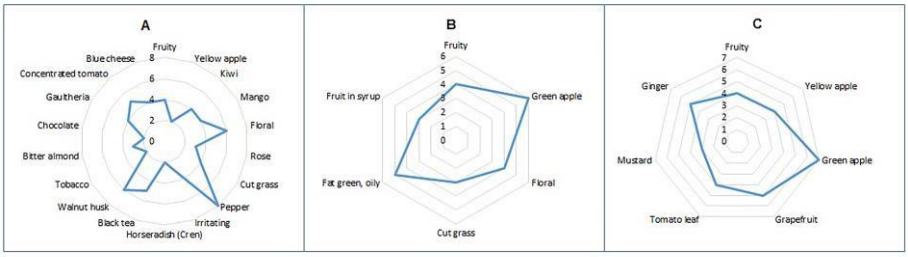


Figure 2. Aroma profile of three different species of pepper. (A) Naga Morich (*Capsicum Chinense*); (B) Calabrian pepper (*Capsicum annuum*); (C) Aji (*Capsicum baccatum*).

Conclusions

In this work, the volatile fraction of 17 varieties of chili belonging to *Capsicum chinense*, *Capsicum annuum* and *Capsicum baccatum* was analysed by HS-SPME extraction method followed by GC analysis. The aromatic diversity found between the cultivars is due to the qualitative and quantitative differences of the volatile odorant molecules, as confirmed also by the sensorial analysis. Moreover, the volatile profile of three chili pepper flavoured extra virgin olive oil has been investigated to evaluate the impact of the addition of the spices on the volatile composition of the oils for food traceability and authenticity.

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C5: A NEW WORKFLOW FOR RATIONALIZING RAW DATA EXPLORATION IN GC×GC: COMPUTER VISION TO UNRAVEL DIAGNOSTIC SIGNATURES

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Introduction

Computer vision is a branch of artificial intelligence (AI) that allows systems to extract useful information from digital images and make predictions based on that information [1].
Chromatographic fingerprinting on patterns obtained through comprehensive two-dimensional chromatography (C2DC) aims to detect, align, and compare features in analyzed samples. By representing C2DC chromatograms as digital images, the process enables higher-level information about unique composition and its relation to the studied phenomenon. Chromatographic images can be processed using different feature types, such as peak features, to profile components across multiple samples. The process of aligning and comparing 2D peak patterns is known as chromatographic fingerprinting, which can also be considered a Computer Vision approach.

Materials and Methods

In this work we focused on the processing of analytical data; in particular on the interpretation of two-dimensional chromatograms to understand the evolution of the *volatilome* of a complex during the production chain. In this field AI techniques such as Computer Vision can help rationalize raw data exploration, leading to an understanding of the biological phenomena related to specific chemical signatures and molecular patterns. A sample set from a study project on artisanal butter was explained to show the efficacy of this procedure. The sample set looks at how volatile patterns vary from raw sweet cream to ripened butter during the production process.

Results and Discussion

This work presents a new approach for computer vision that is based on pattern recognition techniques such combined untargeted and targeted (UT) fingerprinting. This procedure entails a number of steps, beginning with the creation of composite class pictures for representative samples' classes. Then, a feature template with reliable peaks and peak-regions is created using these images. After that, bleeding peaks and artefacts are removed from the feature template while keeping the targeted components. All sample images and composite class images are subjected to the feature template once it has been finalized. In order to highlight quantitative pattern differences and relate them to the chemistry of targeted compounds and monitored features of untargeted compounds, pair-wise comparisons are then performed.

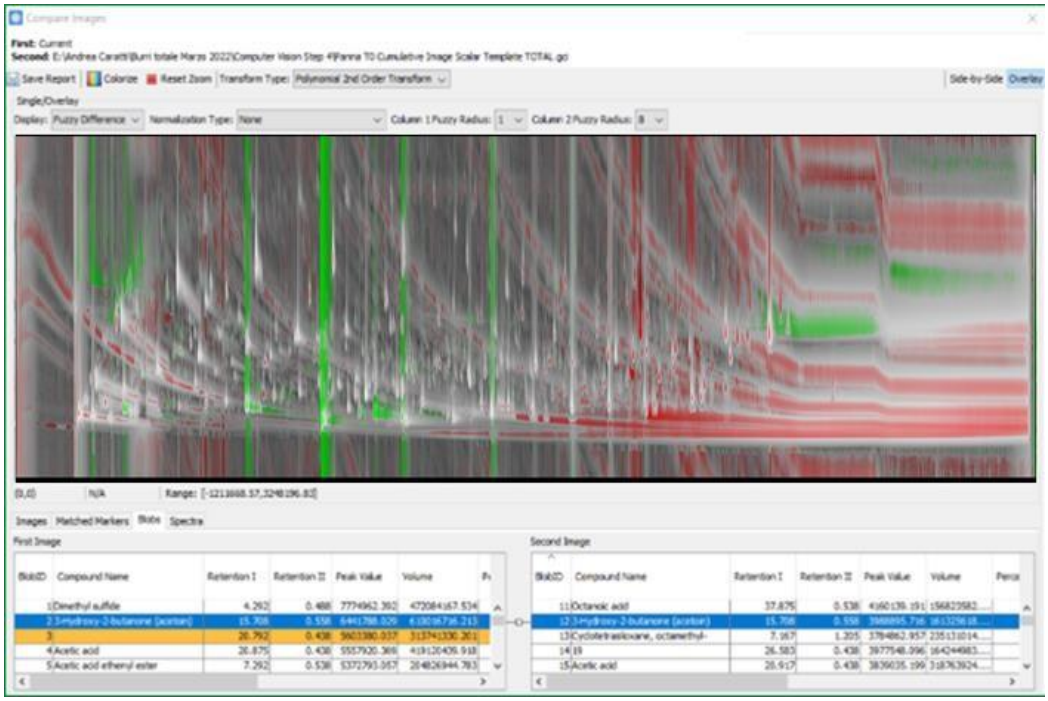

Figure 1. Comparative overlaid visualization between raw sweet cream and ripened butter. Different colorization emphasizes differences between class images.

The workflow's application to this sample set shows how the evolution of the volatile fraction can be tracked throughout the production chain, giving important insights into changes in chemical composition and quality. In figure 1 through the use of Computer vision it is easy to appreciate the compositional difference at the volatilome level between two production steps: raw sweet cream and 40 days ripened butter.

Conclusions

To capture the significant changes in food volatilome throughout processing, GC×GC-TOFMS with thermal modulator proved to be highly successful.

Identification of diagnostic patterns of process and quality markers may be hindered by the interaction of many diverse variables.

Computer Vision allows an effective comparative visualization of Class Images realized by realigning and combining 2D chromatograms from a Samples' Class. Pair-wise differences are conveniently recorded, and UT fingerprinting makes it simple to obtain higher level data.

Investigating at the molecular level is straightforward and reliable.

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C6: CALABRIAN CHILI PEPPER FLAVOURING EXTRACTS: PRELIMINARY CHARACTERIZATION AND VALORISATION OF THIS VARIETY TOWARDS THE PGI MARK

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Introduction

Chili pepper is a relevant spice widely used all over the world; it belongs to the *Capsicum* genus and the *Solanaceae* family. Considering not just its flavour but also its nutritional value, it is a suitable source of compounds (mainly capsaicinoids and carotenoids) with antioxidant and antimicrobial activity useful for functional food production. In fact, chili peppers were probably firstly used as medicinal plants before being exploited as spices. Indeed, not just the potential benefit of capsaicinoids is widely known for the treatment of pain, ischemic heart disease or for the antidiabetic [1] and the recently studied chemo-preventive effects [2], but also the antioxidant activity of carotenoid compounds was proven to be even higher: that can explain their good anticancer activity, as well [3]. These effects are promising, considering the side effects of synthetic anti-obesity drugs, for instance. Thus, the aim of this study is a future valorisation of the studied Calabrian Chili Pepper flavouring extracts, considering that a complete characterization exclusively of this specific variety is still missing in scientific literature.

Materials and Methods

1) Soxhlet Extraction: pre-dried and powdered matrix of Calabrian Chili Pepper, provided by New Flavours® company, was then grinded and extracted using a Universal Extractor in Soxhlet mode. 4 GROUPS of extracts were obtained varying extraction conditions according to the following table and the solvent was evaporated at 38°C using a rotating evaporator, till complete dryness.

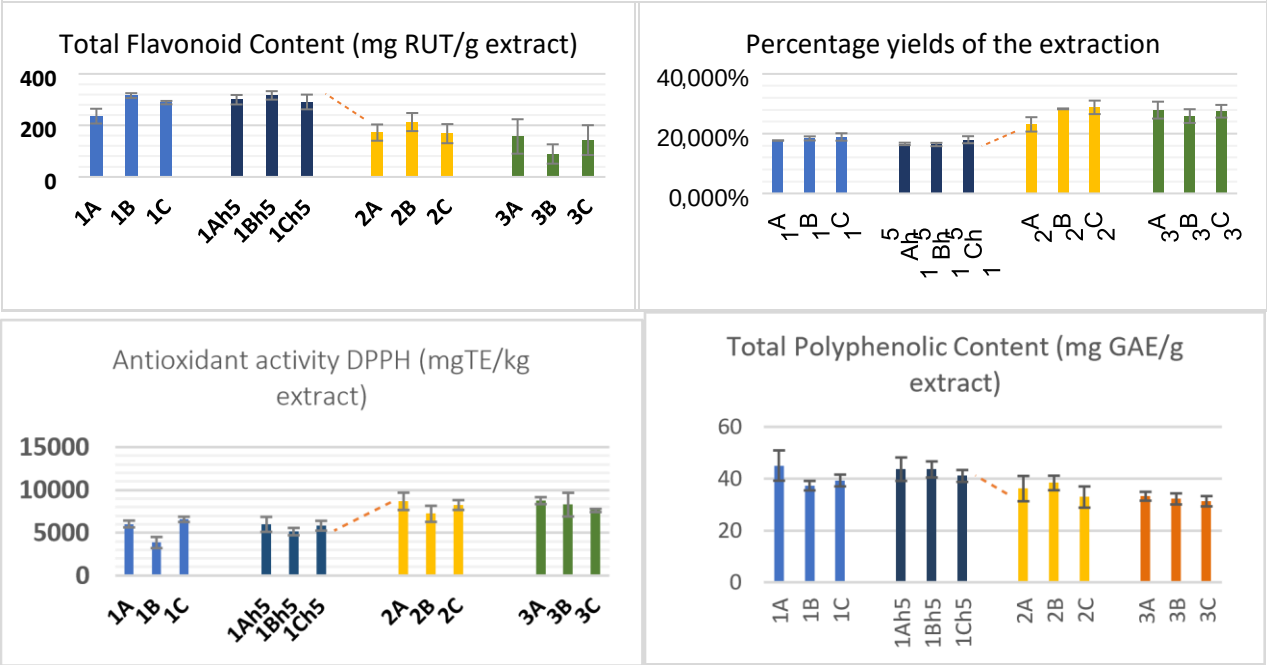
Extraction conditions:	**GROUP 1**	**GROUP 1 h5**	**GROUP 2**	**GROUP 3**
SOLVENT	**EtOH Anhydrous**	**EtOH Anhydrous**	**EtOH/H2O 70:30**	**EtOH/H2O 50:50**
TIME of extraction	3h	**5h**	4h	4h
MATRIX/SOLVENT RATIO	1:25 (extract **1**A)	1:25 (extract **1**A **h5**)	1:25 (extract **2**A)	1:25 (extract **3**A)
	1:50 (extract **1**B)	1:50 (extract **1**B **h5**)	1:50 (extract **2**B)	1:50 (extract **3**B)
	1:100 (extract **1**C)	1:100 (extract **1**C **h5**)	1:100 (extract **2**C)	1:100 (extract **3**C)

2) Spectrophotometric assays

Total Flavonoid and Polyphenolic Content along with the antioxidant activity through DPPH assay were evaluated, using rutin as standard at 5 different concentrations from 200 to 1200 ppm, gallic acid (5 different concentrations from 100 to 600 ppm) and Trolox (10 different concentrations from 1 to 100 ppm) to have the respective calibration curves. The specific details of the experiments are given in previous papers of this research group [4].

All the experiments were conducted in duplicate (total=48 samples for each assay).

Results and Discussion



- As for the percentage yields of the extraction, the significant difference (p value < 0,01) can be noticed just between the two groups of the samples extracted both with EtOH anhydrous, but with different time of extraction (group 1 and 1h5), and the other two obtained using hydroalcoholic solutions (group 2 and 3). The yields of the latters were higher. Instead, no statistically significant difference was highlighted between the two groups of samples both extracted with ethanol anhydrous, but with a quite different time (3 h and 5h). That can suggest that a 3 hours time of extraction or a longer one doesn't significantly change the yields of extraction.
- As for the Total Flavonoid Content (TFC), there is a really significant difference (p value < 0,001) between the two groups of the samples both extracted with ethanol anhydrous and the ohter two obtained using hydroalcoholic solutions. In fact, the ones obtained using ethanol anhydrous had a higher TFC, while it significantly decreases in extracts obtained with EtOH 70% and those from EtOH 50% (p value < 0,01). This difference can be explained by the apparently predominant presence of less polar flavonoids. Anyway, even the lowest value of TFC among all extracts, that was found in 3B, was higher than the average level of TFC in extracts from *Capsicum Annuum* [5]. Furthermore, if no statistically significant difference can be noticed between the TFC of group 1 and 1h5, that indicates that a longer time of extraction doesn't affect the levels of these compounds in the extracts.
- As for Total Polyphenolic Content, entirely the same tendence was noticed and that could be explained by the presence of less polar polyphenols also in this case.
- As for the Antioxidant Activity (evaluation through DPPH assays), also in this case there is a really significant difference (p value < 0,001) just between the two groups of the samples both extracted with ethanol anhydrous and the other two obtained using hydroalcoholic solutions: the latters showed a quite higher antioxidant activity, with respect to groups 1 and 1h5. That could indicate the presence of other powerful antioxidant compounds different from flavonoids and polyphenols. Anyway, in each case all the obtained values can be considered good levels of antioxidant activity in a plant matrix [6].

3) GC-MS and HPLC-DAD ANALYSES of different volatiles, polyphenols, the main capsaicinoids, vitamins and carotenoids are still ongoing to be elaborated, along with the results of the ***COMPARISON WITH THE ULTRASOUND ASSISTED EXTRACTION*** in terms of yields and chemical characterization of the extracts.

Conclusions

All those results of this preliminary investigation are just a promising beginning of a future wider, interesting and more accurate characterization towards a valorisation of the Calabrian Chili Pepper through a possible optimization of its extraction and analyses.

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C7: NEW FUNCTIONAL FOODS: CHARACTERISATION OF PRODUCTS DERIVED FROM *CANNABIS SATIVA* L. BY ADVANCED CHROMATOGRAPHIC TECHNIQUES

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Introduction

The increasing demand for high nutritional and nutraceutical foods has generated, in recent years, a growing interest in *Cannabis sativa* L., a versatile plant with an eco-sustainable culture. In particular, the low ratio of *ω*-6/*ω*-3, together with the high content of polyunsaturated fatty acids, phytosterols, tocopherols, polyphenols, terpenes and phytocannabinoids, make hemp seed foods sources rich in beneficial properties for health [1].

Materials and Methods

Samples

A total of seven hemp seed-based food products were investigated: four different hemp seed oils (1 to 4), two hemp seed flours (1 and 2) and one flour by-product. Oils were provided by different local companies and they were obtained by cold pressing of hemp seeds (without any filtration). Flours were obtained by stone milling of hemp seed after cold pressing and sieving, while the flour by-product represents the coarse residue of the sieving process.

Analytical techniques

After their extraction, all the interesting nutraceutical fractions were investigated by means of different chromatographic techniques. In particular, volatile and sterol compounds were analysed through the use of GC-MS and GC-FID, for qualitative and quantitative purposes, respectively. Cannabinoids and polyphenols were characterized coupling an HPLC system with PDA and MS, while for tocopherols an FLD detector was used.

Results and Discussion

Among tocopherols, *γ*-tocopherol was quantified at the highest level (Tab. 1).

Table 1. Tocopherol content (mg/100g ± standard deviation) in hemp seed-based food products.

Compound	Oils				Flours		Flour by-product
	Oil 1	Oil 2	Oil 3	Oil 4	Flour 1	Flour 2	
***α* -tocopherol**	3.88 ± 0.00	2.91 ± 0.03	2.75 ± 0.01	2.43 ± 0.00	3.67 ± 0.09	3.42 ± 0.01	4.00 ± 0.10
***α*-tocotrienol**	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
***β*-tocopherol**	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
***γ*-tocopherol**	57.10 ± 0.09	54.72 ± 1.59	50.09 ± 0.00	55.13 ± 0.06	59.81 ± 0.11	93.64 ± 0.10	58.70 ± 0.23
***δ*-tocopherol**	1.74 ± 0.01	1.89 ± 0.01	1.99 ± 0.01	1.7 ± 0.00	2.64 ± 0.08	2.67 ± 0.02	1.91 ± 0.07
Total	62.72	59.52	54.83	59.26	66.12	99.73	64.61

Terpenes represented the most abundant compounds among volatiles (Fig. 1). A total of 58 compounds belonging to the unsaponifiable matter was identified only in hempseed oils. Phenols and cannabinoids were also investigated, and a total of 52 compounds were identified and quantified (Fig. 2).

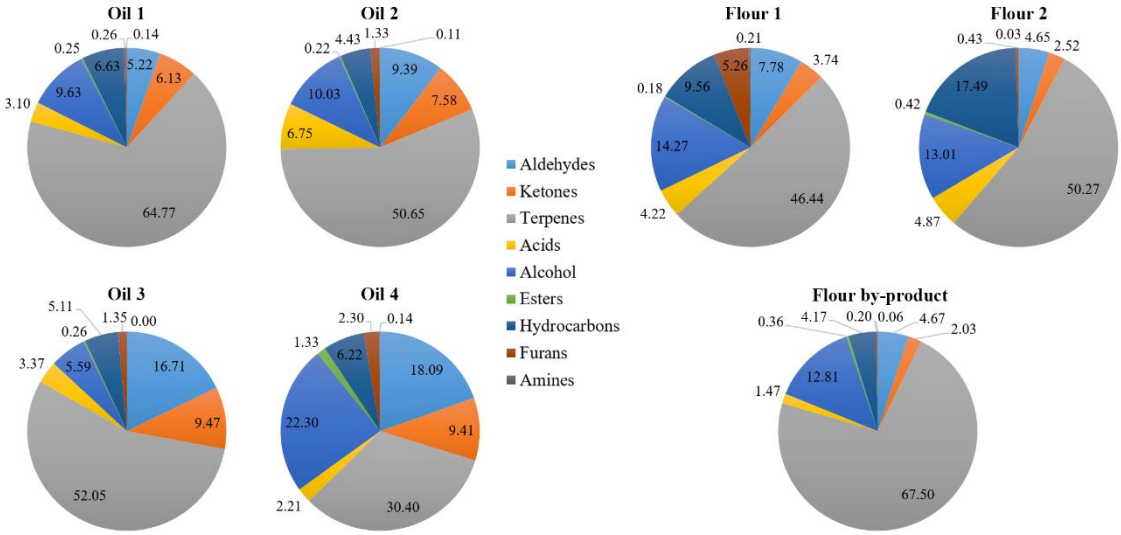

Figure 1. Volatile fraction profiles in hemp seed-based food products.

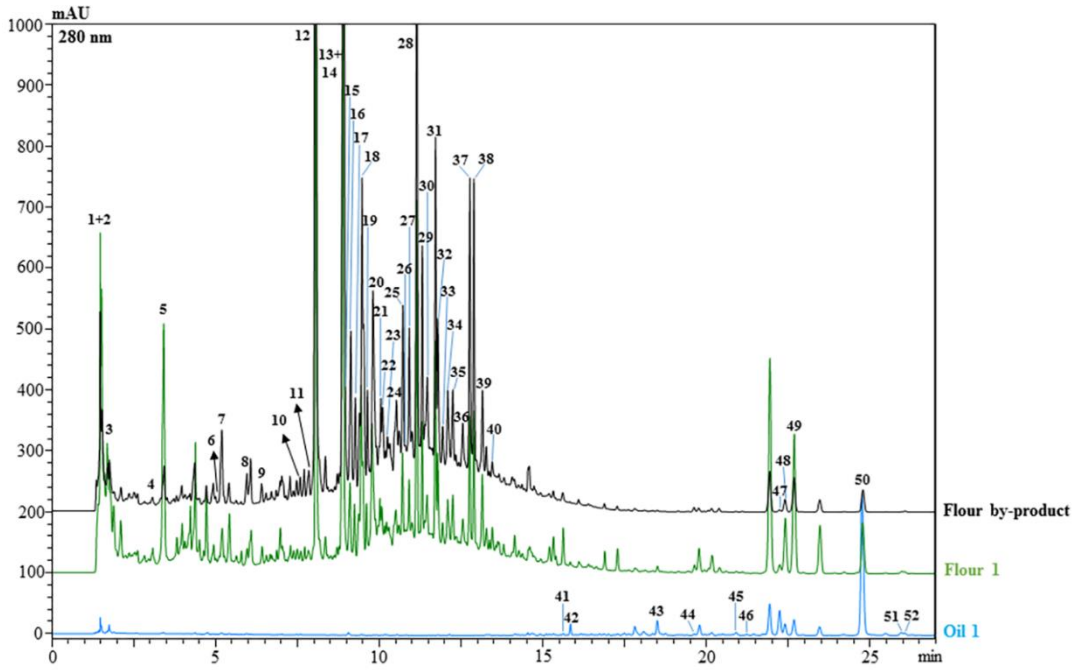

Figure 2. LC-PDA profiles of phenolic and cannabinoid compounds of the oil 1, flour 1 and flour by-product.

Conclusions

All the investigated samples showed the same qualitative profiles with respect to all the chemical classes (except for the phenolic profile), while quantitative differences were detected even within the same type of samples (oils or flours). Within this context, the analysis of secondary metabolites can be extremely useful for the evaluation of storage conditions and the influence of technological processed on the chemical composition, which determines the biological activity of such food products. As an example, the ratio between the acidic form (CBDA) and the neutral CBD confirmed a care in preserving the raw material from

oxidation prior and during the technological process leading to the production of the oil, as well as proper storage conditions.
The results obtained confirm the importance of *Cannabis sativa* L. as a high nutraceutical value source, emphasizing the qualitative-quantitative differences of its by-products.

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C8: GREEN EXTRACTION AND CHARACTERIZATION OF GELATIN COMING FROM UNSORTED DEHYDRATED CANNED TUNA SIDE STREAMS"

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Introduction

This study, funded by the EcoeFISHent project [1], describes a scalable method for the extraction of valuable proteins, such as marine gelatin and Hydrolyzed Gelatin Peptides (HGPs), but also non-collagenous proteins, in order to define future applications in the food, packaging, and cosmetic industries. The process starts from unsorted mixed Yellowfin tuna scraps after a patented industrial dehydration process that enhances the logistics in the valorisation of highly perishable biomasses.

Materials and Methods

Samples made up of raw crude heads, fins, skin, tails, and bones from Generale Conserve's (ASdoMAR®) mixed unsorted canned Yellowfin tuna processing side streams were dehydrated and milled using an industrial process patented by Themis S.p.A. [2] to stabilize them over time as a fish biomass with a low residual humidity. Besides, microbiological activity of samples pre and post dehydration was monitored to ensure the occurred stabilization.

Before gelatin extraction, the starting material was analyzed in terms of proximate analysis to assess residual moisture (AOAC 950.46B), protein fraction (AOAC 981.10), ashes (942.05) and applying Hara-Radin method for the evaluation of lipid content [3]; moreover, amino acid composition was determined by HPLC [4,5].

The gelatin extraction can be divided into three main steps: pre-treatment, extraction, and drying. In addition to the extraction of gelatin, this extraction method also allows the recovery of non-collagenous proteins and HGPs.

The following analyses were proposed for the characterization of the extracted gelatin and non-collagenous proteins: their composition in residual moisture, protein, ashes, and lipid contents (AOAC Official Method 935.46), and amino acidic composition [4,5]. The FT-IR analysis was applied to study the secondary structure of extracted gelatin and, as for its rheological parameters, a solution of 6.67% was prepared and the viscosity was measured at 40°C, while the gelling point was detected in a range of 5-40°C [6].

Results and Discussion

The proximate analysis of the starting material reported low values of residual moisture (under 5%) which means that the industrial dehydration treatment was very effective. As expected for large-sized fish, the crude protein fraction analysis revealed a high percentage of total nitrogen, over 50%; ashes were found to constitute about 30% of the total composition, reflecting a high presence of bones in the biomass, which are considered a good gelatin source. Lipid fraction presented a 13% value and parallel research on its quality and omega-3 composition are currently ongoing. The study of the amino acidic profile revealed that Glycine (13.80%), Glutamic Acid (11.92%), and Alanine (7.33%) make up the majority of the components, Cysteine and Taurine (around 1% each) are the minor ones, while the collagen/gelatin-characteristic amino acids Hydroxyproline and Proline account for 10.57% together.

The extraction process allows to recover both non-collagenous proteins and also collagenous ones (i.e., gelatin and HGPs) with yields equal to 14.6, 1.7% and 3.2%, respectively.

The extracted gelatin was analysed as follows: in terms of stability and shelf life, the low residual moisture value of 2% is considered a good result; crude protein fraction of 88% shows that gelatin has been well purified; implying that the defatting phase was effective (lipids below 1%), although demineralization still needs to be improved, since ashes were found to be 5%. The composition of amino acids was found to be similar to the one reported by Nurilmala et al. [7] for gelatin extracted from skin tuna, showing a high concentration of Glycine (24.57%) and about 9% for Arginine, Glutamic Acid, Proline, Hydroxyproline and Alanine, with ratio OH-Pro/Pro equal to 0.98.
The FT-IR spectrum shows the characteristics peaks of collagen/gelatin: Amide A, B, I, II, and III.
The viscosity and the gelling point were measured obtaining 5.0 mPa s and 14.6 °C respectively; these specific and characteristics results are lower than those reported in literature [8] for gelatin extracted from previously separated fish side streams, nevertheless further investigations using different concentrations are currently ongoing and several studies including different fields of application, such as foods, packaging and cosmetics, are *in fieri* above all considering the different range of the obtained gelatin and HGPs in terms of molecular weight .
As for non-collagenous proteins, the analysis of the composition revealed that they are constituted by residual moisture (5.3%), proteins (67.0%), and ashes (24.0%); the amino acidic profile shows 14.59% for Glycine and 12.18% for Glutamic Acid, followed by about 8% for Aspartic Acid and Alanine, 7.25% for Arginine, while Proline (6.82%) and Hydroxyproline (4.32%) were considerably lower than what was found in gelatin.

Conclusions

The disposal of the side streams caused by the enormous growth in fishing production over the past few years is becoming an urgent environmental and economic issue to solve. In the literature, the extraction of gelatin starting from separated leftovers (skin and bones) has already been widely discussed. Differently, in this work, a scalable process to extract valuable proteins from unsorted mixed tuna scraps coming from canned tuna industry is presented, demonstrating that it is possible to benefit from the entire side stream, avoiding the onerous step of separation which could represent a significant advantage for industry.

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C9: OPTIMIZATION OF THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM GRAPE POMACE

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Introduction

In the last few decades, many attempts have been made to develop methods and find different ways of exploiting fruit and vegetable waste to obtain bioactive compounds: agro-industrial wastes are widely used as fertilizer or livestock fodder but, according to recent studies, these by-products can be used in the production of high-value products like foods, cosmetics, and medicines.[1] The extraction of bioactive compounds (phenolic acids, carotenoids, flavonoids, bioactive proteins, fatty acids, fiber, and many others) from natural matrices represents the first challenge in the recovery of natural constituents with beneficial effects for human health and disease prevention.[2;3] In this study, considering the potential of UAE as an emerging and innovative technique to get polyphenol compounds from plant sources, this approach was selected to perform the extraction of phenolic compounds from grape pomace, the main by-product of wine industries. In this study we carried out a multi-step optimization of the extraction of phenolic compound from grape pomace considering several variables that may influence the recovery of these bioactive compounds, such as solvent composition, time and temperature. In the next phase of the study, we investigated the impact of various drying processes in order to identify the best conditions for stabilizing grape pomace, without compromising the concentration of the phenolic compounds.

Materials and Methods

Samples and Reagents.

Grape pomace deriving from Merlot and Cabernet grapes variety was supplied in 2021 by a local farm, located in the province of Perugia (Italy). Trolox, DPPH, ABTS and Folin and Ciocalteu's phenol reagent were from Sigma-Aldrich (St. Louis, MO, USA). Acetone and ethanol 99.7% were purchased by VWR Internationals (Milan, Italy). Formic acid was purchased by Carlo Erba (Milan, Italy).

Grape pomace preparation and UAE extraction of phenolic compounds.

In a preliminary stage, five aliquots of air-dried Merlot pomace powder were prepared and different content of formic acid (from 0.1% to 1.5%) was added in the extraction mixture (EtOH:H_2O, 50:50). Dried grape pomace powder was weighed and treated with the selected solvent, then each sample mixture was mixed by vortex for 10 s and finally the UAE was performed for 15 min at 30 °C. After sonication, samples were centrifuged at 3500 rpm for 10 min at room temperature. The supernatant was filtered by using a syringe filter (nylon membrane 25 mm, 0.45 µm pore size, VWR International, Milan, Italy), subsequently concentrated and stored in amber glass vials at 4-5 °C until further analysis. Grape pomace extracts were analysed to characterize the phenolic fraction and the antioxidant properties through spectrophotometric assays, such as TPC, DPPH, ABTS and FRAP, as previously reported.[4] Another aliquot of dried grape pomace powder was extracted with acetone:H_2O containing 1% of formic acid as a solvent mixture. Lastly, pomace from both Merlot and Cabernet grapes was divided into fractions and each of them subjected to different drying treatments: the first fraction was dried at room temperature for 8 days (MA and CA samples, for Merlot and Cabernet, respectively), the second was dried in an oven for 28 h at 40 °C (MO and CO samples) and the last fraction was freeze-dried (ML and CL samples). A further aliquot of fresh sample was stored at - 20 °C (MF and CF samples). Dried samples were ground in a blender to obtain a fine powder, which was stored in glass containers, in a dry place at room temperature until the extraction, carried out in the extraction condition optimized by experimental design.

Optimization of UAE extraction by experimental design.

The optimization of the extraction conditions of phenolic compounds from grape pomace was achieved by experimental design, using MODDE 5.0 (UMETRICS AB, Umeå, Sweden) software. The following quantitative factors were considered: Water content (in EtOH), which ranged between 20% and 80%; Temperature, which varied from 35 and 60°C; Sonication time (min), which ranged between 10 to 30.

Results and Discussion

In a first phase of the study, preliminary extraction tests were carried out on an air-dried sample aliquot, using EtOH:H_2O mixture (50:50 v/v), containing different concentrations of formic acid: 0%; 0.1%; 0.5%; 1%; 1.5%. The extracts were characterized by determining their phenol content, and their anti-radical and reducing properties. According to the preliminary findings, formic acid addition to the extraction solvent enhances the extraction of bioactive components from grape pomace. The results obtained from the characterization of the antioxidant, antiradical and reducing activity by *in vitro* assays, indicated a higher antioxidant power for the samples extracted using 1% of formic acid, showing how formic acid is essential for the recovery of antioxidant compounds during the extraction phase.

In order to evaluate the extraction efficiency with different solvent composition, two different solvent systems based on EtOH:H_2O (50:50, v/v) and acetone:H_2O (50:50), containing 1% of formic acid, were compared on air-dried grape pomace powder. Ethanol-based system afforded the best results.

An experimental design approach was adopted in a second stage of the study to further investigate the impact of specific variables on the recovery of phenolic compounds. The software indicated 11 experimental conditions for the extraction and the optimal conditions were: UAE for 30 min at 35 °C using an extraction mixture with 20% of water in ethanol.

In the last part of the study, in order to evaluate different drying pre-treatments on the recovery of phenolic compounds, the characterization of grape pomace from two different varieties was achieved by the measurement of antioxidant and antiradical activity by *in vitro* assays. In this study, air-dried, oven-dried, freeze-dried and fresh grape pomace samples deriving from Merlot grapes, were compared with samples from Cabernet grapes treated under the same conditions. The extractions were carried out under the optimized conditions indicated above. Some differences in the total phenolic content and in the antiradical activity were found for the examined samples: fresh samples showed higher content of phenolic compound and antiradical/reducing activity with respect to the dried samples. Among the drying treatment, freeze-drying proved to be the best for the recovery of phenolic compounds.

Conclusions

The results of the study increase knowledge of the extraction process, which represents the first obstacle to overcome in the promising perspective of the production of high-value foods from agri-food waste. In particular, the results obtained in this work highlighted that grape pomace represents cheap and bioactive-rich starting materials, to be exploited for the recovery of natural bioactives, like phenolic compounds. In this study we also demonstrate that bioactive compounds can be extracted using environmentally friendly extraction techniques (UAE) and solvents (ethanol:water) recognized as safe for the environment and human health (GRAS).

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C10: GREEN APPROACH TO EXTRACT POLYPHENOLS FROM *S. EUROPEA* L.

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Introduction

The recovery of antioxidants, minerals and oils from fresh vegetable matrices and agro-industrial by products is one of the most widely used natural health resources today. In this sense, *Salicornia europea* L., a halophyte plant found along the Mediterranean coast, is the subject of our investigation. Salicornia is utilized in Chinese traditional medicine as well as consumed as a seasoned vegetable due to its advantageous effects in preventing chronic diseases. It has been shown in various studies that *S. europea* contains a high concentration of bioactive compounds, with polyphenols being the most common.[1]
Furthermore, extractions were carried out from *S. europea* in order to recover polyphenols. A green approach based on novel green solvents Natural deep eutectic solvents (NADES) and Ultrasound assisted extraction (UAE) were used to extract bioactive compounds from plant.

Materials and Methods

Salicornia was collected from Apulian Adriatic coast and subsequently, it was dried in an oven at 40°C for 48h. The dried plant was milled to the particle size of 200um.
The Software BIOVIA COSMOtherm 2020 (version 20.0.0. Dassault Systemes, Paris, France) was used to predict the activity coefficient calculation and for calculate the geometric and energetic optimization of HBA, HBD and polyphenols revealed in the extracts. The optimal NADES was synthetized at 50°C for 2h while extractions were performed in an Ultrasound bath at 25°C. To compare the extraction efficiency, an extract was run in a 50% ethanol mixture at 25°C for 10 minutes to identify via HPLC/DAD the class of polyphenols most represented in the plant investigated.[2,3]

Result and discussion

Preliminary studies are in progress. The obtained results will be explained.

Conclusions

Salicornia is a resource of important polyphenols compounds thus the research was focused to recover the most revealed class of compounds in this plant through a sustainable process.
The use of green solvents such as NADES will lead in the future to obtain a Salicornia 'ready-to-use-extract' in order to formulate a nutraceutical supplement.

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C11: CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC CHARACTERIZATION OF PUMPKIN WASTE

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Introduction

Pumpkins, belonging to the Cucurbitaceae family, are well-known edible plants, frequently used as functional food or herbal medicine [1,2]. The agro-industrial processing of pumpkin generates different kinds of waste (i.e., seeds, filamentous strands, and peels), representing an important source for the recovery of interesting bioactive compounds such as carotenoids. After their extraction and purification, it is possible to reuse them to formulate supplements or functional foods with potential health effects [3]. This study aimed to characterize carotenoid-enriched extract obtained from peels and filaments of 8 different varieties of pumpkin. An ultrasound-assisted extraction (UAE) procedure, optimized in a previous study [4], was employed for the extraction of carotenoids. Extraction efficiency and carotenoid profile were monitored by reversed phase-high performance liquid chromatography (RP-HPLC) procedure, while the *in vitro* antioxidant activity of the obtained carotenoid extracts was evaluated by ABTS assay.

Materials and Methods

Pumpkin's peel and filamentous strand preparation

8 different varieties of pumpkin belonging to two different species of pumpkin, *C. Moschata* (Butternut, Violina Rugosa, Moscata di Provenza and Lunga di Napoli) and *C. Maxima* (Hokkaido, Delica, Delica Vanity, Mantovana), were purchased from a local farm (Perugia, Italy). Pulp, peel, and filamentous strand were separated manually, then the seeds were removed. The peel was cut into thin slices of 1.5 cm and subsequently dehydrated in a ventilated oven at 40 °C; the same procedure was followed also for the filamentous strand. After complete dehydration, peel and filaments were separately blended (Oster, model n. 869-50R USA) to obtain a homogeneous powder, and then stored in amber glass bottle at room temperature, until further analysis.

Ultrasound-assisted extraction (UAE)

The extraction of carotenoids by UAE was performed according to a previous study [4]. The extraction was carried out with an ultrasonic bath (model AU-65, ArgoLab, Carpi, Italy), composed of an inox jug with a maximal capacity of 6500 mL. 1 g of dried pumpkin powder (peel or filament) was sonicated in 20 mL of hexane/isopropyl alcohol (60:40, *v*/*v*) for 30 min at 45 °C. Then the extracts were filtered and kept in amber glass vials at −20 °C until further analysis.

Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) analysis

The HPLC measurements were made on a Shimadzu (Japan) system equipped with two LC-10AD high pressure binary gradient pumps, an SPD-10A variable-wavelength UV-Vis detector and a Rheodyne7725i injector (Rheodyne Inc., USA). A Develosil C30 column (250 × 4.6 mm, 5 µm, Nomura Chemical Co. Ltd., Japan) was used for the analytical separation. Data acquisition was performed using Chrom&Spec software (Chromatographic Specialties Inc., Canada).

Antioxidant assay

The *in vitro* antioxidant capacity was spectrophotometrically evaluated by ABTS (2,2-azinobis (3ethylbenzothiazoline-6-sulfonic acid) assay [4]. All the analyses were performed in duplicate, and the values were expressed as mg Trolox Equivalents per gram of dried pumpkin (mg TE/g). A Lambda 20 spectrophotometer (PerkinElmer, Inc.; Waltham, MA, USA) was used for each assay.

Results and Discussion

The extraction efficiency was evaluated by RP-HPLC analysis, by relying upon an optimized gradient program developed in a previous work [4]. The chromatographic analysis showed considerable variability in terms of individual carotenoid content between the different pumpkin varieties, both in the case of peels and filamentous strands. The results of previous analyses conducted on pumpkin pulp [4] were also confirmed in the case of peel and filamentous strand since for all the varieties tested, the chromatographic profiles evidenced the presence of different peaks corresponding to β-carotene, lutein, zeaxanthin, and mono- and diesters-xanthophylls as the major carotenoids. The chromatographic profiles also showed considerable variability in terms of lutein content between pumpkins belonging to different species (*C. moschata* and *C. maxima*). As reported by other works [5], the lutein content is clearly higher in pumpkins belonging to the *C. maxima* species, unlike those belonging to the *C. moschata* species, where only small traces of lutein have been detected. Regarding the differences between the two characterized by-products; for the filaments, βcarotene was the compound with the highest concentration, while for the peels in the specific case of Delica, Mantovana, and Delica Vanity, lutein had higher concentrations than β-carotene. The results of the antioxidant potential, evaluated by ABTS assay, show an interesting activity for the extracted carotenoids both from filamentous strand and peel. For peels, *C. maxima* species showed the highest value, Hokkaido and Delica above all, while for the filamentous strand *C. moschata* and *C. maxima* were comparable between each other when the varieties with the highest value were considered (Violina Rugosa for *C. moschata* and Delica Vanity for *C. maxima*).

Conclusions

These findings showed that UAE is an efficient technique for the extraction of carotenoids from pumpkin peel and filamentous strands. The antioxidant activity shown by the extracts obtained can lead us to hypothesize the use of these food wastes deriving from the processing of the pumpkin as ingredients for the formulation of food with added value or supplements. Further analyses are underway to further characterize the carotenoid profile and the antioxidant activity, to be able to select the species and more specifically the variety or varieties with the most suitable characteristics for future use in the development of value-added foods or supplements.

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C12: INNOVATIVE PLANT DERIVED EXTRACT WITH HEALTH PROMOTING ACTIVITY

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Introduction

Plant products contain a wide range of bioactive compounds (such as vitamin C, glucosinolates and polyphenols) whose major health-promoting effects are achieved due to the antioxidant, anti-inflammatory, antimicrobials, and hypocholesterolemic properties [1]. Nevertheless, bioactive compounds such as phenolic compounds, essential fatty acids, and insoluble vitamins are mostly hydrophobic, poorly soluble and chemically and thermal unstable [2]. Thus, the main challenges for applying these compounds in food formulations and dietary supplements are the low bioavailability and stability. In light of these observations, by using multidisciplinary approaches, the first objective of this study was the development of a reliable protocol to obtain an innovative and stable plant extract for its fast and scalable industrial application. Finally, its biological activities (hypocholesterolemic and hypolipidemic effects) have been investigated in *in vitro* cellular models.

Materials and methods

The plant extract has been obtained by developing an innovative and easily industrially scalable protocol (under patent). By applying multi-omic approaches, the plant extract composition has been characterized. The intestinal transport of the plant extract was assayed on differentiated Caco-2 cells, a well-established *in vitro* model of absorptive enterocytes. Then, to investigate the biological effect of the plant extract absorbed, a co-culture system, including Caco-2 and HepG2 cells, was developed to investigate how the plant derived extract absorbed can modulate cholesterol metabolism in HepG2 cells. In particular, the experiments were carried out on HepG2 cells, since the hepatocyte is the major cell involved in the low-density lipoprotein (LDL) clearance by the LDL receptor (LDLR) activity. The LDLR expression is tuned by changes in intracellular cholesterol levels and a transcription factor, the sterol-responsive element binding protein-2 (SREBP-2), which plays a key role in LDLR mRNA expression [3]. Among SREBP-2 gene targets, the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoAR) is particularly important [4].

Results and Discussion

The results show that this innovative plant derived extract can be easily obtained and stabilized by applying the developed protocol. The plant extract contains a wide range of bioactive compounds (aminoacids, fatty acids, polyphenols and organic acids) and genetic material (miRNAs). Indeed, the experiments performed on the co-culture system show that the plant derived extract does not show any toxic effect on Caco-2 cells and can be uptaken by Caco-2 cells. After the uptake, the extract increases the LDL receptor protein levels in HepG2 cells through the activation of SREBP-2 transcription factor, leading to enhanced ability of hepatic cells to uptake extracellular LDL molecules with a final hypocholesterolemic effect. Moreover, the plant extract regulates the intracellular HMGCoAR activity through the increase of its phosphorylation by the activation of AMP-activated protein kinase (AMPK)-pathways; lastly, unlike statins, the plant extract does not produce any unfavourable effect on proprotein convertase subtilisin/kexin 9 (PCSK9) protein level.

Conclusions

In conclusion, this multidisciplinary study provides a standardised, industrial scalable, and versatile workflow that can be used for obtaining an innovative plant derived extract that overcomes the obstacle of low bioavailability of the phytocomplex. The innovative plant extract shows a pleiotropic and regulatory activity, attributable to the very rich composition in bioactive compounds and miRNAs. Hence, due to the cholesterol-lowering and lipid-lowering properties shown, this plant extract could be considered an innovative functional ingredient for the development of a new generation of dietary supplements/functional foods suitable for the metabolic syndrome prevention.

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C13: RICE NUTRACEUTICALS AND THEIR LOCALIZATION INSIDE THE SEED BEFORE AND AFTER COOKING: AN OVERVIEW THROUGH MASS SPECTROMETRY IMAGING ANALYSIS.

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Rice is one of the most important cereals in human diet. Rice post-harvest processing, such as parboiling and polishing, could lead to changes in the chemical and physical composition by varying the quantities and distribution of its metabolites. The aim of this study is to apply MALDI Mass Spectrometry Imaging technique (MSI) to investigate localization and migration of rice constituents of various classes, after industrial processing and/or domestic cooking. Target analytes in this study are two classes of micronutrients, naturally present in the rice bran, whose positive effect on human health are well known: steryl-ferulates and anthocyanins. At same time, we try to annotate and follow the distribution of other detectable small molecules present in rice seeds, like lipids, phospholipids, phenols, etc, using a high-resolution mass spectrometer.
Samples of rice were analyzed as uncooked, boiled and steamed. Seeds were frozen and embedded into 2% 2-carboxy-methylcellulose solution, then were cryo-sectioned at 8 µm thickness. In the case of uncooked rice samples, a tape-transfer protocol was needed to collect the sections. Dihydroxybenzoic acid (DHB) and 9-aminoacridine were used as MALDI matrices for positive ion and negative ion acquisition, respectively. Mass spectra were recorded in full scan mode at high resolution (100000 at 400 m/z) in the mass range 300-900 m/z in positive polarity and 360-1000 m/z in negative polarity.
Data obtained were entered into Metaspace platform and many molecules of the analyte classes were putatively identified. Interestingly, several classes of annotated compounds showed a peculiar distribution in distinct rice seed compartments, instead of a broad and uniform distribution among the whole seed area. Interestingly, steryl-ferulates and anthocyanins distribution showed a migration from the rice bran to the endosperm in parboiled rice varieties while they are localized in the bran in wild type rice varieties. This difference is maintained, and in some case enhanced, even after domestic cooking like boiling or steaming. This preliminary investigation of rice nutraceuticals in raw seed will continue with the aim of monitoring the analyte migration after other industrial processes and domestic cooking procedures investigating also additional rice varieties.

C14: ACRYLAMIDE CONTENT AND ANTIOXIDANT ACTIVITY AMONG 8 DIFFERENT FILTER COFFEE EXTRACTION METHODS

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Introduction

Coffee is one of the most consumed beverages in the world. During the roasting process, green coffee beans undergo various changes due to different thermal reactions, most of them in the context of Maillard reactions (e.g. Caramelization, Strecker degradation, Pyrolysis) that lead to the development of the desired physicochemical and organoleptic properties of roasted coffee beans and derived beverages, such as flavour, aroma and colour, but also to the formation of undesired compounds [1]. One of the undesired heat-induced contaminants is acrylamide (AA), a substance formed mainly by the condensation of an amino group of amino acids (principally asparagine) and the carbonyl group of reducing sugars (e.g., glucose and fructose) during the Maillard reactions triggered at temperatures above 120 °C [1]. It is known that, despite the presence of AA, coffee is also a rich source of biologically active compounds with significant antioxidant properties. Polyphenols and chlorogenic acids are the characteristic coffee compounds linked to an antioxidant activity whose contents are influenced by the roasting process and by the extraction method [2]. Several health benefits are attributed to these compounds and their role in the prevention of chronic diseases such as cancer and cardiovascular pathologies has been the subject of many scientific research [3]. Therefore, coffee brewing is regarded as a solid-liquid extraction, from an engineering perspective, that takes place between hot water and ground coffee beans when the water passes through a bed of coffee ground [4]. Among the different brewing methods used for specialty and filter coffee applications, recently, Turkish Ibrik (boiling method), French Press (steeping or immersion method), V60, Chemex, Clever (filtration or drip method), AeroPress and Moka (pressured method) have been proposed for mostly [5]. To give a comprehensive overview of the filter coffee world, the new Pure Brew (Victoria Arduino) [5] was compared with these seven extraction methods. Therefore, the present work aimed to develop a comprehensive study and investigate the differences between the newly filter coffee extraction method, Pure Brew, with traditional ones (Turkish Ibrik, French Press, V60, Chemex, Clever, AeroPress and Moka), in terms of coffee extraction yield, AA content and antioxidant activity. To the best of our knowledge, this is the first paper which reports a chemical investigation of filter coffee produced by the novel Pure Brew technique. All analyses were carried out on three diverse coffees differently roasted (i.e., light, medium and dark); in this way, it has been realizable to explore at full the world of coffee, and for this reason, the selection was made among three different coffees with distinct roastings, origins, post-harvesting process and quality lots. This is to assess how the heat treatment can be correlated to the presence/formation of unhealthy compounds, such as AA and healthy compounds, among which are chlorogenic acids.

Materials and Methods

Three different coffees with varying degrees of roast were used for each of the eight extraction methods: Gardelli Specialty's natural, non-classic anaerobic Ethiopia Uraga for a *light roast*, Gardelli Specialty's washed Kenya Thiriku for a *medium roast*, and roasted Blond 100% Starbucks Arabica for a *dark roast*. A specific routine was used for each of the eight preparation methods, keeping some parameters as constant as possible, but without distorting the beverage recipes. For the extraction of AA, purification of samples and its quantitation a previous developed and validated HPLC-MS/MS procedure was used [1] with some adaptations. While analysis of chlorogenic and phenolic acids (antioxidants) and caffeine was performed

according to a previously developed and validated method by HPLC-DAD [6]. Antioxidant activity was determined spectrophotometrically by the DPPH method [7], while TPC and TFC of the different extracts according to a method described by [7], with slight modifications. Statistical analysis was performed by MetaboAnalyst (version 4.0).

Results and Discussion

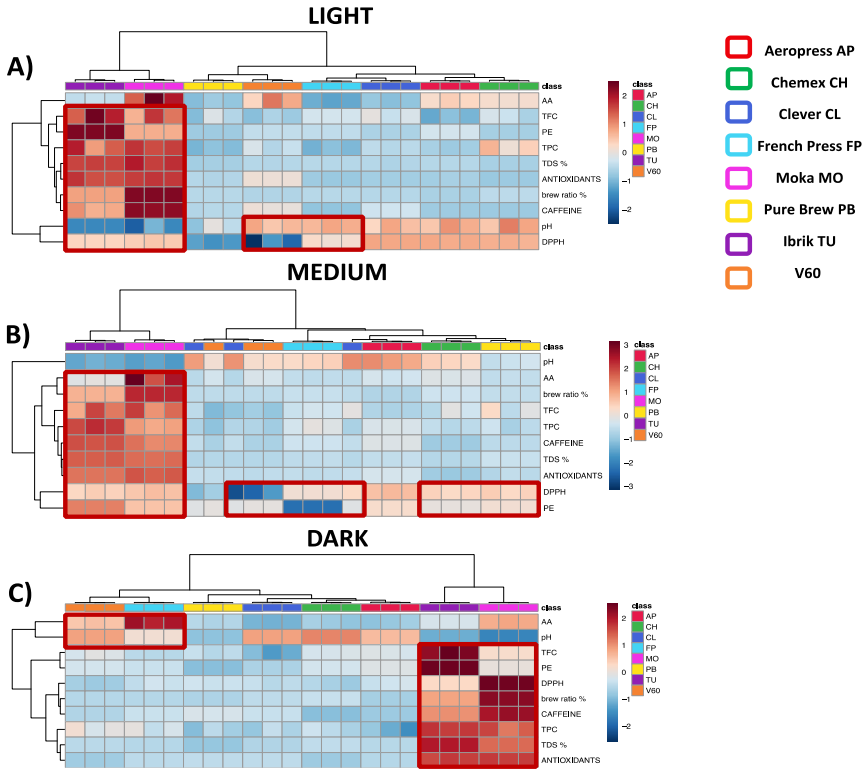

FIGURE 1 Hierarchical Heatmaps clustering comparing the different coffee extractions methods in terms of the significant process terms according to P-values: terms with a P- value 1.5. Colors from red to blue indicate p-values from high to low; and light cells indicate the lack of significant enrichment. **A)** Light roast **B)** Medium roast **C)** Dark roast.

Conclusions

The results confirmed that the AA levels and antioxidant activity reached maximum when the coffee to water's ratio used for the same filter coffee extraction method is higher, consequently also the TDS, and then decreased when this ratio for the same brew is minimum. Moreover, a strong correlation was found between the content of antioxidant compounds such as chlorogenic acids and polyphenols, and the concentration of dissolved solids in the beverage. In conclusion, both healthy and unhealthy compounds can be influenced by the same parameters during the brewing processes, which are coffee mass to water ratio, particle size distribution, brewing time, water temperature, and agitation.

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C15: INTEGRATING STRUCTURE- AND LIGAND-BASED APPROACHES FOR VIRTUAL SCREENING OF NATURAL PRODUCTS.

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Introduction

Computer-Aided Drug Design (CADD) provides a rapid and cost-effective way to develop candidate drugs for the treatment of many diseases types[1]. Structure-based drug design (SBDD) and ligand-based drug design (LBDD) are two possible approaches that use knowledge of the three-dimensional structure of the target protein, and knowledge of the structural or chemical features of known ligands or active compounds, respectively. In many cases, a combination of both approaches may be used to maximise the chances of identifying new active compounds. Furthermore, Natural Products (NPs) databases[2] are an essential resource to conduct investigations into the potential health benefits of natural substances and to develop new natural product-based therapies.
In this study, we undertake our investigation by exploiting natural substances deriving from plants, animals, fungi, and microbes, which are accessible through the Collection of Open Natural Products (COCONUT) database[3]. In detail, we report an application of both docking- and shape-based virtual screening (VS) methods, to identify potential natural candidates able to target the human glutaminyl cyclase (hQC)[4]. Due to its potential role in the formation of pGlu-modified amyloid peptides, hQC is regarded as a promising drug target for Alzheimer's disease (AD).

Materials and Methods

All computational studies were carried out by means of Schrödinger Suite 2018-1[5]. The X-ray crystallographic structure of the hQC in complex with inhibitor SEN177 (PDB code: 6GBX) was used[4]. The receptor structure was prepared with Maestro's Protein Preparation Wizard tool, using the OLPS_2005 force field. Crystallographic buffer components were removed, missing side chains were constructed using the Prime module, hydrogen atoms were added, and the protonation states of the side chains were assigned properly processed by using the LigPrep tool, hydrogens were added, salts were removed, and ionization states were calculated at pH 7.4. Starting from the online COCONUT database, over 500,000 molecular structures of NPs were properly processed by using the LigPrep tool, hydrogens were added, salts were removed, and ionization states were calculated at pH 7.4.
Employing the Phase method, based on the principle of rapid initial alignments using atom triplets followed by refinement and volume overlap scoring, a shape-based screening approach of the natural compound database was performed by using the bioactive conformation of two hQC inhibitors, PBD-150 and SEN177 (PDB code: 4YWY and 6GBX)[4,6]. For each molecule in the database, up to 100 conformers were generated, and the shape of each conformer was compared with that of the two selected query structures. NPs with a shape similarity score over 0.7 for both queries were retained for subsequent docking calculations. All docking and scoring calculations were performed using Extra Precision Glide (XP) algorithm, with default parameters, generating 10 poses for each ligand.

Results and Discussion

In order to identify natural compounds able to inhibit the hQC activity, we screened more than 500,000 small molecules, from COCONUT database, taking into account the PBD150 and SEN177 inhibitors, two previously designed molecules (Figure 1).

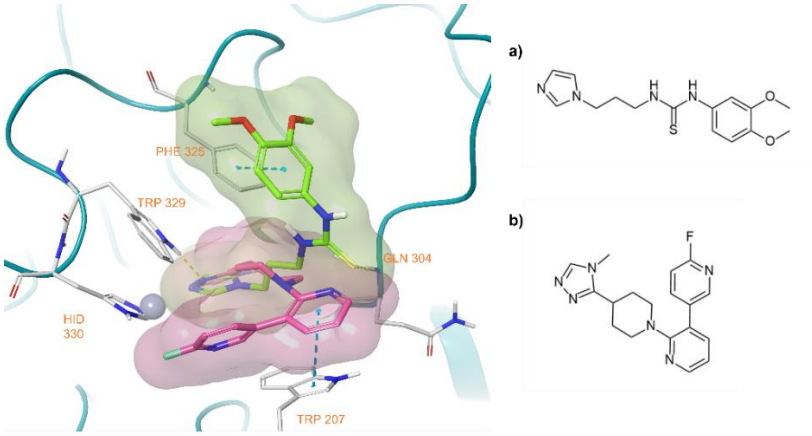

Figure 1. 3D representation of the PBD150 (green sticks) and SEN177 (pink sticks) inhibitors binding to the active site of hQC enzyme. 2D structure of **a)** PBD150 and **b)** SEN177 inhibitors.

From the ligand-based approach, we retained only the shared molecules with a shape similarity score higher than 0.7 with respect to the bioactive conformation of the two selected queries. Subsequently, docking virtual screening was performed to elucidate the binding mode and the interactions between the natural compounds and the hQC binding site. Our molecular recognition results were ranked and analysed based on the Glide score (G-score) value and all the compounds with a G-score value within 1 kcal/mol from the best *hit* were considered. The top-ranking compounds selected by docking were further filtered, by analysing their drug-like properties. Finally, by merging these results, we identified the best 20 shared NPs further tested in terms of their hQC inhibitory activity.

Conclusions

In this study, we combined structure-based and ligand-based drug design to screen a large database of NPs to identify novel hQC inhibitors. The implemented computational workflow allows us to select 20 promising natural candidates for further experimental testing.

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POSTERS

P1: SET UP AND VALIDATION OF IN VITRO APPROACHES TO INVESTIGATE PROTEIN-BASED FOOD WASTE SECURITY

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Abstract

The use of food-derived protein hydrolysates for the development of functional bioactive compounds is becoming an attractive practice with health-promoting effects, requested on the market [1]. Due to the high interest in these compounds, it is important to consider their toxicological implication and safety. It is clear that food security, food safety, and nutritional effects are key aspects of food systems with important implications for population health [2]. In this contest, the present study regards the development and validation of a static *in vitro* simulated digestion approach useful for the evaluation of bioaccessibility, absorption, and toxicity of protein hydrolysates from food waste. INFOGEST 2.0 [3], an *in vitro* static approach, will be used by setting up and adapting it to protein hydrolysates for future food matrices. A standardization of the method is also necessary considering the manner and timing of collection of digested samples [4]. This approach consists of three consecutive digestive phases (oral, gastric, and intestinal) within the incorporation of enzymes (e.g., oral α-amylase, gastric pepsin, and intestinal pancreatin) and bile salts with fixed physical-chemical parameters. In parallel, an in vitro cell-based dynamic model will be conducted on the same protein hydrolysate to investigate the possible correlation with in vivo digestion process [5]. Studies regarding the stability and the toxicity will be also performed to complete the food safety and quality profiles of protein matrices.

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P2: MICROENCAPSULATION OF *THYMUS VULGARIS* EXTRACT BY SPRAY DRYING TO OBTAIN A SUITABLE FOOD AND PHARMACEUTICAL ANTIOXIDANT INGREDIENT

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Introduction

Numerous additives are used in the food industry for several needs, such as to preserve the freshness of food (antioxidants), to inhibit the growth of microorganisms (preservative agents), to obtain an appealing colour or to restore the original colour of the product. Nitrites and nitrates are used as preservative agents, especially for meat-based food, not only for their antibacterial activity but also to maintain the pink colour and a smoked taste. Unfortunately, high levels of these compounds can represent a risk for human health and for the environment, due to the formation of N-nitroso derivatives after the combination with amines. Therefore, substitutes of nitrites and nitrates, possibly from natural sources, are under evaluation by many researchers [1].

Thymus vulgaris is an aromatic and medicinal plant belonging to *Lamiaceae* family, widely known for its therapeutic uses in conventional and traditional medicine. Its essential oil and polyphenolic components have important antioxidant, antibacterial, antifungal and antispasmodic activities [2], and beneficial effects on neurodegenerative, cardiovascular, respiratory tract problems, cancer, inflammatory diseases and others [2-4].

The aim of this work was to evaluate the antioxidant activity of a hydroalcoholic extract of *Thymus vulgaris* and preserve its activity through microencapsulation via spray drying technique, in order to obtain a solid product, in particular a powder, with suitable characteristics for food or pharmaceutical applications.

Materials and Methods

Preparation of the hydroalcoholic thymus extract

Hydroalcoholic thyme extract was obtained by maceration of dry leaves in hydroalcoholic solution (H_2O/EtOH 50:50) with a 1:10 ratio (grams of triturated leaves/mL of hydroalcoholic solution) under magnetic stirring for 2 hours. Then, after a filtration step, the hydroalcoholic extract was collected.

Since the final product is intended to be incorporated into a food product, the filtered liquid was concentrated by Rotavapor® at 40 °C to remove the ethanolic fraction and part of the water.

Production of the microparticles

Spray drying technology was used to obtain solid microparticles starting from the concentrated liquid extract and selecting maltodextrin as carrier excipient. The process conditions were set as followed: inlet temperature = 145 °C, pump = 10-12%, feed rate = 5-6 g/min, aspiration = 100%, pressure nozzle = 45-50 mm, tip = 0.7 mm and cap = 1.4 mm.

Two different starting formulations, with different extract/maltodextrin ratio (F1 = 70/30 and F2 = 85/15), were processed by spray drying obtaining two different powders.

Characterizations

Particle size distribution (PSD) of the powders was evaluated by laser diffraction technology, their flowability properties were determined by the measurement of the angle of repose by a flowability tester, spray drying process yield was calculated. Residual moisture of both liquid extract and final powders and their thermal behaviour were determined by thermogravimetric analysis (TGA), antioxidant activity by DPPH and ABTS and total polyphenols content (TPC) by Folin-Ciocalteu test.

Results and Discussion

Both the liquid formulations submitted to the spray drying process were successfully transformed into dried powder and the high process yields (over 70%) confirmed the good processability of the slurry by the selected technology. The two batches of powder produced were composed of green in colour microparticles characterized by a very small diameter (D90 values < 11 µm). All the characterization data are shown in Table 1. Particle size distribution revealed a non-significant difference between the two batches of powders, indicating the reproducibility of the process regardless of the starting formulation composition. According to European Pharmacopoeia [5], the angle of repose values indicated a discrete flowability of both powders. Residual moisture values of the powders were consistent with data available in the literature concerning spray drying technique. DPPH and ABTS test results confirmed a good preservation of the antioxidant activity of *Thymus vulgaris* extract after microencapsulation. Folin-Ciocalteu assay demonstrated a noticeable maintenance of the polyphenolic content in the powders produced, indicating the appropriateness and the non-invasiveness of the process.

Table 1. Characterizations.

	Liquid extract	***Powders***	
		70:30	*85:15*
Residual moisture (%)	80.67 ± 3.55	5.38 ± 0.80	7.80 ± 1.17
DPPH (mg TE/g extract)	494.83 ± 25.51	438.37 ± 30.03	485.12 ± 2.67
ABTS (mg TE/g extract)	750.68 ± 55.08	374.28 ± 12.10	485.07 ± 2.10
TPC (mg CE/mg extract)	342.54 ± 4.45	222.45 ± 13.25	323.15 ± 11.80
Process yield (%)	--	70.61	77.32
PSD (D90)	--	10.11	10.26
Angle of repose (°)	--	36	37

Conclusions

Thymus vulgaris hydroalcoholic extract was successfully converted into a powder and spray drying technique turned out to be a good strategy to obtain a microencapsulated product. Antioxidant activity and total polyphenolic content were acceptably preserved after microencapsulation process. This powder may be a starting point to develop an attractive natural and food-grade alternative to nitrites and nitrates salts in food industry, even though other studies, such as microbiological tests, are required.

Acknowledgments

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P3: COMPARISON BETWEEN COMMERCIAL AND NON-COMMERCIAL APRICOT (*PRUNUS ARMENIACA*) AND PEACH (*PRUNUS PERSICA*) COLD-PRESSED SEED OILS: A PRELIMINARY STUDY.

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Introduction

The valorisation of by-products and wastes from agri-food chains, as well as the recovery of bioactive compounds from these matrices is a global trend, supporting the circular economy. In 2019, the European Commission announced that the EU Green Deal will transform the EU into a modern, resource-efficient and competitive economy [1]. Plant edible oils are often an important source of food for humans, because they provide essential fatty acids and minor bioactive compounds (e.g., tocopherols). Moreover, they could be used in cosmetic and pharmaceutical applications also due to their anti-microbial, anti-septic, anti-oxidant properties [2], as well as considered "gourmet" oils. The cold pressing process (depending on the processing parameters andthe temperature reached during the production) usually preserve the nutritional quality of the oils,representing an elective method for lipid extraction from food matrices. Apricot (AO) and peach (PO) cold pressed seed oils are remarkable principally because of their composition and their volatile flavouring fraction, but also relating to their potential functional properties, despite their high costand the difficulty to prepare the kernels for the seeds extraction [3]. The aim of this work has been the characterization of some samples of AO and PO, comparing cold pressed oils appositely produced for the study with some commercial samples. This preliminary study reports i) their fattyacids composition, ii) their volatile organic compounds (VOCs) fingerprint obtained by GC-IMS (GasChromatography - Ion Mobility Spectrometry, iii) the presence of cyanogenic glycosides and iv) thethermal impact evaluated by TGA (Thermo-Gravimetric Analysis).

Materials and Methods

Non-commercial peach (NPO) and apricot oil (NAO) samples were obtained by a cold-pressing process and kindly supplied by Fratelli Ruata S.p.A (NUTRAcore Project). Both commercial and non-commercial oil samples were kept in the refrigerator after the production, until their use in the experiments. All the chemicals and solvent used in this study were of analytical grade.

Analysis of fatty acid (FA) composition: the fatty acids in the lipid fraction were trans-esterified intothe corresponding methyl esters (FAMEs), then analyzed by GC-FID. Gas chromatography (GC Thermo TRACE 1300) with split/splitless injector. DB23 J&W Scientific column (30 m x 0.25 mm ID and film thickness 0.25 mm); gas carrier: H_2 (flow: 1.5 ml/min; split ratio 50:1); temperatures: 250
°C as T° max for injector; 350 °C as T° max, using a ramp of 5°C/min; Fatty Acid Methyl Ester mix, provided by Supelco was used as standard. All analyses were performed in triplicate and the resultswere expressed as average ± standard deviation.

VOCs profiling: a HS-SPME-GC-MS (Agilent, Palo Alto, CA) analysis has been made in order to havea fingerprint of volatile compounds both non-commercial and commercial AO and PO. For NPO andNAO samples a preliminary analysis with GC-IMS (FlavourSpec®, G.A.S., Dortmund, Germany) has been done too using an MXT-5 column (15 m × 0.53 mm i.d., 1 µm film thickness; Restek Corporation, Bellefonte, PA, USA), the volatile chemicals were separated at 40 °C.

TGA analysis: these measures were performed using TGA 4000 instrument (Perkin Elmer, Milan Italy), temperature range 30-600 °C, 10 °C/min, nitrogen purge gas.
Cyanogenic glycosides presence: the analysis of these components was performed using a UHPLC- HRMS Thermo QExative (Thermo Fisher Scientific, Waltham, USA); BEH chromatographic column C18 (100x2,1 mm, 1,7 µm 130 Å (Waters, Milford, USA).

Results and Discussion

The FA profile is decisive for the quality of the oil. We identified 14 and 12 fatty acids in NPO and NAO, respectively, of which oleic, linoleic, palmitic and stearic acids were found to be the most relevant (Tab.1).

Tab. 1 More abundant FA in peach kernel oil and apricot seed oil (relative percentage; triplicate; average ± standard deviation).

	NPO	**NAO**
C18:1n-9cis	70.60 ± 0.08	66.01 ± 0.05
C18:2n-6cis	21.41 ± 0.01	26.32 ± 0.03
C16:0	5.32 ± 0.09	5.15 ± 0.09
C18:0	1.45 ± 0.01	1.18 ± 0.01

The parallel extraction (Soxhlet apparatus) of lipid fraction from raw seeds used to produce oils, permitted to confirm the same trend for fatty acids composition (no significant differences were found, when compared to those obtained by cold pressed oils). Moreover, the TDS of commercial samples confirmed a similar fatty acid composition.
In addition, the 2D fingerprints of VOCs obtained by GC-IMS permitted to compare the profile of NPO and NAO, identifying some key flavoring compounds. Peach oil showed a more elaborated profile than apricot; this outcome has been supported by an analysis performed by HS-SPME-GC- MS. This last analysis has been done for commercial samples too, confirming the presence of acrolein, depending on a probable thermal degradative process, when compared to NPO and NAO samples. This difference has been also confirmed by TGA analysis, which highlighted the low stabilityof the commercial samples. Finally, traces of cyanogenic glycosides were found in both NPO and NAO, whereas the commercial oils do not have these compounds, probably as a result of refining processes.

Conclusions

The fatty acids profile was similar for the tested oil samples, confirming the few data reported in literature [4]. Oleic acid showed the highest concentration in both samples, followed by linoleic acid.The presence of HCN far below the European limits [5] and high thermal stability denote how theseoils produced by cold pressing starting from a high value raw material, can be used in formulations for nutraceutical or cosmeceutical purposes. The perspective of this study (on-going research) is theproduction of food micro-emulsions and micellar dispersions using these oils, also considering the addiction of lipophilic vitamins, so creating new functional ingredients.

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P4: UNTARGETED METABOLIC PROFILING OF THREE DIFFERENT CULTIVARS OF BURDOCK (*ARCTIUM LAPPA L.*) ROOTS BASED ON HIGH-RESOLUTION MASS SPECTROMETRY TECHNIQUES.

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Introduction

Metabolomics involves the identification of metabolites produced by intracellular biochemical processes, which constitute the so-called metabolome. In the framework of "omic sciences", metabolomics allows to decipher the correlation between genes and phenotype and understand organisms' development and their response to environmental stresses. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) proved to be a powerful analytical technique for the characterization of complex matrices, due to its very high resolving power, sensitivity, and mass accuracy, which allow for the simultaneous detection of thousands of compounds often present in very small quantities [1]. The untargeted approach, performed in direct infusion of the samples, aims to identify all the major classes of characteristic metabolites.
Within the different biological systems, plants contain the highest assortment of metabolites, many of which are nutraceutical compounds noteworthy in pharmaceutical, cosmetic and food fields. In this study, an untargeted metabolomic analysis was performed to characterize the phytochemical composition of three different cultivars of Burdock (*Arctium lappa L.*) roots, investigated by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS), both in positive and negative ionization modes. Burdock is a plant known as nutraceutical vegetable, traditionally used in Chinese medicine and cuisine in form of infusions, decoctions and tinctures for its excellent nutritional values and bioactive properties. In fact, the extracts obtained from roots fulfil various biological activities in humans, including antioxidant, anti-inflammatory, antimicrobial, antiviral, hepatoprotective, anticancer, gastroprotective, hypoglycaemic actions [2]. Most of these health benefits are due to the high content of secondary metabolites, including phenolic compounds such as flavonoids and lignans [3].

Materials and Methods

Plant material consisted in pulverized roots of three different cultivars of Burdock, named BR1, BR2 e BR3. For the extraction procedure, the Bligh-Dyer protocol was applied, so as to extract both hydrophilic and hydrophobic metabolites. The analysed solutions were prepared by: i) dissolving 1 mg of each dried extract in 1 mL of a H_2O/CH_3OH (1:1) mixture; ii) filtering to remove debris impurities; iii) diluting the filtered solutions in methanol, to a final concentration of 0.1 mg L^{-1}. Each diluted solution was directly infused in the electrospray ionization source coupled to a FT-ICR mass spectrometer (BioApex 4.7 T FT-ICR MS, Bruker Daltonics) for untargeted analyses and investigated in both positive (ESI+) and negative (ESI-) ionization modes. Full mass spectra were recorded in the m/z 100-1000 range and processed with software DataAnalysis 3.4 (Bruker Daltonics), and the lists of accurate m/z values obtained were submitted to the free tool MassTrix (https://metabolomics.helmholtz-muenchen.de/masstrix3/) for metabolite assignment. The tentative annotations were obtained by comparing the experimental high-resolution m/z values with the theoretical exact masses. Supplementary experiments were accomplished using a APCI source coupled with a linear ion trap analyzer (LTQ XL, Thermo Fisher Scientific).

Results and Discussion

Direct-infusion ESI FT-ICR MS analysis has provided an untargeted metabolite profile of hydroalcoholic and organic extracts of BR1, BR2 and BR3. High-resolution mass analyses revealed up to 200 distinct molecular formulas for each extract, except for BR3 which shows a relatively lower compound density. Overall, a larger

number of hits were detected in positive ionization mode and the highest number of metabolites was observed in the BR1 hydroalcoholic and BR2 organic extracts. The molecular formulas gained from each sample were transposed to two-dimensional van Krevelen diagrams, composed by plotting the molar hydrogen-to-carbon ratio (H/C) as a function of the molar oxygen-to-carbon ratio (O/C) for each data point, corresponding to a unique molecular formula (Figure 1). This graphical representation provides a visualization of the main classes of detected metabolites, thus furnishing a qualitative description of the phytochemical composition of the samples. The diagrams of the three cultivars show strong similarities, with a higher compound density in the area of lipids and polyketides, followed by polyalcohols and amino acids, whereas metabolites belonging to the carbohydrate and nucleic acid classes are less represented. BR2 extract shows a slightly higher metabolite density than BR1 and BR3, mostly concentrated in the lipids and polyketides regions.

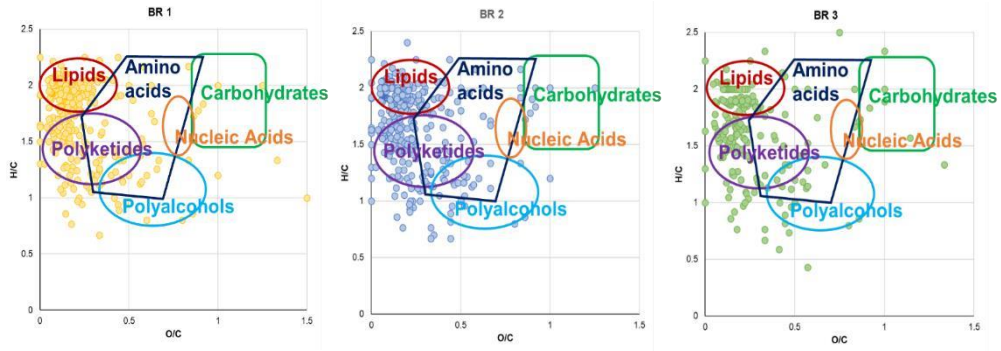

Figure 1. van Krevelen diagrams obtained from the molecular formulas detected by ESI FT-ICR MS analyses of hydroalcoholic and organic extracts of BR1, BR2 and BR3 samples.

ESI FT-ICR MS analyses of Burdock roots revealed the presence of some amino acids, including arginine and succinyl proline, while free fatty acids, counting many SFA, MUFA and PUFA, were detected in all samples. Numerous organics acids, especially phenolic acids, such as quinic, caffeic, and chlorogenic acids, were found in almost all samples; other metabolites include carbohydrates, vitamins, monoterpenes, and some plants hormones. Burdock roots showed a great variety of secondary metabolites, including lignans, such as arctignan, lappaol A and lappaol B, and many flavonoids, mainly detected in O-glycosylated form.

Conclusions

Thanks to the high sensitivity and accuracy of the method applied here, this metabolomics study allowed the identification of all the major classes of compounds present in the extracts, providing a rich phytochemical fingerprinting of the samples.

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P5: IMPACT OF CARBOHYDRASES ON DIETARY FIBER AND POLYPHENOLS OF COCOA BEAN SHELLS

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Introduction
Cocoa beans are the seeds contained in the fruit (cocoa pod) of the cocoa tree ; this product is massively consumed worldwide by producing chocolate and cocoa-associated food and beverage with fine flavour [1]. During the massive production of these delicious ingredients a lot of by-products and wastes are produced, of which cocoa bean shells (CBS) are generated during the roasting phase, when they are de-hulled from the rest of the bean, and discarded [2]. On average, 700 thousand tonnes are produced every year [3]. Despite their sad destiny this valuable by-product is rich in dietary fiber and antioxidant compounds [4]. CBS are characterized by a very high fiber content, ranging from 18.3 to 59% (dry matter) and show a high nutritional value, thanks to their phenolic composition and their residual lipid profile [5]. The high content of these bioactive compounds has led the attention over the CBS functionalization and exploitation as ingredient for their prebiotic and antioxidant activity [4].

Materials and Methods
Hydrolysate generation
CBS were kindly supplied by an Italian chocolate manufacturer; first, they were submitted to lipid removal by Soxhlet extraction with dichloromethane. Both raw and defatted CBS were enzymatically hydrolysed by different commercial enzymes (cellulase, xylanase and pectinase) in different combinations. The enzymatic treatment was performed as follow: i) preparation of each enzymatic solution was carried in phosphate buffer (pH adjusted according to the activity of each enzyme) with a final adequate enzymatic activity; ii) CBS, in a ratio of 10 % (w/v) were mixed with the enzymatic solutions in Erlenmeyer flasks and incubated at 58 C° for 2h under continuous stirring; iii) hydrolysis was finally interrupted by placing the flasks on ice and transferring the content in plastic tube, followed by freeze-drying. All treatments were carried out in duplicate.

Chemical Characterization
CBS treated were then submitted to different chemical characterization. Dietary Fiber content was analysed by quantifying the soluble (SDF) and insoluble dietary fiber portions following the Lee protocol AOAC (991.43).
Spectrophotometric assays were performed to assess i) the Total Phenolic Content (TPC) by the Folin-Ciocalteu assay, ii) the Total Flavonoid Content (TFC) by the Aluminium Chloride assay, and iii) the Antioxidant Activity (AA) by DPPH assay.
RP-HPLC-DAD was used to quantify methylxanthines and flavonoids, as described in Papillo et al., (2019) with modifications [4].

Results and Discussion
The obtained outcomes confirmed a significant impact of the enzymatic treatments on the different chemical profiles of treated CBS, specifically regarding the SDF portion, significantly increased by the enzymatic treatments utilizing cellulase, xylanase and their combination.
The most performing enzymatic treatment resulted to be the cellulase and xylanase treatment on raw CBS, with more than 100% increase in SDF content, when compared to CBS untreated. The combination of cellulase and xylanase did not show a significant quantitative improvement of the SDF, when compared to

the single enzyme treatment. Moreover, the various treatment demonstrated to be less effective on defatted CBS, suggesting that it could be an unnecessary step.
More studies are needed to further evaluate in deep the chemical composition of the SDF fraction, as well as the oligosaccharides profile. Moreover, prebiotic activity should be evaluated with the most advanced protocol in simulated digestion and simulated intestinal microbiota fermentation to further correlate with positive health outcomes.

Conclusions

Enzymatic treatments resulted positively correlated with desired characteristics into the final product, opening a new scenario for the valorisation of this cocoa by-product.

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P6: CHARACTERIZATION OF Se-ENRICHED BLUEBERRY (*Vaccinium corymbosum* L.) AS FORTIFIED FOOD

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Introduction

Blueberries (*Vaccinium corymbosum* L.) are a high-valued plant-based food and good source of bioactive and phytochemicals, such as polyphenols and vitamins, able to contribute to a healthy diet. In the last years the sector of berry fruits is become of great interest for the Piedmont Agro-food system, in relation to the growing market demand **[1]**. Selenium (Se) is an important micronutrient involved in different metabolic pathways such as antioxydant and immune defence **[2]**. This element is an important enzyme cofactor for glutathione peroxidase, and its organic forms are represented by selenocysteine and selenomethionine in animals. Plants represent the main dietary source of this element, but Se content depend by the presence of this element in soils **[2]**. Selenium enrichment can modify the chemical composition of the plants, in particular, the production of Se-enriched crops at a safe level may improve the phytochemicals content (for example, phenolics in tomato plants and glucosinolates in *Brassica* spp.) and increase the antioxydant capacity. Thus, increasing Se content in food crops offers an effective approach to improving human nutrition **[2,3]**. Blueberries could be good candidates for being enriched with Se to obtain a "*fortified food*" that can offer the correct intake of Se in human diet and increase the antioxydant capacity.

Materials and Methods

Two samples of "*Last Call*" blueberry cultivar (control and Se-enriched) were given by Azienda Golden Blu SS Agricola (Tarantasca, CN). The Se-enrichment was done using foliar spray application.
Moisture, solid soluble content, pH, titratable acidity and sugar:acid ratio were performed on homogenized samples. Morphological changes were evaluated on berries diameter measure. Phenolic compounds were extracted with 50:50 (v/v) ethanol:water solution and determined with spectrophotometric assays. Total polyphenols content (TPC) was determined applying a modified *Folin-Ciocalteu* assay **[4]** and the total anthocyanin content (TAC) were evaluated according to the pH-differential method **[5]**. A modified BL-DMAC method **[6]** was used for the quantification of the proanthocyanidins content (PAC) and $AlCl_3$ method has been applied to evaluate the total flavonoid content (TFC). The antiradical and antioxydant capacity were performed using 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$) **[7]** and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) assays **[8]**. Flavonoid and phenolic acids profile were characterized and quantified with HPLC-DAD method using a reversed-phase Luna C18(2), 100 Å LC Column (150 × 2.0 mm i.d., particle size of 5 µm); eluent A was H_2O/formic acid 0.1 % v/v and Eluent B ACN/formic acid 0.1 % v/v. All data are expressed as average ± standard deviation. Statistical analyses were conducted with software "R" (ver. 64.4.1).

Results and Discussion

The results of the blueberries composition are showed in **Table 1**. It is confirmed that selenium enrichment led to a decrease in the average diameter of blueberries compared to the untreated samples from the same cultivar (from 18.21 mm to 14.00 mm).

Table 1 *Compositional data of blueberry samples. Different letters in the same columns indicate samples significantly different ($p < 0.05$).*

Sample	Diameter (mm)	Moisture	pH	TA	SS	SS/TA
Control	18.21 ± 1.08^{a}	84.22 ± 0.30^{a}	2.96 ± 0.01^{b}	1.569 ± 0.047^{b}	12.0 ± 0.0^{b}	7.65 ± 0.23^{b}
Se-enriched	14.00 ± 0.98^{b}	81.39 ± 0.11^{b}	3.04 ± 0.01^{a}	1.668 ± 0.028^{a}	14.6 ± 0.0^{a}	8.76 ± 0.15^{a}

TA = titratable acidity (% citric acid); SS = solid soluble content (°Brix); SS/TA = sugar:acid ratio.

In general, the Se-enriched sample does not show discordant composition values with the control sample, even if the values are significantly different for each parameter. Nevertheless, there is an increase in the soluble sugars content in the enriched sample (from 12.0 to 14.6 °Brix).
Phenolics groups, expressed in mg/g dry matter (d.m.)., and radical scavenging activity are shown in **Table 2**. The statistical analysis confirmed that there are significant differences between control and Se-enriched, in TAC, PAC, $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assays. Instead, the TPC content doesn't show significant differences. There is a decrease in the anthocyanin content in the sample enriched in selenium compared to the untreated sample. Nevertheless, TFC and PAC increase significantly in the enriched sample: TFC turning from 0.89 mg CAE/g d. to 1.38 mg CAE/g d.m., and PAC turning from 0.470 mg PAC/g d.m. to 0.600 mg PAC/g d.m. The antiradical activity of the Se-enriched sample increases more significantly if evaluated in $ABTS^{\cdot+}$ assay, than $DPPH^{\cdot}$ assay.
In **Figure 1** are reported the principal flavonoids and phenolic acids detected with the HPLC-DAD method. Chlorogenic acid, quercitrin, rutin and hyperoside content are increased in Se-enriched sample while the myricetin content is decreased.

Table 2 *Polyphenols composition of blueberry samples. Different letters in the same columns indicate samples significantly different ($p < 0.05$). TPC = mg CAE/g d.m.; TAC = mg C3GE/g d.m.; TFC = mg CAE/g d.m.; PAC = mg PAC/g d.m.; $ABTS^{\cdot+}$ = mg TE/g d.m.; $DPPH^{\cdot}$ = mg TE/g d.m.; CAE = catechin equivalents; C3GE = cyanidin 3-O-glucoside equivalents; TE = Trolox equivalents.*

Assay	*Control*	*Se-enriched*
TPC	14.1 ± 0.32[a]	14.4 ± 0.37[a]
TAC	5.96 ± 0.04[b]	5.53 ± 0.21[a]
TFC	0.89 ± 0.016[b]	1.38 ± 0.061[a]
PAC	0.470 ± 0.009[b]	0.600 ± 0.018[a]
$ABTS^{\cdot+}$	8.90 ± 0.23[b]	15.03 ± 0.44[a]
$DPPH^{\cdot}$	9.47 ± 0.19[a]	9.96 ± 0.24[a]

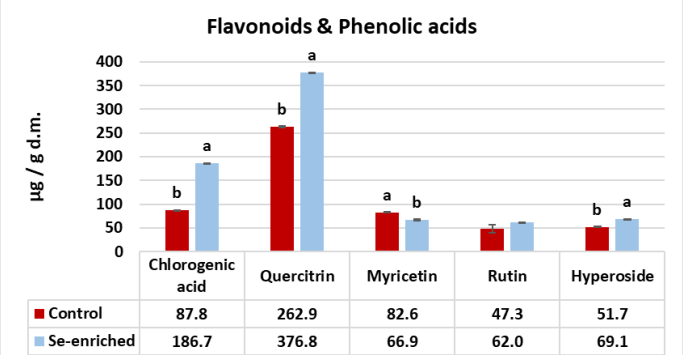

Figure 1 *Differences in the principal flavonoids and phenolic acids content detected in "Last Call" cultivar with HPLC-DAD method. Different letters indicate samples significantly different ($p < 0.05$). The content are expressed as µg / g d.m.*

Conclusions

The parameters of base composition are slightly different after selenium enrichment, nevertheless, sugar content increase in the enriched one and differences in the morphologic parameters were noted. Among phenolics compounds, flavonoids and proanthocyanidins seem to be the classes mostly affected by the Se-enrichment in "*Last Call*" blueberry cultivar and also the antiradical capacity increased in the enriched sample. The HPLC-DAD analysis confirms the increase of each flavonoid except for myricetin. The research is going on with a planned new season of Se-enrichment and the Se content will be evaluated to monitor the concentration in the berries in order to avoid toxicity problems.

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P7: BIODIVERSITY VALORIZATION: EVALUATION OF ANTI-GLYCATIVE ACTIVITY OF SEVERAL PLANT EXTRACTS

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Advanced glycation end products (AGEs), formed through the nonenzymatic reaction of reducing sugars with the side-chain amino groups of lysine or arginine of proteins, are implicated in various diseases, such as diabetes, cardiovascular, and neurodegenerative diseases. Glucose, certainly, is the sugar mainly involved in the formation of endogenous AGEs, followed by fructose, taken in from the diet, and dicarbonyl compounds such as MGO (methylglyoxal) and GO (glyoxal), produced during the glycation reaction and other different metabolic reactions[1]. The constant hyperglycemic condition in diabetic patients accelerates the formation of AGEs and the development of AGE-related complications, as nephropathy, retinopathy, atherosclerosis, and cardiovascular disorders [2]. The further development and discovery of novel food ingredient with anti-glycative activity represents a potential approach to reduce and prevent AGE-related disorders. The Italian flora is known for its large number of species and subspecies, which it is important to preserve and enhance as a source of goods and resources. Therefore, the anti-glycative activity of twelve plant extracts (*Salvia pratensis, Succisa pratensis, Althaea officinalis, Diospyros kaki, Sanguisorba officinalis, Verbaschum thapsus, Beta vulgaris, Castanea sativa, Rosa canina, Butomus umbellatus, Aloysia citrodora, Arthemisia abrotanum*) included in the Belfrit list has been evaluated. A preliminary screening to test their capacity to inhibit the glycation reaction has been carried out by in *vitro* assay. In particular, model systems consisting of glycating agent, such as MGO and a protein, such as bovine serum albumin, has been set up at physiological conditions (37 °C, pH 7.4) to evaluate the intermediate stages of glycation process. With the help of NBT (blue nitrotetrazolium chloride), the capacity of the extracts to inhibit the formation of Amadori products, the initial products of the glycation process, has been evaluated. In addition, the MGO and GO trapping ability of the extracts has been investigated[3]. Most of them show good activity at the intermediate stage and excellent trapping capacity. Therefore, the most promising extracts have been selected to deepen the anti-glycative properties. At first, the anti-glycative activity of *Diospyros kaki and Succisa pratensis* has been also evaluated in the presence of fructose by BSA-FRU assay [4]. In addition, OPA assay has been carried out to evaluate the ability of *Diospyros kaki* to protect the amine groups involved in the glycation reaction [5]. Anyway, the biological activity of the most promising extract will still be studied and other plant extracts with better biological activity will be selected for future studies.

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P8: DEVELOPMENT OF NEW ANALYTICAL METHODS FOR THE QUANTIFICATION OF CAFFEINE, ORGANIC AND CHLOROGENIC ACIDS FROM ESPRESSO COFFEE USING SPE AND HPLC-DAD

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Introduction

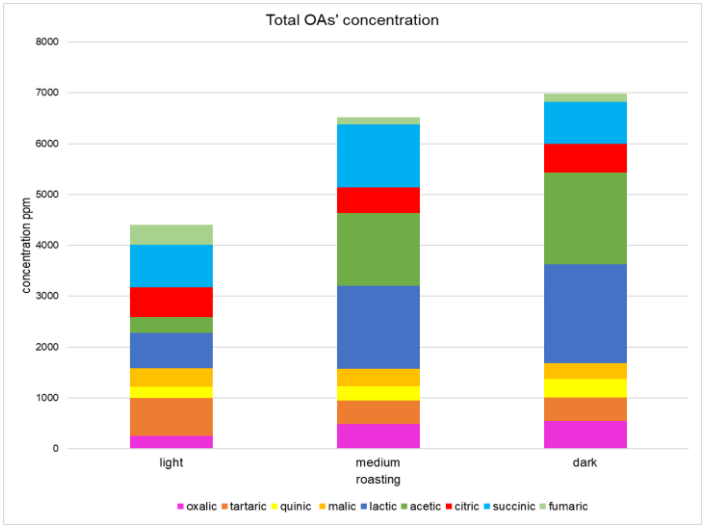
Coffee (*Coffea sp*.) was first cultivated between the fifth and eighth centuries. The coffee beverage consists of over 1000 volatile and non-volatile compounds that are responsible for the taste and aroma [1]. Of these, caffeine (1,3,7-trimethylxanthine) is the most studied compound, responsible for the stimulant effect on the central nervous system [2]. Among the compounds that have the greatest influence on the taste and aroma of coffee, carboxylic acids, specific organic acids and chlorogenic acids were found. There are nearly 38 different organic acids (OAs)[3]. OAs are important because their properties allow us to define the type of acidity perceived in the beverage [4]. Acids account for a large proportion of the total mass, up to 11% in green beans and 6% in roasted beans [3]. In literature, different concentration ranges of organic acids were found in the coffee matrix, analyzed by several sample clean-up procedures and many chromatographic conditions. The term chlorogenic acids (CGA) encompasses many types of quinic and caffeic acids esters (combining quinic and caffeic acids through an oxygen atom), and as such, the structures of CGAs can become quite complex and large. During roasting, CGAs convert to CGA lactones, which contribute to the bitterness of roasted coffee. For this reason, after developing the sample preparation and the ideal chromatographic conditions, it was decided to apply these two new and fast analytical methods to analyze the different compositions of the nine organic acids, four chlorogenic acids, two phenolic compounds and caffeine in three different espresso coffees (EC) made from the same coffee treated with three roasting methods (light, medium and dark) [5], with the main objective of determining how the different roasting can affect the coffee composition.

Materials and Methods

For the EC samples, were used three different roasting grades (light, medium and dark) of the same coffee variety 100% Arabica (Kenya Kaliluni) supplied by Perfero Coffee (Altidona, Italy). The EC samples were prepared using VA388 Black Eagle Maverick machine (Simonelli Group, Victoria Arduino). Before filtration and HPLC-DAD analysis, several methods were tried to purify the samples for OAs analysis, finally the anion exchange solid phase extraction were selected. Then, a new HPLC-DAD was validated for the analysis of OAs, for this was used an analytical column Luna Omega Polar C18 (250 mm × 4.6 mm, 3 µm) and elution was performed in isocratic mode using a potassium dihydrogen phosphate buffer at a pH of 2.5 at a flow rate of 0.8 mL/min. While the new method for analysis of chlorogenic and phenolic acids and caffeine used a Gemini C18 (250 mm × 3.0 mm, 5 µm) and elution was performed in gradient mode using water (A) and methanol (B), both with 0.1% formic acid as the mobile phase.

Results and Discussion

A)



B)

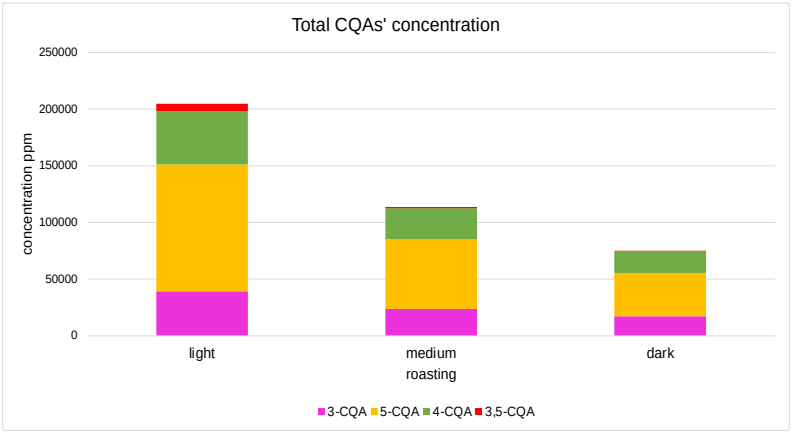


FIGURE 1 Histograms of the concentration of various carboxylic acids in espresso coffees samples, prepared with coffee from three different degrees of roasting (light, medium and dark) analyzed with HPLC-DAD. **A)** Organic acids **B)** Chlorogenic acids.

Conclusions

This research allowed further elucidation of the content of carboxylic acid in the samples of EC by developing innovative SPE purification procedures and HPLC methods. The efficiency of the developed analytical method and the affordability of the equipment used could simplify the determination of OAs, CGAs, phenolic acid, and caffeine in the complex matrix of coffee. With these methods, 9 OAs, 4 CGAs, 2 phenolic acids and caffeine were clearly and accurately detected in an Arabica sample EC. Finally, the caffeine content is relatively stable to heat. While the high concentration of chlorogenic acids decreases due to roasting, and the total organic acid content increases with medium and dark roasting due to the formation of acetic and lactic acids, but each acid shows a different trend based on the degradation or activation of formation processes.
One of the most important innovations of the study is the short purification procedure using anion exchange solid phase extraction followed by direct HPLC-DAD analysis for the OAs. Therefore, this method can be considered as a reference method for the determination of carboxylic acids in coffee.

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P9: QNMR APPLICATIONS FOR THE QUALITY CONTROL OF FOOD SUPPLEMENTS EMPLOYED IN HYPERLIPIDEMIC AND JOINT INFLAMMATION CONDITIONS

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Introduction

The Italian food supplement market was esteemed at 1.3 billion euros in 2008, and in 15 years was 3-fold increased. The increment was induced by the greater attention of people to well-being and easier attainability of food supplements in the market. In Italy, food supplements with benefic effects on the cardiovascular system (e.g., containing monacolin K, berberine, and resveratrol) or on joints and muscles (e.g., containing *Boswellia serrata* extracts, rich in boswellic acids) are among the top ten most used. Due to the fast rising of the market, the quality control of food supplements is gaining more and more relevance to ensure consumers' safety and reproducible active compound content and efficacy. Conformity to legislation is essential in the case of food supplements containing monacolin K, whose maximum levels of daily intake decreased from 10mg/die to 3mg/die. Besides, the quality control of food supplements containing boswellic acids is required since their content in the raw extracts is usually estimated through acid-base titration methods, which are affected by the presence of other organic acids. Thus, the present thesis project considered food supplements containing monacolin K, berberine, resveratrol, and boswellic acids. In the literature, these active compounds are mainly quantified through separative methods which display several drawbacks compared to novel advanced techniques, such as quantitative nuclear magnetic resonance spectroscopy (qNMR). The project aimed to develop, optimize, and validate the extractive method, and qNMR quantification through the ERETIC2 technique.

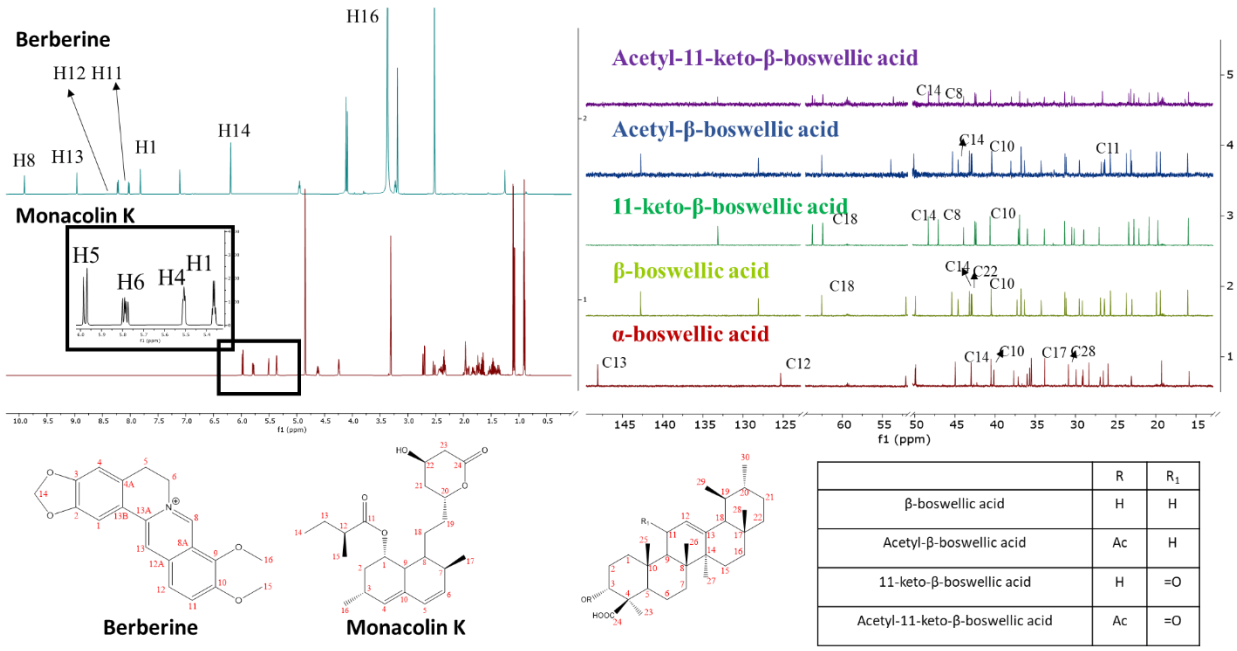
Materials and Methods

Food supplements containing monacolin K, berberine, and resveratrol were purchased from the market. Raw extracts of *B. serrata* employed for food supplement production were obtained from local companies. Qualitative analyses in NMR were carried out on a Bruker FT-NMR Avance III HD 600 MHz spectrometer equipped with a CryoProbe BBO H&F 5mm. 1D and 2D-NMR pulse sequences were performed to correctly identify and assign the resonances of the target compounds. Then, the NMR pulse sequence for quantifying each active compound was optimized on proton and carbon spectra, using pyridoxine as the external ERETIC standard. The quantification of monacolin K, berberine, and resveratrol was achieved on ^{1}H-NMR spectra. Conversely, the quantification of boswellic acids was carried out in ^{13}C-NMR spectra due to the complexity of the proton spectra of the extracts. Finally, the method was validated in terms of intra- and inter-day precision, and recovery.

Results and Discussion

The resonances of monacolin K, berberine, resveratrol, and boswellic acids were assigned, and well-resolved and not overlapped peaks were identified for further quantification (Figure 1).

To verify the reliability of the qNMR method, the intra- and inter-day precision and the recovery were performed for monacolin K and one standard of boswellic acid (Table 1).



	R	R_1
β-boswellic acid	H	H
Acetyl-β-boswellic acid	Ac	H
11-keto-β-boswellic acid	H	=O
Acetyl-11-keto-β-boswellic acid	Ac	=O

Figure 1. ^{1}H-NMR spectra of berberine and monacolin K and ^{13}C-NMR spectra of boswellic acids. The signals used for the quantification are indicated.

Table 1. Recovery and intra- and inter-day precision of qNMR method for monacolin K and one kind of boswellic acid.

	Recovery %			Intra-day precision (RSD%)	Inter-day precision (RSD%)
	High	Medium	Low		
Monacolin K	97.54	95.46	93.48	0.99	5.07
Acetyl-β-Boswellic Acid	80.71	83.05	96.45	5.54	8.36

Once the method has been optimized and demonstrated to be precise and accurate, it has been used to quantify the bioactive compounds in food supplements. The results obtained by the qNMR method were compared with the amounts declared on the label. Overall, the food supplements containing monacolin K contained a lower amount than that declared on the label. In the cases of association of red yeast rice with *Berberis aristate* extracts (rich in berberine), monacolin K could not be determined due to the great amount of berberine.

The analyses performed on the food supplements for the control of lipidemia showed high variability in bioactive compound content in tables/capsules belonging to the same batch, suggesting a lack of standardization in the production of these food supplements.

Concerning *B. serrata* extracts, the total content of boswellic acids, determined by summing the concentrations of single derivatives, was lower than 65% as declared on the label. Moreover, three extracts out of 35 did not display any trace of boswellic acids, demonstrating the occurrence of frauds on the market.

Conclusions

The results obtained from the analysis of food supplements demonstrated the important issue related to food supplement safety and quality. In conclusion, health authorities should impose to producers strict quality controls on the food supplements used for contrasting hyperlipidemic and joint inflammatory conditions.

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