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Quality of olive oils

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(Article begins on next page)



New Advances in EVOO Production Technologies

The day before the GENP2022 Conference (or so-called “pre-Conference”) is dedicated to in-depth workshop on New Advances in Extra Virgin Olive Oil (EVOO) Production Technologies. This is your chance to meet specialist peers and join communities of practice around mentioned specific topic.

The Workshop will start on October 26th at 18:00 hours.

Expected duration of the Workshop is 2 hours.

The entrance to Workshop is FREE of CHARGE!

Register for workshop

THE WORKSHOP PROGRAMME:

Introduction: EIT Food Phenoils Project – **Giancarlo Cravotto**

Section 1: Production and characterization of extra virgin olive oil (EVOO)

- New hybrid technologies for EVOO processing and production
 - **Giorgio Grillo**, Emanuela Calcio Gaudino, Luisa Boffa, Giancarlo Cravotto
- Quality of olive oils
 - **Luisa Boffa**, Arianna Binello, Emanuela Calcio Gaudino, Giorgio Grillo, Giancarlo Cravotto

Section 2: Valorization of waste and by-products from EVOO industry and their added-value applications: a circular approach

- Olive mill wastewater from waste to resource
 - **Emanuela Calcio Gaudino**, Silvia Tabasso, Giorgio Grillo, Giancarlo Cravotto
- Biorefinery based on olive waste. State of the art and future trends
 - **Silvia Tabasso**, Emanuela Calcio Gaudino, Giorgio Grillo, Giancarlo Cravotto

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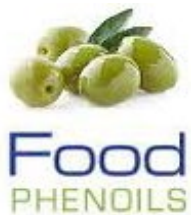
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QUALITY OF OLIVE OILS



Dr Luisa Boffa
26 October 2022
Poreč, Croatia



DSTF
Dipartimento di Scienza
e Tecnologia del Farmaco
UNIVERSITÀ DI TORINO

Green Extraction of Natural Products

27 and 28 October 2022



GENP 2022



Book of Abstracts



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Introductory word

Dear colleagues and friends,

On behalf of the Organizing and Scientific Committee, it is a great honor and privilege to welcome you to Poreč (Valamar Diamant) for the 4th International Congress of Green Extraction of Natural Products - GENP2022, which will take place on October 27-28, 2022.

GENP2022 is organized by the Faculty of Food Technology and Biotechnology University of Zagreb with the support of ISEKI-Food Association, EFFoST and the Energy Efficiency and Environmental Protection Fund.

The congress is divided into 4 sections with 6 main topics: Alternative Solvents for Green Extraction, Sustainable and Clean Extraction Technologies, Innovative Design of Extraction Processes, Valorisation of By-Products and Biorefinery, Industrial and Case Studies, and New Tools for Green Extraction Education and Operator Training.

With its themes, this meeting aims to promote constructive dialog and collaboration between experts from universities, research institutes and industry in the fields of cultivation, extraction, processing and recycling applied to the agrofood, nutraceutical, perfume, cosmetic, chemical, fuel and energy sectors.

GENP2022 will also provide an opportunity to present new insights and ideas in the area of new process technologies at the EIT Food Phenolic Workshop, "New Advances in EVOO Production Technologies."

In addition, the 280 participants from 18 countries who contribute posters and oral presentations to this congress will have the opportunity to publish their work in the proceedings of GENP2022 and in a special issue of the journals Applied Food Research (Elsevier) and Antioxidants (MDPI).

The GENP2022 Scientific and Organizing Committee thanks all conference participants for their participation and active contribution. We wish you a pleasant stay in Poreč and much benefit from the multitude of ideas exchanged, contacts made and successful scientific and professional collaborations.

We would also like to take this opportunity to thank our sponsors and exhibitors for recognizing the importance of this congress and supporting it with their valued participation.

Great thanks are also due to the help and valuable contribution of all members of the Scientific and Organizing Committees.

In addition to the scientific program, the city of Poreč on the Istrian peninsula in western Croatia will ensure that our guests and participants can enjoy the beauties of Poreč and its surroundings.

Thank you very much for joining us!

Chair of the conference
Mladen Brnčić



Program

Workshop program

Wednesday, October 26th 2022

14:00 – 18:00	Registration
18:00 – 20:00	EIT Workshop - New Advances in EVOO Production Technologies
20:00	Dinner
21:30	Welcome cocktail party

Conference program

Thursday, October 27th 2022

8:00 – 9:00	Registration
9:00 – 9:30	Opening of the GENP Conference
Session I 9:30	Alternative solvents for green extraction - HOSTED BY SENSIENT Chair: Antonio J. Meléndez-Martínez Damin Ježek Kristina Kljak
9:30 – 10:15 Plenary Lecture	Giancarlo Cravotto The Industrial Green Revolution in Solid/Liquid Extraction and Processing
10:15 – 10:45 Plenary Industrial	Gian Carlo Leocata - Sensient Subcritical Water Extraction: Cleaner Extracts, Sustainably
10:45 – 11:15	Coffee break
11:15 – 11:45 Keynote Lecture	Christian Cravotto Improvement and greener extraction of grape seeds by 2-methyloxolane
11:45 – 12:00	Ombéline Claux Is it possible to perform oilseeds extraction using bio-based 2-methyloxolane instead of hexane?
12:00 – 12:15	Laura Waldschütz Supercritical CO ₂ - Green Extraction on Industrial Scale
12:15 – 12:30	Manuela Panić Plant-based extracts in natural deep eutectic solvents for industrial application
12:30 – 12:45	Sponsored lecture - Vendor seminar IKA - Božidar Nikić HABITAT - New Generation Reactor



12:45 – 13:00	Sponsored lecture - Vendor seminar RU-VE - Hrvoje Runje Your Lab Partner
13:00 – 14:00	Lunch Break (lunch not included in registration fee)

Session II 14:00	Alternative solvents for green extraction Sustainable and clean extraction technologies Chair: Giancarlo Cravotto Marija Badanjak Sabolović Ivana Radojčić Redovniković
14:00 – 14:45 Plenary Lecture	Axel Schmidt Green Manufacturing Technology For Natural Products
14:45 – 15:15 Keynote Industrial	Leon Skaliotis - Flavourtech Unique Technologies For the Production of Natural Aromas and Concentrates
15:15 – 15:30	Siti Ramli Optimization of the oil extracted from Sardinella lemuru waste with supercritical fluid extraction (SC-CO ₂) using response surface methodology (RSM)
15:30 – 15:45	Lauriane Bruna Supercritical Fluids extraction of bioactive molecules from Apple Pomace
15:45 – 16:15	Coffee break
16:15 – 16:30	Angeles Maron-Ortiz Preliminary studies for the optimization of ultrasound-assisted extraction of microalgal carotenoids
16:30 – 16:45	Marilena Muraglia Combined experimental designs for screening and optimizing the ultrasound-assisted extraction of polyphenols from ripe carob pods (<i>Ceratonia siliqua</i> L.)
16:45 – 17:00	Sónia Santos Green extraction methodologies for the sustainable recovery of linear diterpenes from <i>Bifurcaria bifurcata</i>
17:00 – 17:15	Alberto Alessandro Casazza Cascade recovery of biocompounds from <i>Chlorella vulgaris</i>
17:15 – 17:30	Emilie Isidore Optimization of the Supercritical Extraction of Rosmarinic Acid from Clary Sage and the Antioxidant Activity of the Extracts
17:30 – 18:30	Poster Session 1



Friday, October 28th 2022

Session III 9:00	Innovative extraction process design New tools for green extraction education and operator training Chair: Giorgio Grillo Antonela Ninčević Grassino Sven Karlović
9:00 – 9:45 Plenary Lecture	Francisco J. Barba Nutrient and Bioactive Compounds' Recovery From Mushrooms Assisted by Pulsed Electric Fields, pressurized liquid extraction and SC-CO ₂
9:45 – 10:15 Keynote Lecture	Želimir Kurtanjek Causal Artificial Intelligence Molecular Model of CO ₂ -H ₂ O Extraction Coefficient
10:15 – 10:30	Nenko Nenov Pressurized hot water extraction vs. Energy consumption - are they contradicting?
10:30 – 10:45	Anita Šalić Deep eutectic solvents aqueous two-phase system based protein extraction in a microextractor
10:45 – 11:00	Sponsored lecture - Vendor seminar MILESTONE - Luca Bertoli High-purity extracts from natural products through a fast and solvent-free approach
11:00 – 11:30	Coffee break
11:30 – 11:45	Ana Jurinjak Tušek CFD modelling of the continuously operated microextraction of proteins - a shortcut to new microextractor designs
11:45 – 12:00	Morag Davidson Optimization of an ultrasound-enzymatic assisted extraction for the simultaneous recovery of polyphenols and oil from raspberry pomace using a Definitive Screening Design
12:00 – 12:15	Larissa Knierim; Axel Schmidt Digital twins with process analytical technology under quality by design regulations towards autonomous operation of natural products manufacturing
12:15 – 12:30	Margherita Pettinato Solid-liquid multivariable extraction (SoLVE) of lycopene from tomato waste
12:30 – 13:30	Lunch Break (lunch not included in registration fee)



Session IV 13:30	- Valorisation of by products and biorefinery - Industrial and case study applications Chair: Sandra Voća Jana Šic Žlabur Suzana Rimac Brnčić
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14:15 – 14:30	Pablo Méndez-Albiñana Obtaining pectin of Premium quality From industrial orange juice by-products
14:30 – 14:45	Michał Ochnik The antiviral activity of the blend of double-standardized extracts of black chokeberry and elderberry against human influenza A virus and betacoronavirus-1
14:45 – 15:00	Mia Dujmović Ultrasound as a sustainable technology For the isolation of polyphenols from coffee grounds
15:00 – 15:30	Coffee break
15:30 – 15:45	Matsia Sevasti Biostimulant extraction and analytical characterization of marine organism residual products
15:45 – 16:00	Lavinia-Florina Călinoiu Integrated sustainable pre-treatments approach for cereal bran valorization
16:00 – 16:15	Marilena Muraglia Unripe Apulian carob: a future perspective in nutraceutical and food supplements fields
16:15 – 16:30	Laura Pastare Bioactive cosmetic ingredients From super-critical fluid extracts of Matricaria chamomilla industrial processing by-products
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**PLENARY AND KEYNOTE
LECTURES**



The industrial green revolution in solid/liquid extraction and processing

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Keywords: enabling technologies, process intensification, subcritical water, scaling up, continuous-flow extraction

In the last two decades, the literature has highlighted the considerable effort made by researchers to find more efficient and environmentally friendly extraction processes that comply with the principles of green extraction. Improved heat and mass transfer results in lower solvent and energy consumption, increasing yield and quality of the extract while minimizing degradation. Relevant progress has been made with the use of non-conventional technologies and environmentally friendly solvents. Among the most effective extraction technologies are supercritical fluid extraction (sc-CO₂) and extractions assisted by microwaves, ultrasound, hydrodynamic cavitation, high-shear homogenizers, pulsed electric fields, ohmic heating, cryogrinding and enzymatic treatments. One of the most versatile and environmentally friendly methods exploits the unique properties of subcritical water extraction (SWE) at around 150°-160°C (5-6 bar). Under these conditions, hydrogen bonds are broken and water changes its polarity and dielectric constant, improving extraction performance and mimicking the behaviour of hydroalcoholic mixtures. Current availability of new industrial SWE reactors offered new opportunities for big scale production getting rid to ethanol with big economic and safety advantages. Moreover, the extracts obtained under SWE can be spray-dried without maltodextrins addition. Microwave processing is a leading solventless technology where fresh plant material can be extracted by hydrodistillation or hydrodiffusion and gravity. The volumetric dielectric heating enables a fast distillation of essential oils and volatiles. Worth to be mentioned the new paradigms in plant extraction that in contrast to batch methods, exploits continuous flow processes mainly in cavitation reactors. Another novelty refers to hybrid technologies where the combination of different energy sources and methods offered unexpected synergies. Several enabling technologies have also been applied to downstream processing (concentration, filtration, drying, crystallization, emulsification, pasteurization, etc.), resulting in remarkable process intensification and overall improvement in product quality.



Green Manufacturing Technology for Natural Products

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Keywords: Green Extraction, Waste Valorisation, Digital Twins, Quality by Design, Process Analytical Technology

Climate-neutrality of chemical pharmaceutical industry as being the third largest energy consumer after steel and cement is politically set to be gained in 2045, latest. [1, 2] Essential part of resource and energy efficiency in a biobased world is waste valorisation or cascade utilization, classically recycling. Decarbonisation is not any molecular option, but defossilation. Therefore, as the former discussion plate vs. tank has been decided clearly towards plate, waste valorization has become one key-technology to gain C-sources in addition to main CO₂ capture and recycling. [3, 4]

Application of Green Technologies are set as the manufacturing method of the future. [5, 6] In addition, central processing demand is focussed on efficient utilization of rare resources like land-area farming, qualified operation personal as well as equipment and chemicals [7, 8]

The approach will be explained and demonstrated in detail on exemplified examples like Cocoa Bean Shells (CBS) which contain a high amount of polyphenols like Catechin and Epicatechin. The development of process routes for the use of co-products is essential for the economic viability of these processes [5]. To utilize the full potential of the CBS in the interest of bioeconomy, a process is developed using only minimal amounts ethanol as the only organic solvent. Waste valorisation of polyphenolic parts of essential oils are another component group of general application importance e.g. for natural crop protection agents. The method will be exemplified on those processes as well [6].

In the meantime, a full digital workflow is feasible: Starting from drone and robotics supervised farming including local and event-based feeding, crop-protection and harvesting up to fresh plant manufacturing and at field process manufacturing. Process Analytical Technology (PAT) with in the regulatory demanded Quality-by-Design (QbD) framework is in combination with digital twins the key-technology for success. [7, 8] It will be exemplified that all different technologies are ready for industrialization and business cases are discussed.

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Nutrient and bioactive compounds' recovery from mushrooms assisted by pulsed electric fields, pressurized liquid extraction and SC-CO₂

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Keywords: mushrooms; high-added-value compounds; pulsed electric field; pressurized liquid extraction; supercritical fluid extraction.

The recovery of nutrients and bioactive compounds from natural resources using innovative green technologies is an interesting approach to developing new ingredients and products. In this sense the use of mushrooms could be an interesting source of nutrients and bioactive compounds. In this presentation, different extracts were obtained through pulsed electric field (PEF), pressurized liquid (PLE) and supercritical solvent (SC-CO₂) extraction from mushrooms (*A. bisporus*, *L. edodes*, *A. brunnescens* and *P. ostreatus*). The extracts were characterized by total phenolic content (TPC), total antioxidant capacity (TEAC), phenolic profile (LC/MS/MS) and minerals (P, Se, Mg, Zn, Ca and Fe). Moreover, the solids residues recovered after extraction were analyzed by Scanning Electron Microscopy to better understand the effect of extraction processes on the matrix. After evaluating the extractions, significant increases in the antioxidant compounds, carbohydrates, and proteins, were found after the application of the innovative extraction processes compared to conventional extractions, varying the highest yields of the targeted parameters according to the technology used and type of mushroom evaluated. These results show that PEF, PLE and SFE are promising sustainable technologies to improve the extraction of biomolecules from mushrooms, being necessary to establish which is the best technology used according to the type of mushrooms and the targeted parameters evaluated.



Carotenoids and apocarotenoids: actions in nature, importance for food security and applications

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Key words: Carotenoids, agro-food, health, cosmetics, food security

Carotenoids are classified into carotenes and xanthophylls, depending on the presence or not of oxygenated functions. The term apocarotenoid is commonly used to refer to products obtained from carotenoids by oxidative cleavage. Carotenoids and apocarotenoids are very versatile isoprenoids that intervene in many processes in Nature. For instance, carotenoids are key in photosynthesis, protection against oxidation, communication between species (important for pollination, seed dispersal, mating), modulation of membrane properties, etc. Apocarotenoids can be obtained enzymatically or not and are eliciting increased interest in agro-food and health. Classical examples are retinoids with vitamin A activity, aroma compounds or the phytohormone abscisic acid. Recently there is growing interest in the new phytohormones strigolactones and other apocarotenoid signals that can improve plant production and resilience. Altogether, it is undeniable that carotenoids and apocarotenoids are important for plants and therefore for plant and animal food production. This versatility of actions and properties is in turn important for diverse industries. Thus these compounds have applications in agriculture, foods, feeds, therapeutics, cosmetics and even as textile dyes (1–3).

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Improvement and greener extraction of grape seeds by 2-methyloxolane

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Keywords: 2-methyloxolane, bio-based solvent, oil extraction, polyphenol, hexane.

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Almost all of the 20 solvents approved for food use in Europe (Directive 2009/32/EC) are totally or partially derived from petroleum like hexane. After more than fifty years of extensive use, a valid alternative to hexane is nowadays offered by 2-methyloxolane (2-MeOx), a bio-based solvent that provides high-quality oils and defatted, stable protein-rich ingredients for food and feed. Based on a comprehensive survey of scientific studies, on March 2022 EFSA stated that 2-MeOx is a safe solvent for food application releasing a positive opinion on its inclusion in the list of permitted solvents for food production. In this study, 2-MeOx, both dry (dry 2-MeOx) and saturated with water (2-MeOx 95.5%), was compared qualitatively and quantitatively with hexane for the extraction of grape seed oil, which is widely used in food and cosmetics. The oil extraction yield, the total tocopherol and tocotrienol content, as well as the total polyphenol amount were considerably higher with both dry 2-MeOx and 2-MeOx 95.5% if compared to hexane. The fatty acid and phospholipid profiles were not influenced by the extraction solvent, whereas a slightly higher sterol concentration was obtained in the hexane extract. The whole extraction process with 2-MeOx including downstream steps and crude oil chemical refining, was further investigated at pilot scale. The full characterisation of both crude and refined oil confirmed the excellent scalability of the process. In conclusion, thanks to the high extraction efficiency, the safer toxicological profile and the reduction up to 90% of the carbon footprint compared to petrochemical solvents, 2-MeOx proved to be an excellent candidate to replace hexane in grape seed oil extraction.



Causal artificial intelligence molecular model of CO₂-H₂O extraction coefficient

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Keywords: molecular causal discovery, supercritical, CO₂ extraction

Applied is a causal artificial intelligence model for molecular descriptor prediction of the high-pressure partition coefficients (log K) in the CO₂-H₂O system. The model is based on a set of 447 data points of 45 aromatic organic compounds at various temperatures and pressures. Each molecule is represented by 1875 descriptors and 12 types of fingerprints available from the pharmaceutical PaDEL database and the corresponding octanol-water coefficient. The descriptors and fingerprints are calculated using the Chemistry Development Kit. Applied is a flexible net with LASSO and RIDGE (L1 and L2) metrics with boosted decision tree forest (XGB) for extraction of the principal molecular descriptor and functional effects of process temperature and pressure. Temperature is varied in the range from 18 °C to 90 °C and pressure from 7 MPa to 32 MPa. The space of the model predictors is reduced to process parameters (pressure, temperature), octanol-water partition coefficient, and the three molecular descriptors: MinPartialCharge, PEOE_VSA10, and MaxEStateIndex. Due to nonlinear interactions applied is Hamilton-Schmit independence criteria (HSIC) for inference of causality. The structural causal model (SCM) is presented as a directed acyclic graph (DAG) with the corresponding causal interactions. Functional relations between partition and process parameters logK(T,P) are determined by Bayes neural networks as marginal distributions (partial dependence plots) based on an adjusted set of descriptors. The model is validated by k-fold (k=5) cross-validation relative error of 6 % in the logK prediction. Application of the model for optimization of extraction process is discussed.



ORAL LECTURES



Is it possible to perform oilseeds extraction using bio-based 2-methyloxolane instead of hexane?

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Keywords: oilseeds, vegetable oils, proteins, 2-methyloxolane, hexane

Population growth and the environmental crisis are fostering the emergence of new global challenges. Hence, feeding the world in a sustainable way has never been more crucial. However, our food is largely based on vegetable oils and proteins, which are produced through oilseeds extraction using hexane in spite of its known toxicity and environmental issues. Alternatives have been studied and developed in the past decades, but none was competitive enough so far.

Since 2012, a new bio-based solvent - 2-methyloxolane (2-MeOx) - appeared in the industry with several scientific publications showing its potential for the extraction of various oilseeds. However, only few research was published on major oilseeds such as soy, sunflower, or rapeseed.

Therefore, the possibility to replace hexane with 2-MeOx was investigated with soybeans and rapeseeds as raw materials. First, the extraction products obtained with this new solvent were compared to those typically obtained with hexane. Then, the impact of the solvent switch on the extraction process was investigated, starting from the lab-tests to the large-scale trial.

Overall, results of this study reveal that 2-MeOx has a good potential to replace hexane for the extraction of oilseeds, as this alternative solvent provide extracts with similar composition and exhibits a similar behavior during extraction.



Biostimulant extraction and analytical characterization of marine organism residual products

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Key words: Biostimulants, blue organisms, analysis, green extractions

Marine organisms (fish, algae, mussels, etc.) attract keen interest from the scientific community due to their high content in nutrients. Such nutrients (fertilizers and biostimulants) are widely used in agricultural activities for promoting plant growth. Poised to develop methods identifying molecular and (non)metal ionic components in BlueBio materials, able to prevent plant stress in horticultural crops, research efforts were launched to employ appropriately configured analytical tools in the characterization of Low and High Nitrogen Algal Cake and possibly other marine-derived residual products from specific such materials emerging from marine organism processing industrial activities in Scandinavia. A classical Weende-based approach was incorporated in the investigation of different parameters, such as cellulose content, Macro and Micro Nutrients, including K, Ca, Mg, P, Fe, Mn, etc., as well as potential toxic elements (PTEs) As, Cd, Cr, Hg, Pb determined through Inductively Coupled Plasma (ICP) methodologies. Key to such an endeavor was sample preparation, involving dry ashing as well as microwave assisted digestion. PTEs were also determined via ICP-MS, thus seeking to achieve lower levels of detection. Moreover, screening for organic compounds emerged prominently as a necessity in the study and was pursued through GC-MS techniques on extracts of varying polarity solvents. Using those extracts, the lipid content in the title materials could be explored through GC-FID. The collective results formulate a well-defined profile for both Low and High Nitrogen Algal Cake materials, thus signifying the importance of a) screening of key ingredients in marine-based remnant raw materials, and b) identification of both organic and inorganic components in algal materials, that could be used in agricultural processes for plant growth enhancement. The importance of such an analytically intensive work



rides on the fact that underutilized or poorly utilized remnant materials from industrial marine organism processes carry significant molecular and ionic inorganic and organic components that could be used as naturally-derived fertilizers with potential enhancing effects on growth of traditional crops that have come to exemplify the European Union's strategy on F2F practices in agriculture.

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Pressurized hot water extraction vs. Energy consumption – are they contradicting?

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Keywords: Pressurized hot water extraction, energy efficiency, heat recovery

The use of water as a solvent in pressurized hot water extraction systems in the extraction of alcohol- and oil-soluble ingredients from plants requires an increased temperature regime for carrying out the process - within the range of 150 – 200 °C. Despite the lack of phase change, heating the solvent from ambient temperatures to the extraction temperature requires significant heat consumption. The reason for this is the high values of the specific heat capacity of water - about 4.19 kJ/kg.K. For example, for heating 100 kg of water solvent from 10 to 170 °C requires the use of 18.6 kWh of heat. This fact could significantly impair the attractiveness of using the method on a larger, industrial scale, especially in the light of the recent challenges to the European Union in terms of energy efficiency, uncertainty in energy supply and energy resource prices.

In this regard, the presented article discusses the results of the use of energy saving devices in a pilot plant for pressurized hot water extraction with an effective capacity of 2 l. It was developed and manufactured by InnoSolv llc, Plovdiv, Bulgaria. It has been established that through appropriate design and software control of the plant, between 79 and 94 % heat savings have been achieved during extraction at 150 °C. These savings are achieved through heat regeneration and its value depends on the order of the batch or the replacement of the solvent. Based on the results obtained, recommendations for operation of such plants in terms of minimum energy costs are defined



Supercritical CO₂ – Green Extraction on Industrial Scale

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Keywords: Supercritical, CO₂, industrial, natural, purified

1. Introduction

Organic solvents such as methanol or hexane are commonly used for a variety of extraction and purification process. Nonetheless, supercritical CO₂ extraction is a state-of-the-art alternative regarding extraction efficiency and customer compliance providing volatile active components with a focus of Cosmetics, Nutraceuticals and Food applications.

2. Summary

- Technology

Pressurization and temperature increase modify the density of liquid CO₂ resulting in supercritical CO₂, which flows through a pre-loaded extractor and extracts soluble substances. The extract is separated by pressure reduction that changes the solubility of CO₂. A condenser liquefies CO₂ and introduces it again into the extraction cycle allowing a complete recovery of the solvent.

- Advantages

The CO₂ technology allows the production of concentrated and purified extracts containing high-value ingredients by applying moderate temperatures in an antibacterial and oxygen-free atmosphere and without any harmful solvents. For example, aromatic flavors are preserved by exposing products to minimum strain.

- Applications

A various spectrum of natural substances can be processed with CO₂. Gentle extractions enable targeting essential oils and flavors of flowers or herbs. Moreover, Supercritical CO₂ extraction is a standard industrial scale process for generating hops extracts. These oils are generally used for beer brewing. Furthermore, algae extraction for carotenoid enrichment is possible with large-scale equipment working up to 1000 bar. Oil seed press cake defatting purifies and enriches vegetable protein for meat replacement products.

3. Conclusions

The CO₂ technology is used worldwide for the refinement of natural substances. The background of CO₂ extraction as well as a variety of industrial applications will be shown in the presentation.



The antiviral activity of the blend of double-standardized extracts of black chokeberry and elderberry against human influenza A virus and betacoronavirus-1

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Keywords: antiviral, betacoronavirus-1, chokeberry, elderberry, influenza

Natural products and extracts are known for their health-promoting properties, among them, black chokeberry and elderberry are considered rich sources of bioactive compounds with antiviral potency. In this study the inhibitory activity of the double-standardized blend of extracts from *Aronia melanocarpa* (Michx.) Elliot and *Sambucus nigra* L. or separated extracts of *A. melanocarpa* (EAM) or *S. nigra* (ESN) were assessed against four viruses belonging to four different families: influenza A virus (A/H1N1), betacoronavirus-1 (HCoV-OC43), human herpesvirus type 1 (HHV-1), and human adenovirus type 5 (HAdV-5). Antiviral assays were used to evaluate the antiviral activity of the plant extracts in a cell-present environment with extracts tested before, simultaneously, or after viral infection. The virus replication was assessed using the CPE scale or luminescent assay. The EAM-ESN blend strongly inhibited A/H1N1 replication as well as HCoV-OC43, while having a limited effect against HHV-1 and HAdV-5. This activity likely depends mostly on the presence of the extract of *S. nigra*. However, the EAM-ESN blend possesses more effective inhibitory activity toward virus replication than its constituent extracts. A post-infection mechanism of action of the EAM-ESN makes this blend the most relevant for potential drugs and supportive treatments; thus, the EAM-ESN blend might be considered a natural remedy in mild, seasonal respiratory viral infections.



Deep eutectic solvents aqueous two-phase system based protein extraction in a microextractor

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Keywords: protein extraction, aqueous two-phase system, deep eutectic solvent, continuous microextraction

Nowadays, clean, sustainable and efficient extraction methods for downstream processing of biomolecules are of particular interest. The basic idea is to develop an effective and versatile green technology that allows high extraction efficiency at minimal cost and least negative impact on the environment. The aqueous two-phase system (ATPS) is one of these technologies. ATPSs are liquid-liquid systems usually formed by mixing certain polymers (e.g., PEG) with salts, detergents, ionic liquids, and short-chain alcohols. Recently, the use of deep eutectic solvents (DES) instead of polymers to promote extraction in ATPS has been reported. The preparation of DESs is very simple and cheap. Moreover, DES is referred to as a green solvent because it can be composed of natural components such as sugars, alcohols, and amides, and is biodegradable, nontoxic, and low-volatile. To use DESs for efficient extraction of various biomolecules, their composition must be optimized. Moreover, in order to intensify the extraction process, batch extraction is increasingly replaced by continuous extraction, and the extraction process itself is carried out in microsystems.

In this study, ATPS based on salt and DES was used for the extraction of proteins (laccase and lipase). At the beginning of the study, different DES were prepared and characterized. In the next step, the influence of ATPS composition and salt concentration on extraction efficiency was investigated in a batch extraction performed for 30 minutes. The highest extraction efficiency of 94.70% was obtained for lipase by using the ATPS composed of K₂HPO₄ and betaine:urea DES. The highest extraction efficiency for laccase of 67.21% was achieved for the ATPS composed of K₂HPO₄ and choline chloride:poly(ethylene glycol):zinc chloride DES. For selected ATPSs additional process intensification was achieved by extraction performed in a microsystem. The influence of residence time,



temperature and channel diameter on extraction efficiency was investigated. For the enzyme lipase, an extraction efficiency of 98.50% was achieved for a residence time of 30 s. Once the best process conditions were defined, the method was used for protein extraction from crude samples. Finally, an integrated system with continuous protein extraction and phase separation on a microscale was developed.



CFD modelling of the continuously operated microextraction of proteins – a shortcut to new microextractor designs

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Keywords: protein extraction, aqueous two-phase systems (ATPS), microextractor, CFD

Aqueous Two-Phase Systems (ATPSs) have attracted much attention due to their ability to separate and purify various biological molecules with high yield and purity. ATPS can be used for separation and purification depending on their composition. ATPSs are mostly polymer-polymer or polymer-salt systems, but recently Deep Eutectic Solvents (DESs) have also been used for ATPS separations because they are more sustainable and cost-effective as solvents than polymers. To develop an efficient extraction process, the geometry of the extractor and the operating conditions should be optimized in addition to the composition of the extraction solvent. Due to the high surface-to-volume ratio, higher heat and mass transfer rates, and short diffusion path, continuously operated microextractors are widely used to improve mass transfer between two phases.

In this work, ATPS composed of K₂HPO₄ and betaine:urea DES for lipase purification and ATPS composed of K₂HPO₄ and choline chloride:poly(ethylene glycol):zinc chloride DES for laccase purification in continuously operated microextractors were analyzed using Computational Fluid Dynamics (CFD). CFD simulations were performed to illustrate and visualize the interactions between two phases during extraction. The CFD simulations of the ATPS flow profiles in the microextractor were performed using COMSOL Multiphysics v.4.2 finite element software. A laminar flow model with Lagrangian specification of the field and Dirichlet-type boundary conditions were used. CFD simulations were performed by estimating the fluid properties assuming simple mass balances. The results can be used to determine the position of the enzyme separation in the microchannel. Based on the obtained results, new microextractor configurations can be proposed that would enhance extraction efficiency.



Obtaining pectin of Premium quality from industrial orange juice by-products

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Keywords: Orange by-products; pectin; characterisation; bioactivity; techno-functional properties

The food industry generates a huge amount of wastes, being a problem for many sectors, but at the same time, a research area of great interest. These residues, with proper management, can provide benefits both at environmental and economic levels, since they can be a source of products with different applications. Regarding the vegetable processing sector, some of its activities are the production of fruit nectars, juices, frozen products, etc. Compounds of great interest such as pectin, obtained from the by-products of vegetable origin, can be used as functional ingredient for human health, but also as an additive (E-440) for the stabilization of foods such as juices, jams and dairy products.

Despite the multiple ways of obtaining pectin from vegetable by-products (acid hydrolysis, enzymatic hydrolysis assisted extraction using emergent technologies), to achieve high extraction yields from the intricate cell wall, acid hydrolysis, is necessarily used at the industry, having a negative impact in the environment. In addition, it is also important the choice of the best extraction conditions for the maintenance of pectin structure and, consequently, the techno-functional and bioactive properties.

In the present work, we have optimised the conditions of pectin extraction from industrial by-products derived from orange juice. Different initial material, extracting agents and conditions have been evaluated. Measured parameters have been yield, molecular weight, degree of methyl esterification, neutral sugars and galacturonic acid content. Optimal conditions (nitric acid 0.29%, 3.5:20 substrate:extracting agent, 95 °C, 30 min) have been reproduced at pilot plant scale using a 20 L reactor and a purification membrane system by ultrafiltration. In summary, antioxidant activity and technological functionality assays demonstrated the suitability of the process for the extraction of



pectin from orange juice by-products to contribute to the circular economy.

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Integrated sustainable pre-treatments approach for cereal bran valorization

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Keywords: cereal bran, phenolic compounds, ultrasounds, thermal processing, bioaccessibility

From ancient times, cereals have represented the base nourishment in human nutrition all over the world. Wheat and oat represent staple foods for the worldwide population [1]. The European Flour Millers report [2] from 2016 pointed out that in Europe, more than 45 million tons/year of wheat and oat are processed, generating more than 6.5 million tons of bran, perceived as by-products. Contrary, they are abundant in valuable compounds with a positive impact on health [1], whereas phenolic compounds are insoluble, being bound to the cellulose and hemicellulose structures. Therefore, an integrated sustainable pre-treatments approach via food processing thermal treatment (TP) (10 min, 80 °C) and ultrasounds technology was applied to valorise the wheat bran (WB) and oat bran (OB) for human consumption by extracting and intensifying the bio-accessibility of the existing bioactive phenolic compounds, and their antioxidant, antimutagenic, and antimicrobial activities. Thermal processing enhances bran's solubility by making the compounds bio-available [3], reducing their complex structure, whereas ultrasound-assisted extraction (UAE) [4] represents a novel and low-cost technique that can improve the bioactive compound extraction rate and efficiency.

The results showed that the integrated pre-treatments approach improved the total phenolic content of WB by +22.49%, and of OB with +25.84%. The phenolic concentration showed a significant relative percentage increase in the case of pre-treated WB (ferulic acid +39.18%, apigenin–glucoside +71.96%) and of pre-treated OB (avenanthramide 2c +52.17%, dihydroxybenzoic acids +38.55%). The best antioxidant capacity was registered by pre-treated OB followed by WB. The strongest antimicrobial inhibition was attributed to the pre-treated WB sample. Both pre-treated matrices had strong antimutagenic activity toward *S. typhimurium* TA100.

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Plant-based extracts in natural deep eutectic solvents for industrial application

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Keywords: biological activity, Graševina grape pomace, plant-based extracts, natural deep eutectic solvents

We all like to choose products declared natural since we are more aware that a healthy lifestyle is imperative. But little do we know that these products are not always manufactured ecologically; harmful organic solvents are often used during production. Organic solvents are toxic, flammable, explosive, volatile, result in air pollution, and are responsible for the >40% of global CO₂ emissions from industry. Numerous directives regulate organic solvents, such as the Kyoto protocol and other EU directives. Therefore, the global mission is to find alternatives for organic solvents.

To overcome these barriers, green and sustainable technologies and their application in different industries is of growing interest. Within green chemistry principles, finding and development of a promising alternative to traditional, toxic organic solvents used in chemical and biotechnology industries is of great importance. Due to the properties such as non-volatility and non-flammability, in the past decade, natural deep eutectic solvents (NADES) have attracted attention as potential new green solvent. NADES are mixtures of cheap, natural, non-toxic and readily available components, as choline chloride alcohols, organic acids, sugars, vitamins and amines. Since the number of possible chemical structures of these solvents is vast, the possibility of their design for specific applications makes them very interesting for use in various industrial fields such as synthetic chemistry, electrochemistry, making nanomaterials, biochemistry, biocatalysis and isolation and analysis of various compounds such as biologically active compounds from plants.

This work aimed to produce fully characterized plant extracts from Graševina grape pomace in NADES solvents. Prepared extracts were fully characterized and analyzed. According to the results prepared extracts are safe for application in the food and cosmetic industry.



Digital Twins with Process Analytical Technology under Quality by Design regulations towards autonomous operation of Natural Products Manufacturing

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Keywords: Digital Twin, PAT, QbD, green extraction, process simulation

In the plant processing industry, especially in regulated industries, traditional extraction processes are widely used. The regulatory environment sets the framework, as traditional approvals are tied to manufacturing processes, which limits the possibilities for further development of the processes or optimization of operations. The associated dependence on traditional manufacturing processes can lead to the fact that raw materials are not optimally utilized through the selection of suboptimal process parameters. Traditional extraction process can furthermore lead to high process variabilities and a bad process stability. In steam distillation processes traditional processes often lead to long distillation times which can be shortened significantly.

Innovative methods such as Quality by Design (QbD) including Process Analytical Technology (PAT) offer opportunities for manufacturers to meet regulatory requirements such as low product variability between production batches.

In addition to already established, exclusively experimental methods, digital twins based on validated physicochemical models can be used in process design to improve the stability of the extraction results, deepen the understanding of certain substance systems and to scale up laboratory processes by in silico piloting studies. A Digital Twin can also be used as a part of Advanced Process Control (APC) concepts. Therefore it is possible to make minor changes to the extraction parameters by characterizing the input material on the basis of the Digital Twins in order to guarantee a constant product quality in case of fluctuating input material quality.



Optimization of the oil extracted from *Sardinella lemuru* waste with supercritical fluid extraction (SC-CO₂) using response surface methodology (RSM)

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Keywords: Optimisation, supercritical fluid extraction, soaking technique, omega -3,6, sardine oil

This research aimed to determine the optimum the total oil yield extracted from sardine (*Sardinella lemuru*) waste. Supercritical fluid extraction (SC-CO₂) of sardine oil was performed to optimize various parameters, such as soaking (X1) and extraction time (X2) in order to obtain the highest yield of extracted oil with the highest amount of EPA and DHA. At the constant temperature (60 °C), pressure (350 bars) and a flow rate of 5ml/min, the optimal values were obtained at 1.7 soaking time and 3.7 hours of extraction time, with corresponding responses achieved at 3.114%, 15.47% and 15.65% for yield, EPA and DHA, respectively. Of the two independent variables, soaking time had a significant effect ($p < 0.05$) on a yield while EPA and DHA ratios were notably not influenced by any extraction method. The experimental values in this study were reasonably comparable to their predicted counterparts. This result was compared with a traditional solvent extraction and the SC-CO₂ solvent extraction yield where SC-CO₂ extracted yield was better quality.



Unripe Apulian carob: a future perspective in nutraceutical and food supplements fields

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Keywords: unripe carob, antioxidant activity, ultrasound assisted extraction, polyphenols

Ceratonia siliqua L., a Mediterranean carob tree, has recently been the focus of attention due to the presence of a significant percentage of potentially beneficial polyphenols for human health.^{1,2,3} While much scientific evidence is available on the bioactive properties of mature carob (R-CAR), few investigations have been conducted on unripe carob (U-CAR) as a possible source of functional health foods.

In this work, for the first time, two unripe varieties of Apulian carob (*Amele* and *Selvatica*) were evaluated and compared for extraction, characterization, and profiling of the antioxidant potential of polyphenolic compounds. The extraction methodologies adopted were aimed at improving polyphenol extraction yields and the quality preservation of bioactive compounds. Environmentally friendly extraction approaches such as ultrasound-assisted extraction (UAE) and the traditional maceration process (MAE) were selected, using an aqueous mixture with ethanol in different proportions. The total phenolic content and antioxidant profile of U-CAR extracts were determined using Folin Ciocalteu, flavonoid, DPPH, and ABTS tests. High-pressure liquid chromatography was performed to detect polyphenols in the extracts.

The highest polyphenolic content and best antioxidant capacity were obtained using the UAE process and water as the extraction solvent.

As part of this study, the cytotoxic activity and effects of U-CAR extracts on H₂O₂-induced cell viability against SH-SY5Y and MCF7 cells were investigated.⁴ According to our data, U-CAR extracts result in significant reversal of H₂O₂-induced damage on SH-SY5Y and MCF7 cells, with viability restoration rates ranging from 73% to 110%.



Overall, our results provide a valuable starting point for planning a research program aimed at *in vitro* and *in vivo* screening of the polyphenolic antioxidant profile of the Apulian U-CAR fruit as a potentially useful bioactive resource for human health.

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Supercritical fluids extraction of bioactive molecules from Apple Pomace

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Keywords: supercritical fluids extraction, antibacterial tests, apple pomace, waste valorisation, circular economy

Apple (*Malus domestica* Borkh.) is one of the more common fruit in Europe and the fourth most consumed fruit in the world (Musacchi & Serra, 2018). According to the Food and Agriculture Organization of the United Nations, more than 86 million tonnes were produced in 2020 around the world and 11.8 million tonnes in the European Union (FAOSTAT). According to Kammerer et al. (2014), apple juice is the most value-added product with an annual production of 25% to 30% of total crop. Apple pomace (AP) is a left-over obtained after pressing the apples. It contains peels, flesh, seeds, and stem. About 12 million tonnes of AP waste is produced in the world (Martău et al., 2021). This left-over found several applications and uses, namely in animal feeding, biofuel and ethanol production (via fermentation), compost and biogas (Kennedy et al., 1999). According to International Energy Agency, Europe is the main user and producer of biogas, 18 Mtoe in 2018 against 7 Mtoe for China, 4 Mtoe for USA and for the rest of the world. Yet, in most countries, AP is discarded, and buried in soil (Lyu et al., 2020). Clearly these procedures cannot valorise the valuable phytochemicals present in AP. The main phenolic compounds are phloridzin, quercetin glycosides, and chlorogenic acids, all molecules with high added value (Suárez et al., 2010). Besides antioxidant and anti-ageing properties, these compounds showed interesting antibacterial and antifungal activity.

This work focuses on the valorisation of AP by extraction with supercritical CO₂. The extraction experiments could provide optimized yield preserving the biological activities of the extracts. The extracts were tested on *Escherichia coli*, and their minimum inhibitory concentrations and diameter of inhibition zone were evaluated.



Combined experimental designs for screening and optimizing the ultrasound-assisted extraction of polyphenols from ripe carob pods (*Ceratonia siliqua* L.)

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Keywords: carob kibbles; experimental design; phenolic content; UAE; valorization; HPLC-DAD

The pulp of the carob fruit has recently received considerable attention due to its high content of polyphenols, which have a lot of health-promoting effects.^{1, 2}

In this work, ultrasound-assisted extraction (UAE) was optimized sequentially using a screening Plackett-Burman design and non-standard central composite design coupled to response surface methodology and desirability function statistical tools, to find the best conditions for the extraction of 9 polyphenols from carob pods. The gathered mathematical models showed that the highest significant factors influencing the extraction of all compounds were solid-solvent ratio, solvent concentration, and particle size, with the optimal results obtained at values of 0.2 g/mL, 40% ethanol, and 0.3 mm, respectively. Whilst, extraction temperature and time and sonication power and frequency were not significant, even though they were fixed to 35 °C, 15 min, 100 W, and 37 kHz, respectively, for reducing the energy costs and providing the best possible extraction of polyphenols.

In conclusion, the findings from this study confirm the potential of carob pods as a natural source of polyphenols and contribute to giving new insight into their optimal extraction conditions. Furthermore, they propose UAE as an effective and sustainable technology for the revalorization of this agri-food waste.

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Ultrasound as a sustainable technology for the isolation of polyphenols from coffee grounds

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Keywords: waste management, polyphenols recovery, antioxidant capacity, ultrasonic bath, ultrasonic probe

Coffee is one of the most commonly consumed beverages, but after brewing, a large amount of residue is produced, that remains unused and is discarded as waste. One of the priorities of the European Green New Deal is to convert and reuse materials into new value-added products thus significantly reducing waste disposal and its negative impact on the environment and human health. Since the phytochemicals are not completely extracted during the brewing process, coffee grounds is very abundant in bioactive compounds (carbohydrates, proteins, lipids, oils, alkaloids, minerals, phenols, etc) some of which may have antioxidant properties. Therefore, it shows a high potential for recovery and can be used as multipurpose material in mushroom growing, soil enrichment, pellet production and different industries (pharmaceutical, agricultural, food, cosmetic).

Using new sustainable technologies, such as ultrasound-assisted extraction (UAE), which enables low energy consumption, faster extraction, and higher yields of antioxidants also helps minimize negative ecological impacts and therefore is classified as a green technology.

For this study, coffee residue was collected after brewing with a catering coffee machine. Samples were prepared from 5 g of coffee grounds and as a green solvent 50% and 80% ethanol (v/v) was used. The aim was to optimize the extraction method by determining the influence of different variations of probe and bath UAE on the content of polyphenolic compounds and antioxidant capacity of coffee grounds extracts. The type of equipment, ethanol concentration, time and amplitude were tested. The highest total phenolics content (TPC) and total non-flavonoid content (TNFC) (835.68 and 477.62 mg GAE/100 g, respectively) were determined during 10-minute extraction with 50% EtOH and a probe system with 60% of amplitude.



For the determination of antioxidant capacity, the FRAP method proved to be more suitable than the ABTS method for this type of raw material, and the highest value was observed when using the probe system (20% amplitude) with 50% EtOH (v/v) and 5-minute extraction.

In general, the UAE with probe system proved to be a better solution for the extraction of polyphenols from coffee grounds than the bath system. This research has shown that different treatments of UAE can have influence on polyphenol extraction, and instead of being treated as waste, coffee grounds can be utilized as a source of bioactive compounds. It is important to conclude that coffee grounds recovery can have many applications, can greatly reduce waste accumulation and be significant by-product for further reuse.



Bioactive cosmetic ingredients from super-critical fluid extracts of *Matricaria chamomilla* industrial processing by-products

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Keywords: Super critical fluid extraction, DPPH assay, Matricaria chamomilla

Sustainable resource management is one of the main challenges of any industry and society in general. Biorefinery approaches and valorisation of by-products are gaining popularity due to environmental and economic benefits. In this work high value-added bioactive compounds for potential use in cosmetics are obtained from by-products of *Matricaria chamomilla* herb processing activities by super critical carbon dioxide extraction and fractionation with ethanol as co-solvent.

The effect of extraction pressure (5-25 MPa) and temperature (35°-55°C) on extraction yield was evaluated using Response Surface Methodology. Extracts obtained with SCFE were chemically characterized and their safety and efficacy profiles were determined. Apigenin, ferulic acid and coumarin derivatives were quantified to determine the relationship between their content and antioxidant activity. The quantitative DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay indicated that *Matricaria chamomilla* SCFE extracts showed antioxidant activity in the range of 3.9%-55.5%. The results show that coumarin derivatives in the obtained extracts have the highest correlation with antioxidant activity. Cytotoxicity and phototoxicity assays showed minimal toxicity and high safety profile in cell culture-based test systems. Findings within this research suggest by-products of *Matricaria chamomilla* processing are a potential source of valuable bioactive compounds, with potential use in the field of cosmetics.

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Optimization of the Supercritical Extraction of Rosmarinic Acid from Clary Sage and the Antioxidant Activity of the Extracts

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Keywords: Supercritical carbon dioxide extraction; rosmarinic acid; optimization; clary sage; polyphenols

This study investigates the extraction of bioactive compounds of clary sage (*Salvia sclarea* L.) with carbon dioxide in supercritical conditions (SC-CO₂). The target component is rosmarinic acid (RA), a natural phenolic compound (PC), with strong antioxidant activity (AA). The aim is to determine the optimal operating conditions to extract RA from sage using the SC-CO₂ extraction technique. Response surface methodology was applied to optimize the operating temperature, pressure and the co-solvent composition. Indeed, the polar nature of RA requires the addition of a co-solvent to increase its affinity and solubility.

The extraction was performed on clary sage residue obtained after distillation. Extractions were carried out using supercritical fluid extraction system. 25 g of clary sage were placed into a 500 mL cell. The SC-CO₂ had a downward flow in the extraction vessel and the flow rate was kept constant at 60 g/min during the experiments. The separator temperature was set at 60 °C. The pressure and temperature ranged between 100 and 600 bar, and 40 and 100 °C respectively. The co-solvent ranged between 0 and 100% ethanol in water (v/v), added to the supercritical fluid at 10% (w/w).. A Box-Behnken experimental design was employed to evaluate the effect of the studied factors, and determine the optimal conditions to obtain a high RA yield (quantified by HPLC) and AA (measured using DPPH assay).

Higher RA yields were reached at low pressures (100-420 bar) and moderate temperatures (40-85°C). The extraction rate is mainly influenced by the solvent composition. The presence of water in the co-solvent is required to achieve important yield of RA.

AA is affected by ethanol and the interaction of pressure and temperature. Increasing pressures at low temperature induced a higher AA. The opposite observation was made for high temperature. Pressure has no effect on AA at medium temperature. Ethanol in the co-solvent has a significant effect, 30 to 65% allowed to reach the highest AA.



Optimal conditions for maximizing RA yield are a pressure of 100 bar, a temperature of 65 °C and 35% ethanol in co-solvent. The RA yield reached 8.30 ± 0.10 mg/gDM with an AA of 29.07 ± 0.10 mg trolox equivalent (TE)/gDM. The second-order polynomial model fit the experimental data for responses prediction. SC-CO₂ is a good solution for the sequential extraction of the essential oil followed by the PC extraction. This way would provide a sustainable and additional valorization of clary sage.



Green extraction methodologies for the sustainable recovery of linear diterpenes from *Bifurcaria bifurcata*

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Keywords: Macroalgae; Bioactive compounds; Green extraction methodologies; alternative solvents; sustainability

Macroalgae (~10000 species) has proven to be a rich source of structurally diverse and complex compounds exhibiting numerous interesting biological effects, which can be exploited for both pharmaceutical and nutraceutical applications [1]. Among the variety of promising high-value macroalgae compounds, linear diterpenes commonly found in the Sargassaceae family, and particularly in *Bifurcaria bifurcata* species have attracted attention due to their health-promoting properties such as anti-inflammatory, antibacterial or antiproliferative activities [2] which makes them very promising for nutraceutical or pharmaceutical fields. However, these applications have been hindered due to the lack of eco-friendly and efficient extraction methodologies.

In this vein, different methods were evaluated to extract linear diterpenes from *B. bifurcata*. Indeed, high-pressure assisted extraction (HPE) [3], microwave-assisted extraction (MAE) and extraction with alternative solvents, namely switchable solvents were used to obtain diterpenes enriched fractions from *B. bifurcata*. Extracts were evaluated regarding their total extraction yield and diterpenes content. The different extraction methodologies were optimized by response surface methodology and finally compared. In conclusion, these results pointed out different promising green methodologies, comparatively to conventional ones, to obtain bioactive diterpenes enriched extracts from the macroalga *B. bifurcata*.

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Cascade recovery of biocompounds from *Chlorella vulgaris*

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Keywords: Chlorella vulgaris, Green extractions, Biorefinery, Chlorophyll.

Microalgae are unicellular photosynthetic microorganisms that can grow at high rates, showing the possibility to accumulate different kind of nutrients inside their cells changing the growth conditions. Microalgae can be used to produce feed, bioenergy, healthy food, and can be exploited for the treatment of wastewaters.

In this work, the microalga *Chlorella vulgaris* was studied as source of biocompounds, like proteins, antioxidants and chlorophyll, intended for food industry applications.

C. vulgaris was grown in a 6 L column at 20 °C under continuous light exposure (70 $\mu\text{mol/m}^2\text{s}$). The biomass was collected by centrifugation at 3000 rpm for 10 min after 14 days of cultivation and freeze-dried for storage purposes.

A new extraction patented method, named Solid-Liquid multi Variable Extractor (SoLVE), developed by the Food Engineering Laboratory of the Department of Civil, Chemical and Environmental Engineering of the University of Genoa was used for the extraction of high added value compounds. SoLVE is a new versatile technology that allows to perform extraction in continuous mode varying several extraction parameters, like temperature, solvent flow rate and ultrasounds amplitude. Extraction conditions of SoLVE for the recovery of biocompounds from *C. vulgaris* were optimized by using experimental design and Response Surface Modelling (RSM). A Box-Behnken type factorial plan with 15 runs was used. All the extractions were performed for 60 min and samplings were done every 4 min to evaluate the kinetic of the process. The effects of independent variables on the extraction rate and yield are described through RSM.

The validation of the results was done performing a new extraction process under the optimized operating conditions (65 °C, 1.5 mL of ethanol per min and 80% ultrasounds amplitude). The comparison of the results with the expected values given by Design Expert and those found in literature showed that extraction using SoLVE system assisted with ultrasounds gave valuable results and an improvement for what concerns the extraction yield of the product desired.



Solid-liquid multivariable extraction (SoLVE) of lycopene from tomato waste

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Keywords: green technologies; antiradical power; ultrasounds; high pressure, waste valorization

Italy processes about 5.5 million tons of tomato annually and the related industry generates wastes generally used as animal feed, soil improvers by composting, or for biogas production. Nevertheless, waste from the agri-food sector can be an economic and renewable resource for the production of naturally-derived bioactive compounds, in agreement with the bio-economy concept. Lycopene is an interesting carotenoid that can be recovered from tomato waste, showing excellent antioxidant properties and beneficial effects on human health. To maximize the recovery of this functional ingredient preventing losses of bioactivity, process intensification is essential. In this work, solid-liquid multivariable extraction (SoLVE), a new patented technology able to combine high pressure, ultrasounds and temperature, is proposed as innovative method for the recovery of lycopene from tomato peels. A study on extraction kinetics coupled with response surface methodology was elaborated to simultaneously investigate the effect and the interactions of process variables. The dependence of kinetic parameters on extraction temperature, time, ultrasound amplitude, ultrasound on/off pulsed ratio, solvent flow rate, and liquid-to-solid ratio was investigated. Furthermore, process optimization was carried out by desirability method, to maximize process yields and extract antioxidant activity. Results of the experimental plan showed a strong dependence of lycopene recovery from the investigated variables. Reduced quadratic models of response variables were subjected to analysis of variance and indicated as temperature, ultrasound amplitude, and on/off pulsed ratio are the variables that significantly affected the extraction performances ($p < 0.05$). In addition, the modeling of extraction kinetics, allowed to observe the dependence of kinetic parameters representing the initial extraction rate and the equilibrium value on operating conditions. Mathematical modeling, integrating extraction kinetics and response surface modeling, enabled to simultaneously evaluate of the dependence of response variables from multiple input variables available by this technique representing a powerful tool for process optimization.



Preliminary studies for the optimization of ultrasound-assisted extraction of microalgal carotenoids

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Keywords: Carotenoids, microalgae, green solvents, micro-mill, ultrasound

The interest in carotenoids in food science and nutrition is steadily growing due to a great amount of scientific evidence indicating that these can be beneficial for human health. Microalgae are sustainable sources of carotenoids and the main provider of them in the food chain in the marine ecosystem, although their role in the human diet is still negligible.

In addition to sustainable sources, the food industry requires the use of sustainable technologies, such as ultrasound-assisted extraction (UAE), which reduces the time spent and consumption of solvents. The demand for using green solvents such as 2-methyltetrahydrofuran (MeTHF), or ethyl lactate, has also increased. These solvents can replace others commonly used for the extraction of carotenoids, such as methanol or ethanol, which are flammable, volatile, often toxic, and responsible for environmental pollution.

This study aimed to investigate the capacity of green and food-grade solvents to extract microalgal carotenoids by UAE and determine the effect of milling in the extraction. Dimethylsulfoxide (DMSO) a solvent widely used for the extraction of carotenoids from microbial and other sources for research purposes was used for comparison.

Chlorella Sorokiniana (fresh, freeze-dried, and encapsulated) and fresh phytoenrich *C. Sorokiniana* were extracted by UAE (30% amplitude, 2 min) using ethanol, methanol, ethyl lactate, MeTHF, or DMSO, with or without a micro-mill pre-treatment (30 kHz, 5 min).

The micro-mill in freeze-dried and encapsulated *C. Sorokiniana* resulted in a significant ($p < 0.05$) increase in the carotenoid extraction, likely due to the breaking of the microalgae cell wall, which favors the release of carotenoids. However, no significant results were found in the fresh matrices, which may be related to the matrix structure and its water content.



The highest carotenoid concentration in the extracts from fresh *C. Sorokiniana* and phytoene-rich *C. Sorokiniana* were found with methanol, ethanol, or MeTHF. No significant differences were found between them. In the freeze-dried matrix, there were no significant differences between methanol, ethanol, ethyl lactate, and MeTHF. All solvents showed no significant differences regarding total carotenoids extracted in the encapsulated matrix. The highest concentrations of β -carotene and phytoene were obtained with ethanol, methanol, and MeTHF, with no significant differences between them.

MeTHF appears as a promising green solvent to replace organic solvents for the extraction of microalgal carotenoids.



Optimization of an ultrasound-enzymatic assisted extraction for the simultaneous recovery of polyphenols and oil from raspberry pomace using a Definitive Screening Design

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Keywords: berry fruit pomaces, ultrasound-assisted enzymatic extraction, DSD optimisation, phenolic & lipidic compounds recovery

Since 2010, 100 million tons of red fruits are produced each year. Their industrial transformation leads to the waste of around 20% (w/w) of the fruits, as the stems, the peels and the seeds are discarded. These by-products are called the pomace and are mostly burnt, generating CO₂. Yet, the pomaces are rich in bioactive compounds, either hydrophilic (polyphenols) or lipophilic (unsaturated fatty acids, phytosterols and tocols). These components are known for their beneficial impact on human health. This research aims to design an innovative green extraction process to extract simultaneously the hydrophilic and lipophilic compounds from a raspberry pomace and to assess their synergic effect on intestinal homeostasis.

First, several enzymes, such as carbohydrases & proteases combinations, were assessed alone or associated with a physical treatment (ultrasounds (US)). US/enzyme combinations were screened in sequential or combined mode. The extracts were investigated for their total phenolic content, antioxidant activity and lipidic content. The simultaneous combination of an alkaline protease and ultrasounds was selected as the best extraction system due to its high yields and ease of implementation. The process was then optimized using a Definitive Screening Design (DSD), including six factors: temperature (°C), extraction time (min), US amplitude (%), enzyme concentration (%), pH and solid to liquid ratio (%). Three responses were considered in the DSD: the total polyphenol, antioxidant activity and oil-extraction yields. The regression analysis of each obtained response is still in progress, but up to 99% of the total polyphenols, 51% of the active polyphenols and 87% of the oil were recovered into the aqueous media compared to conventional extractions (SLEs in methanol/acetone/water & hexane respectively).



The morphological changes in the raspberry pomace will be visualized thanks to a SEM study. The optimized parameters will be transposed to two other berry pomaces (strawberry & blackberry) and the obtained extracts will be fully characterized.



POSTER PRESENTATIONS



Green extraction of bioactive compounds from bilberry pomace

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Keywords: Bilberry, by-product, pressurized liquid extraction, green extraction, sustainability

Extractions engineered from a concept of environmental sustainability, as pressurised liquid extraction (PLE), were developed as a "green" solid-liquid technology for obtaining bioactive compounds (BACs) from various fruits, and other herbal (by) products. The main advantages of PLE vs. traditional Soxhlet extraction includes improved yield, reduced time and solvent consumption, while inflicting less deterioration to thermolabile compounds. Bilberry pomace (*Vaccinium myrtillus* L), which is a by-product of its juice production, is a valuable alternative source of BACs.

The aim of this work was to evaluate the use of PLE to valorise bilberry pomace. PLE employed water as solvent, varying the static extraction time (SET; 5, 10, 15 min), temperature (40, 80, 120°C) and number of cycles (NC; 1, 2, 3). HPLC-DAD profiled phenolic compounds from the aqueous extracts. Among the seven identified polyphenols, quercetin derivatives predominated in the samples (>60%). The average mass fraction of total phenols (19.49 mg/100 g), which is the sum of total flavonoids (12.70 mg/100 g) and total hydroxycinnamic acids (HCA; 6.80 mg/100 g), was used for PLE optimization. The total phenolic content (TPC) was significantly affected ($p \leq 0.01$) by all studied PLE parameters (i.e. temperature, SET, and NC). The optimal conditions for PLE to obtain an extract with the highest TPC (31.19 mg/100 g) and HCA (10.45 mg/100 g) included $T=40$ °C, SET=5 min, and NC=1. However, to obtain extracts with the highest content of flavonoids, parameters included higher temperature (59.74 °C), SET=5 min, and NC=1. Since pomace is rich in various flavonoids and that they are much more abundant than HCA, a higher temperature probably favours faster diffusion and better solubility of a broader range of analytes in water, hence the need for higher temperature. In conclusion, the use of water as an extraction solvent during PLE shows good prospects for the isolation of BACs from bilberry pomace, which can be further used in the development of sustainable functional food production.



Water-based methods for the solubilisation of rutin

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Keywords: Water Solvents; Water Solubilisation; Methods comparison; Rutin; Natural Products

Water is considered as the greenest solvent. Nonetheless, the water solubility of natural products is still an incredibly challenging issue. Indeed, it is nearly impossible to solubilise many natural products properly using solely water due to their low solubility in this solvent. For instance, the flavonoid rutin – which is theoretically quite polar according to its partition coefficient ($K_{ow} \approx -0.47$) – is only sparingly soluble in water ($S \approx 140$ mg/L). Researchers have tried for decades to tune water properties to enhance its solvent potential in order to be able to solubilise such low-water solubility compounds. Several methods involving the use of solubilisers were described in the early 2000s, namely the use of: salts and/or pH modification, cosolvents, surfactants, complexing ligands, inclusion complexes, stacking complexes and hydrotropes. In recent years, two additional methods have been described as useful to ensure the effective green solubilisation of natural products using water as a solvent. These techniques are water-based natural deep eutectic solvents and switchable water. Up to now, very few studies compared different techniques for the solubilisation of a given natural product, especially not for rutin. The present work aims to compare and analyse all the above-mentioned methods (total of 9), focusing on the rutin case-study. Each method was applied to solubilise rutin and the quantitative results were then compared to determine which is the best for this natural product. Thanks to this comparative study, researchers and industrials will know how to efficiently solubilise a flavonoid such as rutin, using water as solvent.



Isolation of *Thymus Vulgaris* L. Leaf polyphenols by microwave-assisted extraction

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Keywords: Thymus vulgaris L., polyphenols, green extraction, microwave-assisted extraction

Thyme (*Thymus vulgaris* L.) is a Mediterranean herb belonging to the Lamiaceae family well known in the folk medicine for its various health beneficial properties. These properties can be attributed to the high content of biologically active molecules, such as polyphenols. In recent years, the growing demand on functional products enriched with naturally derived polyphenols has brought the need for more sustainable isolation technologies that would allow for their fast and effective isolation. Microwave-assisted extraction has emerged as a green extraction technology due to its main advantage of reducing the extraction time and solvent consumption while achieving higher extraction efficiency and lower degradation of targeted compounds compared to conventional extraction techniques. In the present study, the influence of different microwave-assisted extraction parameters on the isolation efficiency of *Thymus vulgaris* L. leaf polyphenols was examined in two phases. In the first phase, different percentages of aqueous ethanol (0, 30, 50, 70 and 96%) and sample:solvent ratios (1:10, 1:20 and 1:30) were varied while other extraction parameters (temperature 60°C; microwave power 800W; irradiation time 10 min) were kept constant. The total phenolic content (TPC) of the extracts was determined by Folin-Ciocalteu spectrophotometric method. Both ethanol percentage and sample:solvent ratio have shown significant influence on the TPC of the *Thymus vulgaris* L. leaf extracts, and the highest TPC (10.59 mg GAE/g leaf) was achieved when 30% ethanol and a ratio of 1:20 were applied. In the second research phase, the ethanol percentage and sample:solvent ratio were kept constant (30% ethanol and 1:20), while the temperature (40, 60 and 80°C) and irradiation time (5, 10 and 15 min) were varied. The results of Folin-Ciocalteu analysis showed that temperature had a significant influence on the TPC of the *Thymus vulgaris* L. leaf extracts, while the influence of irradiation time was not significant. The highest TPC (11.99 mg GAE/g leaf) was achieved at 60°C after 5 min of irradiation. The results showed how different parameters of microwave-assisted extraction influence the TPC of *Thymus vulgaris* L. extracts, proving the importance of establishing optimal isolation methodologies that would result in the highest recovery effectiveness and utilization of the plants' beneficial properties.



Monitoring the stability of bioactive compounds in strawberry juice treated by high power ultrasound through chemometrics

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Keywords: strawberry juice, high power ultrasound, chemometrics, bioactive compounds, storage

Fruit juices are short-lived products and thermal pasteurization ensures their storage, even if this reduces their quality. Alternatively, non-thermal processing at shorter times is also able to extend their shelf-life while maintaining nutritional and sensory value (e.g. measured by stability of bioactive compounds; BACs). For instance, use of high-power ultrasound (HPU) has great potential as acoustic energy is transmitted instantly through the entire juice volume, thus significantly reducing treatment time and heat exposure. Chemometrics is statistical approach able to provide conclusions from large datasets with utilisation of various multivariate analysis. Hence the aim of this work was to employ chemometrics for choosing HPU parameters for juice processing while monitoring BACs stability during storage as measure of nutritive value (total phenols, monomeric anthocyanins, hydroxycinnamic acids, flavonols, and condensed tannins). Extracted strawberry juices at two ripening stages (75% vs. 100%) were subjected to HPU, with varying the amplitude (25, 50, 75, and 100%), pulse (50 and 100%), and treatment duration (5 and 10 min) that were stored at 4 °C for 7 days. The samples were firstly analysed by exploratory hierarchical Ward's cluster analysis. Samples with different ripeness, pulse, amplitude, treatment duration, content of all bioactive compounds, SSC (%), and pH, showed that ripeness of 100%, a pulse of 50%, an amplitude of 50%, and treatment durations of 5 and 10 min were the most similar to the controls. Other samples, that were similar to controls had maturity of 100%, a pulse of 100%, an amplitude of 25%, and a treatment duration of 5 minutes. HPU samples had similar contents of all polyphenolic groups except condensed tannins (they were higher for HPU samples). Since temperature changes are essential for the retention of majority of bioactives, it was observed how ΔT associated with the HPU parameters (ΔT was strongly positively associated with all HPU parameters). The only exception was the HPU time, with same pattern as the other HPU parameters, except that the correlation was slightly weaker. In conclusion, HPU showed significant potential for industrial juice production.



Microwave Extraction of Polyphenols from Olive pomace of Montenegrin olive variety Žutica as the initial step in waste valorisation strategy

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Keywords: Microwave extraction, alperujo, polyphenols, valorisation, antioxidants

The Spanish term "alperujo" stands for the olive pomace produced by the olive-oil two-phase extraction process. The olive pomace represents a large environmental problem in Mediterranean countries since it contains hazardous materials, such as phenolic compounds, that are harmful pollutants. On the other hand, polyphenols, known for their high antioxidant properties, are getting more and more attention because of their potential in preventing various oxidative stress-related diseases, such as cancer and cardiovascular diseases. Therefore, the extraction of polyphenols is considered one of the solutions for preventing environmental pollution, but also in getting extra profit for valued components in the food and pharmaceutical industries. Having the importance of the phenolic compounds found in olive pomace, the main goal of the research focuses on the implementation of the new extraction technology - the microwave-assisted extraction (MAE) of the polyphenols and the determination of its antioxidant activity.

A sample of the olive pomace was taken from the traditional Montenegrin olive variety Žutica. The sample was dried in a tunnel dryer at 40 °C until the moisture content dropped below 5 w/w%. Central Composite design of experiment (CCD) was selected, where three factors were evaluated- the microwave power (100-800 W), the time (30-180 s), and the solid concentration (1-6 g/50 ml). Twenty extractions were done with the ethanol-water mixture (52,7 %), which was found to be the optimal ratio for the extraction.

The Total Phenolic Content (TPC) of the extracts was determined by the spectrophotometric Folin-Ciocalteu method, while the Antioxidant activity (AA) was done by Ferric Reducing Antioxidant Power (FRAP) method. The highest polyphenol concentration of 1040.687 mg of gallic acid eq per liter (mg GAE/l) was generated after 180 s at 800 W with a solid concentration of 6 g/50 ml. The highest antioxidant activity of 1276.102 mg ascorbic acid equivalents (mg ASE/l) was obtained with the same conditions. Additionally, the response surfaces of the factors were generated, where the used model was reduced quadratic for both responses. The most relevant influence has power and solid ratio. A positive correlation between TPC and AA was found with a correlation coefficient of $r=0.995$

This study could contribute to the research of the efficacy of MAE for the green extraction by comparing the results with different traditional methods, but also other new-technology methods, such as Ultrasound Extraction (UAE).



Fractionation of lipophilic components from rowanberry pomace by SFE-CO₂ extraction and separation at subcritical conditions

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Keywords: Sorbus aucuparia, berry pomace, supercritical CO₂, co-solvent, separation

The aim of current study was the separation of rowanberry pomace lipophilic extract into 'heavier' (HF, 1st separator, S1) and 'lighter' (LF, 2nd separator, S2) fractions. The supercritical CO₂ (scCO₂) extraction with three different concentrations (3%, 5% and 7%) of a co-solvent ethanol (EtOH) was performed at the previously optimized conditions (42.4 MPa; 53 °C) for the recovery of the highest yield from the rowanberry pomace. The separation into 2 fractions was achieved by decreasing the temperature in S1 from 0 to -20 °C at the constant pressure of 7 MPa, while S2 was kept at ambient conditions. The major part of lipophilic compounds always precipitated in the S2, while the contents of fractions were strongly dependent on temperature and the concentration of the co-solvent. In case of -20 °C and 7% of EtOH the recovery of lipophilic compounds in S2 was 6.8 times higher than in S1. The higher recovery from the total amount of β-carotene in the pomace was determined in LF compared to the HF. The highest β-carotene recovery of 31.85% was found at minimum S1 temperature (-20°C) and with maximum co-solvent concentration (7%). The contents of tocopherols and phytosterols in LF exceeded their contents in HF. For instance, the content of β-sitosterol in LF was two-fold higher than that in the HF. The majority of aroma compounds were collected in the LF fraction as well. It may be concluded, that variation in co-solvent concentration and parameters in S1 enable obtaining the products with increased concentrations of different classes of lipophilic compounds.



Valorization of spent coffee grounds by 2-methyloxolane as bio-based solvent extraction. Viable pathway towards bioeconomy for lipids and biomaterials

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Keywords: Up-cycling, 2-methyloxolane, spent coffee grounds, lipid, caffeine

This study attempts to shed light on the efficacy of the solvent 2-methyloxolane (2-MeOx) as an alternative for hexane in defatting spent coffee grounds (SCG). Higher lipid yields were obtained with the bio-based solvent dry 2-MeOx (13.67%) and water-saturated 2-MeOx (15.84%) compared to hexane oil yield which is of petroleum origin and is a known neurotoxin. Palmitic acid and linoleic acid were the principal fatty acids identified. The fatty acid profile of coffee oils obtained with hexane, dry 2-MeOx and aqueous 2-MeOx were similar. Lipid hydrolysis was observed in oils extracted with 2-MeOx which warrants further investigation. The residual caffeine content in the defatted SCG was highest when hexane was used highlighting better solubility of methylxanthine compounds in the solvent 2-MeOx. Within the purview of the biorefinery framework defatted SCG was pressed (Atelier Luma, Tarbes, France) to obtain a board-type material which when optimized can be used as a packaging alternative. Even stationaryaries such as enclosures for pens were fabricated where 50% of the SCG was used as ingredient in the formulation.



Influence of microwave-assisted extraction parameters on brown algae *Dictyota dichotoma* polyphenols

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Keywords: Dictyota dichotoma, polyphenols, green extraction, microwave-assisted extraction

Dictyota dichotoma is a species of brown algae found in rocky littoral and sublittoral zones in a wide range of habitats, from the Atlantic Ocean and the Mediterranean Sea, to the western Indian Ocean. Research has shown that *Dictyota dichotoma* contains a variety of chemical compounds, including pigments, phenols, flavonoids, alkaloids, steroids, tannins, coumarins, quinones, and glycosides. The presence of these biologically active molecules, especially polyphenols, is often associated with numerous health-promoting properties, which is why they are frequently used to fortify functional foods. Microwave-assisted extraction (MAE) is one of the more sustainable isolation techniques that enable rapid and more effective isolation of these substances. In this study, MAE was applied to isolate *Dictyota dichotoma* polyphenols in two research phases. In the first phase, the influence of different percentages of aqueous ethanol (0, 30, 50, 70, and 96%) and sample-to-solvent ratio (1:10, 1:20, and 1:30) were investigated, while temperature (60°C), irradiation time (10 min), and microwave power (800W) were kept constant. The total phenolic content (TPC) of the extracts was determined by the Folin-Ciocalteu spectrophotometric method. The choice of solvent and sample-to-solvent ratio showed significant influence on *Dictyota dichotoma* TPC, and the highest TPC (6.42 mg GAE g⁻¹) was achieved when 30% ethanol and a ratio of 1:60 were applied. In the second phase of the research, temperature (40, 60, and 80°C) and irradiation time (5, 10, and 15 min) were varied, while the ethanol content (30%) and sample-to-solvent ratio (1:60) were kept constant. The results of the statistical analysis showed that temperature had a significant influence ($p \leq 0.05$) on *Dictyota dichotoma* TPC, while the influence of irradiation time was not significant ($p > 0.05$). The highest TPC (3.33 mg GAE g⁻¹) was achieved at 40°C after 5 min of irradiation. The results of this study showed the influence of various MAE parameters on *Dictyota dichotoma* TPC and confirmed the importance of developing optimal isolation method that can lead to the most effective polyphenol recovery.



Optimization of pectin enzymatic extraction from tomato by-products using CALLUCLAST®

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Keywords: Pectin extraction, Celluclast, ANN

Tomato (*Lycopersicon esculentum* L.) is the world's leading vegetable with a production of about 200 million tonnes [1]. The industrial transformation of tomato results in the accumulation of large amounts of by-products composed by tomato peels, seeds and small amount of pulp. Because of the abundance of bioactive compounds in these residues, the appearance of some strategies for using them to obtain compounds of interest, as pectins has been particularly relevant [2]. Pectins are a complex family of polysaccharides, which are present in the cell wall of vegetables and fruits, used as functional food ingredient due to their biological and technological properties. They are composed of three domains i) a linear chain of α -1,4-D-galacturonic acid (GalA) called homogalacturonan (HG) comprising approximately 70% of pectin, partially methylesterified at C-6 or may be O-acetylated at O-2 or O-3; ii) side chains of GalA and α -(1, 2) linked L-rhamnose with galactose and/or arabinose branches, rhamnogalacturonan I (RG-I) and iii) RG-II, a very complex chain consisting of 12 different sugars and over 20 different linkages [3].

Pectin is used in the food, pharmaceutical, medical and cosmetic industries. Different methods have been developed to obtain maximal yield and quality of pectin and among all of them; enzymatic extraction is a methodology which is part of the principles of sustainability and circular economy currently in force. Therefore, in this study, pectin extraction was optimised from tomato pulp as pasteurised pulp (PP) and freeze-dried pulp (FD-PP) with Celluclast®1.5L, using an experimental design analysed by artificial neural networks (ANN), that allows modelling complex and highly non-linear biotechnological processes. Pectins obtained at optimal conditions were characterised determining their monomeric composition (GC-FID), methyl-esterification degree (FT-IR), and molecular weight (HPSEC-ELSD).



The independent variables (corresponding to the input layer of the ANN) were Celluclast dose (30-60 U/g) and extraction time (6-24 h), and the dependent variables (corresponding to ANN output layer) were pectin yield, GalA and glucose content as non-pectic sugar, determined by GC-FID. In all cases, the analysed variables by ANN presented high regression values (R^2 and $R^2_{adj} > 90\%$). The optimised conditions to maximize extraction yields and GalA level while minimised glucose content were for PP 24.8 h and 50 U/g obtaining 12.1% of pectin with 60.3% of GalA and 17.6% of glucose. The conditions for FDP were 27.6 h and 30.5 U/g, obtaining a 6.0% of pectin with 56.2% and 22.0% of GalA and glucose respectively.

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Comparative evaluation of cyclodextrin enhanced microwave and ultrasonic-assisted extraction of polyphenols from olive pomace

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Keywords: olive pomace, microwave-assisted extraction; ultrasonic-assisted extraction, cyclodextrin, polyphenols

Only 2 % of phenolic compounds are transferred to olive oil during production process and this is why olive pomace has recently been targeted as a valuable and highly available source of characteristic secoirridoids - tyrosol, hydroxytyrosol and oleuropein. The aim of this research was to develop fast, efficient green procedures for their extraction and for that purpose cyclodextrin (CD) encapsulation was combined with either microwave (MAE)- or ultrasound-assisted extraction (UAE).

Major independent variables tested were: time of extraction, ethanol concentration, microwave/ultrasound power, probe diameter (UAE) and the type of CD used. Major responses observed were total antioxidants (TA), hydroxytyrosol (HTS), tyrosol (TS) and oleuropein (OLE) that were determined by spectrophotometric method (TA), high performance liquid chromatography coupled with a fluorimetric detector (HTS and TS) and ultra-performance-liquid chromatography-electrospray-ionization-tandem mass-spectrometry (OLE).

The optimal MAE conditions, resulting in the highest yields were 700 W, 10 min and 20% ethanol and was additionally improved by complexation with hydroxypropyl- β -cyclodextrin (HP β CD). The optimal UAE conditions was 20-minute pulsed extraction with 60% ethanol using 12 mm probe at 100% output intensity and it was also additionally improved by HP β CD (probably by increasing solubility of targeted compounds and protecting them from degradation during extraction).

Generally, UAE was found to be more potent technique for extraction of polyphenols from olive pomace in comparison to MAE (UAE yields were: 887 mg/kg HTS, 1117 mg/kg TS, 1744 mg/kg OLE and 55.2 mg TE/kg versus MAE yields of 954 mg/kg HTS, 180 mg/kg TS and 40 mg/kg TE). OLE was undetectable in MAE extracts, probably due to degradation under more severe extraction conditions. Obtained results will contribute significantly to improved management and further investigation and utilization of olive polyphenols in food and pharmaceutical industry.



Enabling technologies and green solvents for lignin valorisation

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Keywords: Lignin, Microwaves, Ultrasound, Oxidation, Biorefinery

Lignin represents the most abundant by-product of the biorefining process of lignocellulosic biomass. However, its variable, complex and irregular structure makes it recalcitrant to chemical and biochemical degradation processes. Furthermore, the wide distribution of molecular mass still limits its scope of application mainly to the energy supply of the refining plant. However, the economic and environmental competitiveness of biorefineries can be improved by lignin valorisation and conversion into aromatic monomers and other value-added products. The use of enabling technologies such as microwaves and ultrasounds, in combination with green solvents, can make the delignification and conversion processes more sustainable, reducing treatment times, energy consumption and environmental impact. In this poster presentation lignin extraction processes from residual biomasses mediated by ultrasounds and microwaves, in the presence of NaDES (Natural Deep Eutectic Solvents), will be described. Furthermore, microwave-assisted aerobic catalyst-free oxidation of native and extracted lignins will be presented, showing the formation of different value-added products such as antioxidants and bioaromatics, but also long-chain compounds such as fatty acids (LCFA), alcohols (LCA) and hydrocarbons (LCH), potential precursors for the synthesis of biofuels. The synergy between technologies exploiting the formation of high-energy micro-environments and NaDES therefore allows the development of sustainable and versatile processes for the conversion of lignin into different classes of products, for different applications.



Variability in antimicrobial activity of *Satureja montana* subsp. *montana* based on different extraction methods

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Keywords: Satureja montana subsp. montana, Extraction, Antimicrobial activity

S. montana is a well-known plant in traditional medicines of many Mediterranean countries, as well as spice and flavouring agent. Its high biological potential, due to established antibacterial, antifungal, antioxidant, etc. properties, makes it exceptional raw material for pharmaceutical and food industries. In order to achieve high-quality extract/product and fit into the circular economy principles, green technologies found their place.

Green technologies use a new approach in the extraction of important bioactive compounds from different sources. These techniques are recognized to have significant potential in the extraction process regarding less requirement of time, reduction of energy, and allow the use of alternative solvents. In this research, besides essential oil and hydrolate, the novel and green methods such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and subcritical water extraction (SWE) were used. Since in the literature there is no reported information about different extraction methods of *Satureja montana*, the present study has aimed to evaluate antimicrobial activity against a wide spectre of microorganisms. The in vitro antimicrobial activity of *S. montana* was tested by inhibition zone diameters using the disc diffusion method and quantified by determining the minimal inhibitory concentration (MIC) using the microdilution assay, as well as, defining kinetics of antimicrobial effect using pharmacodynamics test.

Differences in the chemical compositions not only reflect the variability of the genus *Satureja*, but also the variability within the same species, which is in correlation with the results of antimicrobial activity of tested sample. Gram-positive bacteria were more sensitive to the effect of tested samples, especially in the case of essential oils and hydrolate. Essential oil of *Satureja montana* had relatively high antimicrobial activities against almost all tested bacterial strains, as well as two yeast representatives. Hydrolate revealed antibacterial activity only against cocci bacteria and tested yeasts, while this sample was not effective against *B. cereus*, Gram-negative bacteria, and fungi. On the other hand, tested SWE sample revealed antibacterial activity against only *B. cereus*. Different UAE and MAE extracts did not exhibited antimicrobial activity, except



methanolic UAE extract of *S. montana* subsp. *montana* which inhibited *S. cerevisiae* and *C. albicans*. The obtained results suggest synergistic and combined activity of the present dominant constituents in *Satureja montana* extracts, while their content was directly connected to the applied extraction technique.



Utilization of different extraction methods to isolate β -carotene produced by *Rhodotorula mucilaginosa*

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Keywords: β -carotene , carotenoid- producing yeasts, extractions, *Rhodotorula mucilaginosa*

Carotenoids have found application in food, cosmetics and pharmaceuticals industries, and the production itself is usually performed by chemical or physicochemical methods. According to recent findings, these methods are limited in scope and high a production cost, which directs research to biotechnology production of carotenoids from various sources. Therefore this research presents the isolation of targeted β -carotene from *Rhodotorula mucilaginosa* strain isolated from a Jerusalem artichoke sample. This study aimed at investigating differences in β -carotene yield based on different extraction methods (conventional, ultrasound, and their combination) using acetone as a solvent. For this purpose, the yeast culture was incubated for 5 days on Sobouraud Maltose Agar at 30 °C, obtained biomass was resuspended in phosphate buffer, centrifuged and dried to the constant mass using lyophilisation. Then, three different cell lysis methods were performed on dried yeast biomass (chemical treatment, ultrasound, osmotic shock). Based on the obtained results, the selection of the extraction method plays a crucial role in the β -carotene yield. Briefly, the best extraction method involves a two-step process including vortex and ultrasound treatment during the contact with the selected solvent, while the best lysis method was chemical treatment using sodium carbonate. In summary, *Rhodotorula mucilaginosa* represents a good source of β -carotene, but the obtained yield strongly depends on extraction methodology.



Graševina grape pomace by-product as a rich source of biologically active compounds

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Keywords: extraction, grape pomace, green technology, NADES, polyphenols

Agro-industrial sustainable economy indicates further exploitation of waste disposal because industry by-products are a source of esteemed bioactive compounds. Grape pomace is a major by-product coming from the winemaking industry and is generated in large quantities every year. To improve the environmental impact of the wine production process, grape pomace is used as a source for the isolation of phytochemicals such as phenolic acids, flavonoids, and tannins. Optimization of extraction techniques is an important step to achieving a maximum level of phenolic compounds from the source. Extraction processes favour the implementation of novel green technology methods as an alternative to energy-consuming and aggressive techniques that are still used. Solid-liquid extraction is a classic method for extracting polyphenols made by agitation at a certain temperature using different solvents. Natural deep eutectic solvents (NADES) fulfil principles of green chemistry, making them a green alternative to conventional solvents, usually different organic chemicals. NADES are an easy-to-formulate mixture of naturally occurring primary metabolites with hydroxyl, carboxyl, or amino groups that provide hydrogen bond interactions, resulting in structured liquid solvents. NADES have adjustable viscosity, a broad range of polarity, and most importantly, the capacity to solubilize a wide variety of bioactive molecules. NADES increase extraction efficiency due to the possibility of NADES being tailor-made depending on the physicochemical properties of desired extracts. The formation of additional hydrogen bonds between NADES and solutes increases the solubilization strength of NADES. While using NADES as a solvent, extraction can be further improved by using a microwave or ultrasound-assisted extraction process. NADES are not only used for extraction but also as a solvent for organic synthesis, biocatalysis, separation process, electrochemistry, etc. In this work, we used NADES as solvents of choice for the polyphenols extraction of Graševina white grape pomace. We also compared different NADES with conventional types of solvents and different extraction techniques. Using alternative green technologies for the extraction of bioactive molecules results in a higher yield in a short extraction period with minimized energy consumption.



Semi-industrial subcritical water extraction: a step forward in green extraction

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Keywords: Solvent-free extraction, subcritical water, semi-industrial process, by-product valorization, polyphenols

The strong hydrogen bonds among water molecules at room temperature determine a high dielectric constant and a high polarity. One of the most versatile and eco-friendly methods exploits the unique features of subcritical water extraction (SWE) over the boiling point up to 150°C to 160°C (max pressure 5-6 bar). In these conditions, hydrogen bonds are broken, and water changes the polarity and dielectric constant, improving the extraction power and mimicking the behaviour of hydroalcoholic mixtures. Though several scientific articles demonstrated the huge potential of SWE, only few studies have been focused on scaling up and industrial applications [1].

In the last few years, our group investigated this method from lab to pilot scale [2]. More recently thanks to the collaboration with Tropical Food Machinery srl, we scaled up the lab-optimized protocols to semi-industrial level developing a new patented prototype extractor. The latter is equipped with two 100-liter pressurized extraction tanks with five metallic cylindrical baskets where biomass can be placed and subjected to a radial and vertical flow of pressurized hot water. After 15 to 30 minutes, the solution is transferred to an expansion tank and subjected to flash evaporation with a rapid temperature drop. In this work, we present the valorisation procedures of three agri-food chain by-products: hazelnut pericarps, blueberry pomace, and pomegranate peels. These matrices were subjected to subcritical water extraction protocols to obtain polyphenol-rich extracts. The lab scale optimized procedures were scaled up for the industrial production.

The data obtained, compared with conventional extraction methods using organic solvents, such as hydroalcoholic solution (ethanol/water mixtures), confirmed the efficacy of SWE as a valuable green method to replace conventional procedures. All the extraction at semi-industrial level resulted in comparable yields and total phenolic content giving encouraging outcomes for the industrial production.



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Integrated green approach to extraction of bioactive compounds from orange peel dust-waste generated in the filter tea factory

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Keywords: Orange peel, herbal dust, ultrasound-assisted extraction, supercritical extraction, bioactive compounds

Orange peel waste (OPW), as a by-product of the citrus processing industry, and it represent a rich source of bioactive compounds. During processing in the filter tea industry, a large amount of material with a particle size <0.315 mm remains, which is eventually disposed of, as it can not be used in the further production of filter tea form. This fraction is called herbal dust of orange peel (OPD) and is a raw material that can be valorized and used as a potential source of various high-value compounds (polyphenols, carotenoids, dietary fiber, sugars, essential oils, etc.). Conventional extraction methods can be used to isolate these compounds, however, due to the insufficient efficiency and cost-effectiveness of these methods, there is a problem of low recovery of citrus agro-industry waste. Therefore, in this paper, the emphasis is on "green", attractive extraction methods. The use of supercritical carbon dioxide (SFE-CO₂) extraction is widely recognized as a safe and effective solution to the problems that accompany conventional methods. According to the green concept, SFE-CO₂, in addition to being faster and more efficient, also prevents the decomposition of thermolabile compounds, avoiding the use of toxic solvents, enabling the production of solvent-free extracts. This method was used as a pre-treatment for the ultrasound-assisted extraction (UAE). The UAE is considered a cheap, simple and efficient extraction technique for obtaining extracts of better and more uniform composition in a significantly shorter time and lower energy consumption. The efficacy of UAE for the isolation of bioactive compounds from OPD with SFE-CO₂ pretreatment was investigated using an ultrasonic probe. During the SFE-CO₂ process, the extraction time was 4 h, the temperature was kept constant (40°C), and the extraction pressure (100-300 bar) varied. After that, UAE was performed where the sonication amplitude (20-100%) was varied, while the temperature was defined as a constant (50°C). Changes in extraction time, ultrasonic power and energy consumption were observed. The chemical composition of the obtained extracts



was assessed by HPLC. Compounds identified as dominant are hesperidin, naringin, narirutin and rutin. Hesperidin stands out as the most dominant compound, whose concentration ranged between 848 ± 6.50 to 884.64 ± 21.59 (ug / ml) depending on the applied process parameters. The results show that pre-treatment of SFE-CO₂, followed by the use of UAE using an ultrasonic probe can significantly improve the extraction of valuable compounds from plant material, thus proving to be a value-added process and a potentially efficient technique used at the industrial level.



Recent Advances in Microwave-Assisted Extraction: A Case Study Roadmap

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Keywords: Microwave, Polyphenols, Essential Oils, Food wastes, Pilot reactor

Waste reduction is one of the key principles of circular economy, aiming to transform products that are at the end of their production chain into resources for other purposes and thus close loops in industrial ecosystems. This dissemination of sustainability awareness means that ever more attention is being paid to the reduction of waste and pollution in the agro-food industry. Hence, the recovery of biologically active compounds such as polyphenols and terpenes, from residues or even from fresh matrixes, is a crucial step for sustainable applications in pharmaceutical and food industries. In particular, essential oils market saw a huge demand increase, due to flavouring and therapeutic uses, led by the *Cannabis sativa* L. liberalization in several USA states.

The abovementioned processes are typically performed under conventional solid-liquid extractions, such as maceration, percolation or Soxhlet and hydro/steam distillation. Old-fashioned and conventional methods require high energy consumption and long extraction times, with partial recovery of the desired compounds. Over the last decade, attention has shifted to the development of innovative enabling extraction techniques aiming to overcome these issues. The use of Microwave-Assisted Extraction (MAE) offers a number of advantages: rapid heating, shorter process time, reduction in solvent usage, higher reproducibility, higher extraction rates, and increases in yield. Extraction rates and yields, in particular, can be increased by the enhancement of heat and mass-transfer phenomena, working in synergy.

In this contribute, the focus is to highlight the extreme versatility and the technological readiness of MAE techniques. With this aim, different matrixes are reported to be fruitfully processed taking advantage of MW-assisted hydrodistillation (MAHD), such as orange peel, blueberry peels, chestnut peels as food wastes, but also *Cannabis sativa* L., hops and microalgae as fresh materials. Metabolites quali/quantification (i.e. polyphenols, terpenes/sesquiterpenes, cannabinoids, tannins, poly-unsaturated fatty acids) together with extract activity (antioxidants and biological) fully supported the worthiness of this technology. In addition, example of pilot scale reactor paves the way for industrial applications and commercial perspectives.



Protective effect of *Pistacia atlantica* Desf leaves on Mercury-Induced Acute Nephro-toxicity in Rats

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Keywords: Mercury, kidney, antioxidant enzyme, Pistacia atlantica, rat

Mercury is known to accumulate in living organisms, causing serious damage. An important characteristic of mercury toxicity is the generation of free radicals. The purpose of this study is to evaluate the protective effect of the aqueous extract of *Pistacia atlantica* against mercury-induced oxidative stress in rats. Albinos Wistar rats were treated with mercury chloride (HgCl₂) (at 2.5mg/Kg/1 times per week) combined or with the aqueous extract of *P. atlantica* (at 150mg/Kg weight) over a 32-day period. Our results showed that HgCl₂ administration significantly decreased the glutathione level and the enzymatic activity of the antioxidant system CAT, GPx, GST at the renal level. These changes were associated with an increase in the lipid peroxidation as expressed by a high level of renal MDA and hydroperoxides. However, supplementation with the aqueous extract of *Pistacia atlantica* has modified the toxic effects of mercury by improving certain disturbances. These findings may indicate an antioxidant and protective effect of this plant's extract against the harmful effect of mercury.



Grinding and extrusion treatments: new trends in green extraction

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Keywords: Reactive extrusion; Process intensification; Green chemistry

Mechanochemical treatments are the oldest humankind's processes. By grinding with a mortar and a pestle, the first extractions of natural pigments from biomasses were performed in prehistorical time. Today, after thousands of years, mechanochemical processes are exploited again as frontiers in extraction and synthesis due to their advantages in terms of economic and environmental sustainability. Grinding and cryogrinding in particular are unique procedures to prepare biomass for following extraction or direct use[1].

Reactive extrusion belongs to mechanochemical methods. While extruders are widely exploited in food and polymer industry, their use extraction and biomass conversion is just at the beginning[2]. This technology has been recently applied in biomass valorisation and different synthetic procedures (APIs, MOFs, nanomaterials).

In contrast with conventional methods which have high solid/liquid ratio, extrusion process use wet matrices or solventless conditions, saving energy and reducing wastes[2].

Objective of this work is showing different and innovative applications of reactive extruders in biomass valorisation. Bioactives such as polyphenols can be extracted from residual biomass with other enabling technologies and included in a polymer structure, obtaining a functional polymer with antioxidant properties. Such polymer can be used in the production of active packages, allowing for greater product shelf-life while avoiding the addition of chemicals such as BHT[3].

Biomass delignification can improve the yield of subsequent processes such as the conversion to platform chemicals or fermentation to bioethanol. Reactive extrusion with the use of NaOH - by wetting the biomass or by using the base as a solid - is suitable for lignin removal, resulting in an enriched holocellulose fraction[4] which can be easily treated with higher yields.



Recent applications of reactive extrusion in green processes will be studied in the next years, while further investigating and optimizing the ones described above. It's potential in developing greener processes still remains largely unexpressed and ready to be unleashed.

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Application of ultrasonic probe for the extraction of polyphenols from ginger (*Zingiber officinale*) herbal dust

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Keywords: ginger, herbal dust, valorization, ultrasound-assisted extraction, polyphenols

Application of green extraction technologies in order to valorize by-products and waste generated in various branches of industry has been in the focus in the last decade. One of the sectors where by-products and wastes of plant material, e.g. ginger (*Zingiber officinale*) are generated in high quantities is the herbal filter tea sector. During the herbal filter tea production, a certain amount of plant material with particles lower than 0.315 mm and smaller than the pore size of filter tea bags is generated. This fraction called herbal dust could not be used further in the production of filter herbal tea and is considered as a by-product or waste.

Ginger herbal dust has potential positive health benefits due to the anti-inflammatory, antioxidant, antitumor, and antibacterial activities of ginger, as well as its positive effect on preventing nausea. Ginger is rich in several functional compounds, including terpenes, lipids, organic acids, and fibers, but the phenolic compounds are the major contributors to its bioactivity.

A number of researches have been devoted to developing reliable, and economically and ecologically feasible green methods for the extraction of bioactive compounds from the targeted plant material. In the present study, the efficiency of ultrasound-assisted extraction (UAE) with an ultrasonic probe for isolation of phenolic compounds from ginger herbal dust was determined. The conventional solid-liquid extraction was applied as a reference technique in order to select the optimal extraction solvent (50% ethanol). The sonication amplitude (20, 60, 100%) was varied, while the temperature was the limiting factor (50 °C). Changes in extraction time, ultrasonic power and energy consumption were observed. The highest extraction yield (7.49%) was obtained using the amplitude of 20%, while UAE with the highest amplitude provided the lowest yield (6.57%). Maximum values of total phenolic (112.26 mg GAE/ml extract) and flavonoid (80.45 mg CE/ml extract) contents were obtained using 100% and 60% amplitude, respectively. Results described in this work demonstrated the good possibility of using UAE as an efficient technique for the valorization of by-products from agro-industry. The present study also highlights the potential application of ginger herbal dust extracts as an ingredient in new functional foods, nutraceuticals and cosmetic formulations.



Cavitation Technology - The Future of Greener Extractions?

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Keywords: Ultrasound (US), Hydrodynamic cavitation (HC), Pilot Scale Polyphenols Recovery, Residual Biomass.

Low frequency ultrasound (US) and hydrodynamic cavitation (HC) are key enabling technologies for greener process intensification and are unique non-thermal techniques that see widespread use in food extraction and processing [1]. The advantages of cavitation-based extractions are to eliminate the application of toxic solvents, reduction of extraction time and to achieve better extraction yield, as well as purity. The cavitation phenomena enhance the extraction efficiency via increased mass transfer rate between the substrate and solvent, following the cell wall rupture, due to the intense implosion of bubbles. The cavitation-based extractions, that include mainly the ultrasound-assisted extractions (UAE) and the hydrodynamic cavitation extraction (HCE), have recently been implemented at pilot scale for the extraction of high-value-added compounds both from native and residual biomasses [2]. In fact, the huge amount of material discarded by the agri-food production chain lays down a significant challenge for emerging technologies that can provide new opportunities by recovering valuable by-products and creating new applications. For instance, antioxidants present in plants, vegetables, and fruits can prevent cardiovascular diseases, cancer, premature aging and hence making them as one of the valuable ingredients of extraction. As antioxidants can prevent the cell oxidation process, there is a high demand in food, pharmaceutical and cosmetic industries and looking into the usefulness of bioactive compounds, their effective extraction is crucial.

Herein we report different cavitation-based extraction protocols (from lab to pilot scale) for the efficient (in water) recovery of polyphenols starting from different agri-food residual biomasses: grape stalks, wheat straw, and orange peels [3]. The extracts were characterized using HPLC-DAD, UPLC-ESI-MS/MS, DPPH• assay (2,2-diphenyl-1-picrylhydrazyl) and TPC assay (total phenolic content). The flow-mode cavitation-based extractions were carried out in a 15 L UAE or a 10 L HCE reactors. A semi-industrial decanter unit and a bag-filter were the keys units of the downstream operations. The resulting particle-free solution underwent nanofiltration on a membrane pilot skid, providing a final polyphenols-enriched stream as shown by the antioxidant activity and TPC.



The design of an efficient industrial recovery of polyphenols from agro-food by-products requires data from pilot plant experiments. The flow-mode extraction processes using cavitation reactors, enable an easier scaling up to industrial production.

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The effect of essential oil and extract from sage herbal dust on the shelf-life of grounded meat

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Keywords: sage herbal dust, extraction, meat, oxidation, safety

The effect of essential oil and extract obtained from sage (*Salvia officinalis* L.) herbal dust (food industry by-product) (SEO; SE) on microbiological and oxidative stability of fresh pork grounded meat stored for 8 days at 3 ± 1 °C was investigated. Conventional (hydrodistillation) and novel (supercritical fluid extraction – SFE) extraction techniques were used for recovery of sage essential oil. The grounded meat, back fat and other ingredients were blended for approximately 5 min, until the homogenous batter was obtained, and the temperature of approximately 7 °C was recorded. The resulting mixture was divided into seven batches. Sage essential oil (SEO) and sage extract (SE) were added separately in six of them, at concentrations of 0.05 µL/g (SEO1; SE1), 0.075 µL/g (SEO2; SE2) and 0.1 µL/g (SEO3; SE3), representing different treatments. pH, microbiological analysis, TBARS value and sensory panel scores were assessed. Oxygenated monoterpenes (α -thujone, camphor and eucalyptol), oxygenated sesquiterpenes (viridiflorol) and diterpene polyphenols (epirosmanol) were the most abundant compounds present in SEO and SE samples. SEO addition resulted in significant ($p<0.05$) inhibition of microbial growth, while the lowest microbial counts was obtained in the treatment SE3 (0.1 µL/g). The addition of SEO and SE significantly ($p<0.05$) reduced the TBARS values of fresh pork grounded meat. Moreover, SEO and SE had a positive effect on sensory properties of this meat product. Hence, the results of this study showed significant antioxidative and antimicrobial activities of essential oil and extract obtained from sage filter tea processing by-products and potential of its utilization in production of fresh pork grounded meat in order to enhance their stability and safety.

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Green-based microwave-assisted extraction for isolation of anthocyanins from black elderberry pomace

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Keywords: elderberries pomace, anthocyanins, microwave-assisted extraction, valorization

Due to the growing influence of oxidative stress on the development of many diseases, there is a tendency to isolate and select natural compounds with antioxidant activity. Anthocyanins have been described as compounds that prevent or inhibit oxidation by scavenging free radicals and reducing oxidative stress. The main compounds of the black elderberry (*Sambucus nigra* L.) are anthocyanins mostly cyanidin-3-sambubioside (C3-Sam), cyanidin-3-glucoside (C3-Glu), and cyanidin-3-galactoside (C3-Gal)¹. These anthocyanins remain in the elderberry pomace after juice pressing, being discarded.

In order to isolate valuable compounds from elderberry pomace, green-based microwave-assisted extraction (MAE) has been applied. MAE is emerging as a good alternative to the conventional extraction of bioactives, mainly due to the relatively high extraction yield, short extraction time, and simplicity². MAE has been performed at temperatures from 40 to 120 °C, for 5 and 10 min, with 30% aqueous ethanol solution as solvent. Solid-liquid extraction (SLE) was used as a reference extraction technique for the selection of optimal extraction solvent. The contents of C3-Gal, C3-Glu, and C3-Sam were determined and quantified by high-performance liquid chromatography with a diode-array detector (HPLC-DAD).

The most dominant anthocyanin was C3-Sam, followed by C3-Glu and C3-Gal. By increasing the temperature from 60 to 120 °C during 10 min, the concentration of C3-Sam significantly decreased from 904.00 to 116.18 µg/mL extract. The highest concentration of C3-Glu (724.18 µg/mL of extract) was obtained at extraction conditions of 60 °C and 10 min, while the most suitable MAE conditions for C3-Gal (55.72 µg/mL of extract) were the extraction temperature of 100 °C and extraction time of 10 min. The concentrations of all three anthocyanins were higher during 10 min of MAE in comparison to 5 min, at temperatures from 40-80 °C, while at higher temperatures, the



trend was opposite.

Based on the obtained results, MAE was shown to be a suitable technique for the isolation of anthocyanins from elderberry pomace. In addition to significantly reduced extraction time, higher content of the target compounds was obtained.



Phenolic composition of Algerian *Tetraclinis articulata* (Vahl) Masters Leaves : A promising source of bioactive compounds

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Keywords: Tetraclins articulata (Vahl) Masters, HPLC-DAD-MS/ESI, aqueous extracts, organic fractions, phenolic compounds.

The last decade has seen several important developments with respect to herbal medicines and many natural products have been widely used as medicinal agents for preventing and treating different types of diseases. These matrices are rich in phenolic compounds that are responsible for a wide range of health benefits, finding important applications in the food, cosmetics and pharmaceutical industries (Rached et al., 2018). In this context, *Tetraclins articulata* (Vahl) Masters crops are one of the most important medicinal herbs in the world, described as having antitumoral, antioxidant, antibacterial and anti-inflammatory activities, directly related with the presence of phenolic compounds. Therefore, the separation and identification of phenolic compounds of the crude aqueous extract of the leaves of *T. articulata* and its subsequent organic fractions (ethyl acetate and butanol) were studied by using high-performance liquid chromatography (HPLC) with diode-array detector (DAD) and electrospray ionization mass spectrometry (MS). The analysis of the obtained results showed that B-type (epi)catechin dimer and catechin were the most abundant molecules, among the nine different flavonoids identified,



namely: ((epi)catechin, myricetin, quercetin and kaempferol glycoside derivatives). The concentration of phenolic compounds in the ethyl acetate fraction (93.1 mg/g extract) was higher than the one of the aqueous extract (21.2 mg/g extract) and also than the one of the butanol fraction (43.87 mg/g extract). These findings provide a scientific basis for the traditional uses of *T. articulata* and promotes its potential use in several industrial sectors as the food, cosmetics and the pharmaceutical ones.



Effect of supercritical CO₂ extraction parameters on the fatty acids composition of graševina grape seed oil

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Keywords: supercritical CO₂ extraction, fatty acids, grape seed oil, Graševina.

Grape pomace, generated during winemaking, represents an important raw material for obtaining valuable products. Among them, seed oil has great potential in food industry as an alternative source of vegetable oils, due to the high content of essential fatty acids. The aim of the proposed research was to investigate the effect of supercritical CO₂ (SC CO₂) process parameters, in comparison to cold pressing, on the fatty acids composition in Graševina grape seed oils. The parameters varied during the SC CO₂ extraction were: pressures (300, 400 and 500 bar), temperatures (35, 45 and 55 °C) and gas flow (15, 30 and 45 g/min). The fatty acids composition was analysed by GC-FID according to the ISO 5509:2000 method. Dominant fatty acid in all extracted samples was linoleic acid, followed by oleic and palmitic acid. Moreover, saturated fatty acids accounted up to 12.8%, monounsaturated up to 22.6% and polyunsaturated fatty acids up to 66.02%. Although the differences among samples were slight, statistical analysis of variance (ANOVA) showed that most of them were significant ($p < 0.05$). Cold pressed oil was characterized with significantly higher concentration of linoleic acid, and significantly lower concentration of oleic acid. On the other hand, significantly higher concentrations of palmitic acid were detected in SC CO₂ extracted oils. Regarding the treatments applied, significantly higher concentrations of most of fatty acids were obtained after application of pressure at 400 bars, temperature at 55 °C and flow rate of 15 g/min of CO₂.



Recovery of pumpkin by-products by hybrid vacuum drying and ultrasound pre-treatment

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Keywords: bioactive compounds, vacuum, ultrasonic bath, antioxidant capacity, organic residue

According to the new guidelines for waste management, procedures to prevent the generation of waste and techniques to recover it take precedence over recycling and disposal. Plant food waste (organic residues) and by-products from fruits and vegetables represent a nutritionally valuable source rich in numerous specialized metabolites important for human health. Therefore, the recovery of such by-products into valuable resources is crucial for the production of new food products, especially in order to reduce food waste.

The pumpkin by-product is also a valuable source of numerous metabolites, especially vitamins, dietary fibers, polyphenolic compounds and carotenoids, and therefore represents a valuable material for further utilization. One of the ways to produce new products is the drying process, which very often has a negative impact on the nutrient composition of the plant material due to the high temperature and is also energy consuming.

For this very reason, innovative pre-treatment methods such as ultrasound and hybrid drying techniques (vacuum-conduction) are being developed. Vacuum drying uses low pressure to evaporate water at lower temperatures, preserving heat-sensitive bioactive compounds such as vitamins, carotenoids, and other phytochemicals, while ultrasound pre-treatment also offers a number of benefits, most notably significantly shortening the drying process and preserving nutrients.

The aim of this study was to determine the influence of temperature (60 and 70 °C) and ultrasound pre-treatment (37 kHz, 380W, Elmasonic P 300H, ultrasonic bath 100%) during hybrid vacuum-conduction drying (100 mbar) of pumpkin organic residue on the content of specialized metabolites. Drying processes were performed in a vacuum drying oven VO 200 (Memmert, Germany) at a pressure of 100 mbar.

The pumpkin slices were dried to a final water content of 14%. The highest vitamin C content (439.14 mg/100 g DW) was determined in pumpkin samples pre-treated with



ultrasound for 5 min and dried at 70 °C; total phenolic content (1655 mg GAE/100 g DW) was also determined by vacuum drying at a temperature of 70 °C without ultrasound pre-treatment. The β -carotene content was best preserved by vacuum drying at 60 °C with ultrasound pre-treatment for 10 min.

In general, all dried pumpkin samples are characterized by high antioxidant capacity, proving that hybrid vacuum drying combined with ultrasound pre-treatment has a positive effect on nutritional potential. In conclusion, ultrasonic pre-treatment had a significant effect on the preservation of bioactive compounds in the dried pumpkin samples, while drying by vacuum (100 mbar) at both combined temperatures (60 and 70°C) showed no negative effects on the nutritional composition of the pumpkin samples.

Therefore, it can be concluded that this method of drying and ultrasonic pre-treatment is an efficient way to preserve the nutritional composition of the raw material, thus giving the organic residues the possibility of additional recovery and further use and utilization.



Circular-based alternative protein extraction from grape seeds

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Keywords: Circular economy; grapeseed protein, EIT food, Caviro

The project has the aim of the production and commercialisation of circular-based environmentally and economically sustainable new protein, named PROSEED, from the grapevine seeds, which is an abundant by-product of the wine production processes. PROSEED alternative protein product focus on being higher value circular applications within a several production chains and being a product usable as primary ingredient for new nutritional additive in healthy food industry. The new product will be the result of an innovative industrial bioprocess of protein recovery in full scale plant scale up designed by Caviro SCA and Caviro EXTRA, based on pilot system based on detannified grape seeds. An important goal is to replace the animal-based and foreign vegetable – based proteins currently used in wine clarification, achieving a vegan and gluten – free products by employment of the grapevine – seed proteins as well their introduction as ingredients in the food and beverage industry, especially in nutraceuticals field.

In addition, the project will contribute to the reduction of the environmental footprint, as the CO₂ emission, coming from the use and the production of the current primary ingredients e.g. in comparison to the animals protein, actually in use, we estimate a reduction of 82%.

Grape seed's protein could satisfy broadly increased demand of alternative proteins in European Community considering the acute feed – protein deficit in EU.

Supported by the European Institute of Innovation and Technology of Food (EIT Food), a body of the European Union, we invest in projects, organisations and individuals that share our goals for a healthy and sustainable food system. We unlock innovation potential in businesses and universities cooperation to bring new technologies and products to market. We link entrepreneurs and professionals with the skills needed to transform the food system and reconnecting them to the origins of their food.



A comparative exploration of the phytochemical profiles of *Allium ursinum* extracts obtained by sustainable extraction techniques

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Keywords: Allium ursinum, ultrasonic assisted extraction, subcritical water extraction, polyphenolic compounds, sulphuric compounds

The growing awareness on implications related to the use of harsh chemicals and non-sustainable extraction methods of bioactive compounds from plants is causing a shift towards a more eco-friendly approach. Therefore, this study present extraction of bioactive compounds from *Allium ursinum* by applying modern, sustainable and green (ultrasound assisted extraction- UAE and subcritical water extraction- SWE) extraction techniques. Main extraction parameters for SWE (temperature, acidifier content, extraction time) and UAE (temperature, ethanol concentration, extraction time and ultrasonic power) were optimized, in order to obtain the extracts with the highest content of targeted bioactive compounds. Chromatographic analysis (HPLC) was used to detect phytochemical composition and quantify polyphenolic and sulphuric compounds in obtained optimized extracts.

Comparing the polyphenolics profiles of the analysed extracts, the SWE extract is higher in total polyphenolic compounds, than UAE extract. However, more polyphenolic acids were extracted by UAE, while SWE showed to be was more suitable for flavonoids extraction. Based on the spectrum of dominant components in the tested extracts, it can be assumed that the extract obtained by SWE contains compounds such as phenylpropanoids and flavonoids.

Analysing sulphuric compounds content in obtained extracts, UAE showed to be more effective towards the extraction of allicin, diallyl disulfide and diallyl trisulfide, while a smaller amount of allyl sulfide, diallyl disulfide and S-methylphosyl methanethiones was detected in extract obtained by SWE.

Based on chromatographic analyses of extracts obtained under optimal conditions of UAE and SWE, it can be concluded that SWE is more suitable for the extraction of polyphenolic compounds, while UAE is more effective for the extraction of sulphuric compounds from *A. ursinum*.



According to phytochemical composition of *A. ursinum* extracts they may be utilized in food products as a natural antimicrobial additive, or can improve the nutritional profile of foods. Nevertheless, extract may be used as a dietetic supplement. Therefore, in accordance with obtained results, the appropriate extraction technique should be selected to obtain the maximal yield of the target compounds and determine further application of the extract.



β -carotene extraction from *Rhodotorula glutinis* yeast – a step forward for the valorisation of olive mill wastewater

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Keywords: β -carotene; extraction; ultrasound; microwave; modelling

The idea of a circular economy approach based on reducing waste and recycling products has shaped recent policy efforts since it responds to both environmental and economic challenges. Thus, materials that are currently considered low-value waste must be treated as a renewable feedstock, for instance olive mill wastewater (OMW). OMW is an abundant and highly pollutant bio-waste so it is imperative to sustainably manage this residue.

Nevertheless, there has been an impetus search for natural colorants to be applied in food industry due to the growing consumers' demand for natural ingredients, as well as peoples' awareness for health hazards associated with synthetic dyes. Therefore, natural colorants have received considerable interest to replace the commonly used synthetic pigments. In this sense, considerable efforts have been dedicated to the development of an efficient, cost-effective and sustainable extraction process for its resource recovery.

However, there is a limited number of approved natural pigments. Carotenoids are a well-known class of natural pigments, found in microorganisms and responsible for many promising properties, such as antioxidant, anti-inflammatory and anticancer activities. Hence, it is of great interest to develop environmentally friendly extraction procedures for these high-added value compounds. This can be achieved through bioremediation using *Rhodotorula glutinis* yeast, which has the ability to produce carotenoids, such as β -carotene. Thus, using *R. glutinis* for the biodegradation of OMW allows the valorisation of a residue while being an attractive solution for the production of natural colorants. Still, the choice of extraction type, process parameters and the suitability of solvents remains challenging.

Herein, we compared conventional solid-liquid extraction (SLE), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) for the recovery of β -carotene using different solvents, including greener and environmentally friendly solvents, acceptable in food industry. Furthermore, the effect of a pretreatment was studied, as well as the influence of various process parameters. Alongside the experimental analysis, a diffusion-based mathematical model has been implemented



and numerically solved. It enables the prediction and understanding of experimental results, while decreasing the expenses, because of process optimization and design on both scales, industrial and laboratory. Furthermore, it offers β -carotene mass transfer phenomena, including rate and efficiency of the process. In summary, we believe that the recovery of natural pigment represents an important research field, showing a greener approach with valuable data.



Valorisation of vineyard pruning residues by using microwave and ultrasound extraction technologies

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Keywords: microwave and ultrasound-assisted extraction; biomass valorisation, vineyard waste, polyphenols and resveratrol.

Viticulture generates a significant amount of biomass residues containing valuable bioactive compounds (polyphenols, e.g. resveratrol), which have high potential to use in medicine, agriculture and plant protection, as well as in the food industry. Ultrasonic extraction and microwave extraction, named as green extraction technologies due to their low energy consumption and the safe solvent use, can be applied to separate polyphenols and resveratrol from the solid residue. This study evaluated the effect of these two technologies as well as the grapevine cultivar and the cane dormancy phase on the content of polyphenols and resveratrol in the obtained extracts.

The experiment was conducted in the laboratory of modern extraction technologies of Polli Horticultural Research Centre of Estonian University of Life Sciences in Polli, Estonia. Pruning residues were collected from two hybrid grapevine cultivars ('Zilga', 'Hasansky Sladky') during two dormancy phases (endo-, eco-dormancy). Microwave (5 min, 10 min, power 100W) and ultrasound assisted extraction (power 20%, 40%, 5 min) methods were applied to obtain polyphenol-rich extracts. The content of total polyphenols and resveratrol (mg/ 100 g dw) in the extracts was determined by HPLC.

The results confirmed the significant impact of grapevine cultivar and dormancy phase on the content of polyphenols and resveratrol in pruning residue extracts. The best time to collect pruning residue for high levels of polyphenols and resveratrol was during cane endo-dormancy. The microwave-assisted extraction (10 min, 100 W) showed the best results when compared to ultrasonication treatment.



Antioxidant potential of by-products extracts from traditional Mediterranean productions

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Keywords: valorisation of by-products, green solvent, microwave-assisted extraction

Recently, research has focused on the reuse of by-products from the food industry to achieve sustainable production and reduce waste generation. The aim of this study was to investigate the potential utilisation of by-products from selected traditional Mediterranean productions as antioxidant agents.

Samples of blackberries, cherries and aronia fruits were collected from local Dalmatian juice and tea producers. The samples were shade-dried and extracted in a green solvent (ethanol-water mixture) to ensure that the extracts were safe for food use. The extracts were obtained in an advanced microwave extraction system at 600 W for 5 minutes. Ethanol was evaporated from the extracts, and the remaining water was freeze-dried.

Total phenolic content (TPC) and chemical profile were determined as well as antioxidant potential. The profile was analysed by high-performance liquid chromatography (HPLC), and for antioxidant potential, 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH), ferric reducing capacity (FRAP), and oxygen radical absorbing capacity (ORAC) were used.

The highest TPC results of the samples ranged from 601.9 ± 13.9 to 3186.1 ± 96.6 mg gallic acid equivalents/L, with the highest concentration found in aronia by-products. All extracts had high inhibition of DPPH radicals, ranging from 80.1 to 84.0%. The highest reducing activity was found for the extract from blackberry by-products. The extracts were diluted 1000-fold before ORAC analysis.

The results ranged from 36.1 ± 1.1 to 9.5 ± 0.7 μ M Trolox equivalents, with the highest result for aronia by-products. A correlation was observed between the TPC and ORAC results. The predominant constituents of the extracts were rutin, protocatechuic acid, chlorogenic acid, and gallic acid, all phenolic acids known to be potent antioxidants, with protocatechuic acid dominating in aronia by-product extracts. The composition of phenolic acids was most diverse in the extract of aronia berries, which exhibited high antioxidant activity.



Blackberry by-product extract was rich in rutin, while gallic acid and protocatechuic acid were detected in lower amounts. Cherry by-product extract showed the lowest antioxidant potential, and rutin was the only phenolic compound identified in the sample. These results show that by-products from traditional Mediterranean fruit juice/tea production could be used for the production of extracts with high antioxidant activity that can be reused in the food industry.

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Application of ultrasound-assisted extraction in combination with ethanol to improve antioxidant activity of diatom extracts

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Keywords: diatoms, ultrasound, ethanol, antioxidant activity

Marine microalgae are cellular factories for a wide range of bioactive compounds. Among them, diatoms are an unconventional group that is attracting increasing attention due to their high content of fatty acids, sterols, pigments, etc. In this study, 150 mg of freeze-dried biomass of *Skeletonema grevillei* and *Thalassiosira rotula* were extracted in 6 mL of 50% and 70% ethanol assisted with ultrasound for 1 hour. In the extracts, total phenolic content (TFC) and antioxidant activity was determined. Antioxidant potential was tested by two methods, ferric reducing/antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC). Prior to the determination of TFC, FRAP, and ORAC, the extracts were concentrated to a volume of 3.5 mL. In 50% ethanol extracts of *S. grevillei* and *T. rotula* the TPC was 2.53 and 3.178 mg GAE/g of dry diatom biomass, respectively. The TPC of the 70% ethanol extracts was higher, 2.65 and 5.80 mg GAE /g. Similar activity was determined for the 70% ethanol extracts of *S. grevillei* and *T. rotula* by FRAP, 707.69 and 766.67 $\mu\text{m TE}$, respectively. Similarly, the antioxidant activity of *T. rotula* extracts tested by ORAC showed higher results for 70% ethanol extract, 39.82 to 58.87 $\mu\text{m TE}$ in comparison to 58.87 obtained for 50% ethanolic extracts. On the other hand, the extracts of *S. grevillei* prepared with 70% ethanol did not show higher antioxidant activity by ORAC. Overall, the antioxidant activity and phenolic content were higher in *T. rotula*. The obtained results showed that diatoms may be considered as a source of antioxidants, and the knowledge from this study will contribute to the further biotechnological application of diatom and microalgae.



Mathematical modelling of avocado waste drying

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Keywords: modelling, avocado, waste, drying

By-products such as seeds and peels are currently considered a waste in the industrial processing of avocados. Such material is rich in phenolic compounds which can be further exploited for obtaining new high-value products. Drying milled seeds and peels is an excellent method for prolonging the shelf life of those by-products, while simultaneously minimizing the loss of antioxidants and other nutritionally important compounds. The avocado mix (peels and seeds) was dried using a convective dryer at temperatures of 50, 60, and 70 °C and airflow of 1 ms⁻¹.

Optimization of the drying process is performed, and mathematical models of drying were obtained. Based on the ratio of dimensionless moisture number and drying time, 5 mathematical drying models were tested. Correlation of drying parameters and model coefficients was analysed using non-linear regression modelling in Statistica software. Obtained RMSE and other statistical parameters were used to determine the goodness of fit. The best quality of fit was obtained using Page and Midilli-Kucuk models with $R^2 > 0.95$. and Analysis of data shows a direct correlation of temperature to drying time, with 60 °C independently of mix ratio and airflow, and that those two models were optimal for prediction of the performance of avocado peels and seeds drying and optimization of intrinsic variables such as drying temperature and air velocity.

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Textural and rheological testing of jams treated with high-intensity ultrasonics

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Keywords: texture, rheology, jam, mechanical properties

Jam is food with intermediate moisture, and it is usually microbiologically stable due to large amounts of sucrose. However, it can be spoiled due to osmophilic acid-tolerant flora, and from the industrial standpoint to ensure the safety of the final product during packaging, hydrogen peroxide is usually used as an antimicrobial agent. This leads to changes in surface appearance such as colour and can also change some textural and rheological properties.

Textural characteristics as one component of the sensory properties of food have a very important role in the acceptability of food, as it influences mouthfeel. Mechanical properties of food and foodstuff are specific for the type of the food, but most important are hardness, elasticity, stress, and strain, as well as several others relevant to gel-like materials such as extrusion force, adhesion, spreadability, and stickiness. Those properties have a very important role in further processing steps in the food industry such as pumping, extrusion, drying, baking, and others. Rheological properties such as yield stress and shear stress are also in direct correlation to jam quality and its functionality in the development of final products. Therefore, it is important to retain desired properties while introducing novel food processing technologies for microbial inactivation.

Based on the power, amplitude, intensity, and other parameters such as the amount of liquid in the processed system, ultrasonic waves can produce a cavitation effect, which disperses large amounts of thermal and mechanical energy in the system. High-intensity ultrasonic treatment can thus introduce several changes in the cellular structure of treated material, which can consequently exhibit significant changes in textural and rheological properties.

Methods for determination of the textural properties of jams and eventual changes after ultrasonic treatment are based on the instrumental texture analysis with various probes such as conical spreadability probe, and back and front extrusion cells. Rheological properties of jams are measured using a viscometer at different temperatures (20 °C



to 60 °C) and different shear rates (form 0.1 s⁻¹ to 150 s⁻¹) and calculation of the relationship between shear stress and shear rate is performed using Herschel – Bulkley model. The time dependency of jams at those parameters is calculated based on the kinetic mathematical models by various authors such as Weltman, Figoni, or Hahn.

Based on the correlation of ultrasonic parameters such as power and sonication time to measured textural and rheological parameters, optimal processing parameters can be determined to obtain desired log₅ reduction of microorganisms and thus ensure microbiological safety, while retaining optimal functional properties of jams.

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Valorization of grape pomaces: Green extraction of grape seed oil using supercritical CO₂

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Keywords: supercritical CO₂, green extraction, grape seed pomace, grape seed oil, antioxidant capacity

The grape pomace obtained after pressing is a valuable by-product, containing about 7-22% grape seed oil. This oil is a rich source of essential unsaturated fatty acids, especially linoleic acid, as well as other bioactive compounds with strong antioxidant activity such as vitamin E active compounds, phytosterols and polyphenolic compounds. In contrast to conventional solvent extraction, supercritical CO₂ extraction (SC CO₂) of oil from grape seed pomace can be used as an environmentally friendly alternative. Namely, by using green, non-toxic, non-flammable and low-cost CO₂ as the extraction fluid, the negative environmental factors of conventional solvents can be reduced. The aim of this research was to study the effect of SC CO₂ extraction (pressure, temperature and CO₂ flow rate) on the extraction efficiency of grape seed oil and its antioxidant capacity. The extraction process was monitored, and samples were taken every 10 min during the 90-min extraction period, and the extraction yield (%) was calculated for each sampling point. The antioxidant capacity of the extracted oils was analyzed using the lipophilic ORAC method. The results showed that the application of higher pressure (500 bar compared to 300 bar) at 45 °C and a CO₂ flow rate of 15 g/min during the first 30 min of SC CO₂ extraction contributed to a higher grape seed oil yield. However, the observed trends afterwards tended to disappear, since no significant differences were obtained among samples. Moreover, the same trend was noticed when a higher CO₂ flow rate of 45 g/min was applied. Interestingly, the application of a high temperature (55 °C, 400 bar) did not have a favorable effect on the extraction yield, as significantly higher values were obtained when a lower temperature (35 °C, 400 bar) was used. Moreover, the effect of temperature was more pronounced at a lower CO₂ flow rate of 15 g/min during the first 30 min of extraction. Finally, the CO₂ flow rate of 15 g/min compared with 45 g/min affected the extraction kinetics and contributed to lower extraction yields at the beginning of the process, but the final trend obtained among counterpart samples depended primarily on the applied pressure and temperature. Moreover, the highest extraction yields were obtained at 400 bar and 35 °C, regardless of the applied CO₂ flow rate. However, the highest antioxidant capacity was achieved employing 400 bar, 35 °C, and 15 g CO₂/min.



Valorization of sea buckthorn berry (*Elaeagnus rhamnoides* (L.) A. Nelson) oil as a potential source of lipophilic bioactive molecules

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Keywords: sea buckthorn pulp oil, α -tocopherol, fatty acids, sterols, antioxidant capacity

Production of natural compounds from medicinal plants with numerous biological effects has become very popular due to increasing concerns about the safety of using synthetic substances and the effects of COVID -19 on health. Sea buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson), (SB) is known as an important source of natural antioxidant and antimicrobial bioactive molecules (BAM), but also, when added to foods, improves sensory properties, microbiological stability, and shelf life of products. The best-known sea buckthorn product is berry oil (SBO) (Yu et al., 2017), which contains large amounts of lipophilic BAM such as unsaturated fatty acids, tocopherols, carotenoids, and sterols (Koskovic et al., 2017), but differences in composition have been found depending on the cultivar, growing conditions and extraction methods used (Christe et al., 2020; Cenkowski, 2006). Supercritical CO₂ extraction of oil as an advanced method is gaining popularity due to its better extraction efficiency compared to conventional extraction methods. Moreover, CO₂ as an environmentally friendly and safe solvent (GRAS) can be easily removed from oil. Therefore, the aim of this study was to determine the content of fatty acids, sterols, and α -tocopherols, as well as the antioxidant capacity (AC) of SBO obtained by supercritical CO₂ extraction from SB berries from Croatia. The tocopherol content of the SBO extracts was determined by HPLC/FLD, the content of fatty acids and sterols by GC-MS and AC by the lipophilic ORAC method. A total of 16 sterols were determined, with sitosterol (589.28 mg/100 g oil) being the most abundant, followed by campesterol (31.97 mg/100 g oil), uvaol (21.79 mg/100 g oil), and obtusifoliol (12.99 mg/100 g oil). High content of unsaturated fatty acids such as omega-7 palmitoleic acid (34.54%) and omega-6- γ -linolenic acid (10.78%) was



also found. SBO was characterized by high content of α -tocopherol (275.63 mg/100 g oil) and high antioxidant ORAC capacity (1676.90 μ mol TE /100 g oil). The results suggest that the SBO obtained by supercritical CO₂ extraction is a rich source of various lipophilic BAM with high antioxidant capacity, which could be used for the production of natural products, functional foods and as food additives.



Green volatile compounds in olive pest controlling

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Keywords: green volatiles, olive fruit fly, pest control, sustainable pest management

Olive fruit fly (*Bactrocera oleae*, Rossi) and olive moth (*Prays oleae*, Bern.) are the most economically important olive pests, causing yield losses in all olive-growing areas where they are detected. For years, the use of pesticides was the main method of controlling them, and their intensive use had negative effects on the environment. Therefore, the EU aims to reduce the use of pesticides by 50% by 2030 and 100% by 2050. For all these reasons, more effective non-pesticidal baits are needed for monitoring and/or control of *B. oleae* and *P. oleae*. Current knowledge indicates that several insect species are attracted to natural volatiles, including pheromones, host plants, yeasts, and bacterial volatiles.

Since the interaction between olive trees and olive pests has not been adequately studied, the objective of this study was to identify olive tree volatiles that might be responsible for attracting *B. oleae* and *P. oleae* and to test them in olive orchards. During the study, about 70 different volatiles were identified from olive flower buds, 4-6 mm fruits and leaves, and 100 different volatiles were identified from olive fruits at three stages of ripeness (green, semi-ripe and ripe), mainly belonging to the groups of esters, saturated hydrocarbons, aldehydes, alcohols, terpenes and sesquiterpenes. These results are used to identify volatiles that could be responsible for olive moth attraction. Therefore, selected volatiles will be tested in delta traps in several olive orchards. Volatiles that could be responsible for olive fruit fly attraction will also be selected and tested on yellow sticky traps in other olive orchards. The results of our research could provide answers to many questions about the relationship between olives and pests. Finding volatiles that could be responsible for attracting harmful species could lead to the development and improvement of new attractants that could be a useful tool for monitoring and/or controlling *B. oleae* and *P. oleae* in the future.



Small-scale external consumer testing on the acceptability of beverages based on nades extract

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Keywords: Small-scale consumer testing, Beverages, NADES extract

The aim of this study was to provide a research team with a cheap way to get valuable information of beverages based on NADES extract using a small small-scale external ranking test. Five types of similar samples were included in the test of which three were commercially available beverages on the local market: peach-flavored iced tea (IT1), black elderberry-flavored iced tea (IT2) and forest-flavored iced tea (IT3).

The remaining two research samples had the following characteristics: a beverage to which 0.5 g of NADES raspberry extract (NR) was added and another to which 2 g of NADES thyme extract (NT) was added. A convenience sample of subjects was used: they were essentially passers-by taking part in the larger city event. In total, 93 subjects, willingness to take part in testing, without aversion to the ingredients used for beverage and without any known food allergy, took part in the study. Demographic information collected were gender, age spanned unevenly from 18 to over 50 years old, and frequency of ice tea drinking (at least once a week; once a month; never). For all ranked attributes, significant ($p < 0.05$) differences were evidenced among five samples, indicating that consumers' preferences contributed to obtaining differences between the ranked samples. Each ranking test was split for the attributes of color, odor, sweetness, sourness, flavor, and overall likeability. The lowest rank (1) corresponded to the least likeability, whereas the highest rank (5) corresponded to the most likeability. No ties were allowed, but consumers were allowed to restate any sample.

NR was the best ranked compared to the remaining test samples in relation to color. The colour of NT products was the least acceptable in relation to the samples of iced tea samples from the market. Furthermore, consumers showed a smaller difference in likeability of NT sample for odor property compared to IT1 as the best ranked sample as well as for sourness of NR sample compared to IT3 and IT1, the best ranked samples.



The obtained results showed that the ranking method can be a valuable tool for sensory comparison of own products with corresponding competing products during their development. It also provides the possibility to identify possibly poor performing products and outstanding good performing products, why expensive big scale consumer tests can be limited into only including products that likely needs improvements or that in a larger scale test could be proven “best in rank”.



Application of natural deep eutectic solvents coupled with ultrasound-assisted extraction for the attainment of *Lavandula stoechas* extracts

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Keywords: *Lavandula stoechas*, green solvents, deep eutectic solvent, extraction

Lavandula stoechas L. is an evergreen shrub widely applied in ethnomedicine worldwide. This herbal specie is a rich source of bioactive components which represent significant ingredients and materials in the food, cosmetic, and pharmaceutical industries. Therefore, the potential of *L. stoechas* is confirmed, as well as its application in various areas. However, continuous research is necessary to establish efficient and environment-friendly procedures for the attainment of high-quality and safe *L. stoechas* products.

In this study, alternative extraction solvents, the natural deep eutectic solvents (NaDES), and ultrasound-assisted extraction (UAE) were applied to obtain extracts of *L. stoechas* abundant in bioactive components. Three different NaDES mixtures were used: betaine:ethylene glycol (Bet:EG) (1:3), betaine:glycerol (Bet:Gly) (1:2), and glycerol:glucose combination (Gly:Glu) (4:1), while the UAE was conducted at the temperature of 30 and 60°C and constant extraction time of 60 min. The content of polyphenolic compounds in obtained extracts was determined by high-performance liquid chromatography (HPLC) analysis with UV detection. Moreover, antioxidant activity was measured using three assays: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and FRAP (ferric reducing antioxidant power assay). Also, UAE was conducted with conventional solvents (water and 96% ethanol) for comparison.

The most dominant compound in the extracts was flavonoid rutin (78.17-355.34 µL/mL), followed by herniarin (5.63-81.35 µL/mL). In addition, coumarin and phenolic acids (ellagic, caffeic, ferulic, syringic, and sinapic acid) were detected. Furthermore, the highest polyphenolic content was recorded in extracts obtained by using the Bet:EG mixture. Additionally, extracts obtained with the same NaDES mixture exhibited the highest antioxidant activity (DPPH: 0.351174 µL/mL; ABTS: 0.500329 µL/mL; FRAP: 0.697028 µL/mL). Conversely, the extracts obtained with conventional solvents had significantly lower antioxidant activity and polyphenolic content.



The obtained results demonstrated that new generation solvents can provide extracts rich in bioactive components with high antioxidant activity and represent a promising alternative to the conventional solvents.

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Organic acid and biomass production of propionic acid bacteria grown in lactose and lactate-based broths

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Keywords: dairy, whey, fermentation, green extraction, valorization of food by-products.

The valorization of food production waste into new products is an actual topic nowadays. Five strains of propionic acid bacteria (PAB) (*Acidipropionibacterium acidipropionici*, *Propionibacterium cyclohexanicum*, *Propionibacterium freudenreichii*, *Acidipropionibacterium jensenii* and *Acidipropionibacterium thoenii*) were investigated for their ability to produce organic acids and biomass in laboratory-scale fermentation. The production of propionic acid and acetic acid was considerably influenced by the added carbon source (Na lactate or lactose), but did not differ between PAB strains ($p \geq 0.05$). The concentrations of propionic acid and acetic acid produced in the lactate broths were 7.7–8.6 g L⁻¹ and 3.5–3.9 g L⁻¹, respectively. Lactose broth fermentation resulted in a significant pH reduction, and propionic and acetic acids' outcomes were significantly lower ($p < 0.05$) – about two times less – which was most likely due to acid-induced inhibition. The results also indicate that lactose consumption, substrate conversion efficiency as well as biomass production are strain-dependent. Stirring reduced propionic acid outcome. Biomass production in lactose-based broths was significantly higher ($p < 0.05$) than in lactate-based broths. Neutralization of the environmental acidity during acid production could be a suitable solution to the above-mentioned inhibition problem, as well as to achieve a higher concentration of acids or their salts with respect to the stability of the fermentation product (for example, to prevent the evaporation of undissociated volatile acids).

Since lactose-rich dairy waste is a significant threat to the environment, its conversion into new products is of great interest, and the knowledge of this study can help to model PAB metabolite and biomass recovery by valorizing dairy waste through an environmentally friendly process.



Pulsed electric field-assisted extraction of nutrients and antioxidant bioactive compounds from mushrooms

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Keywords: Pulsed electric field; mushrooms; extraction; bioactive compounds; antioxidant; optimization

Pulsed electric field (PEF) technique has been used over the last years as an alternative, innovative and sustainable technology for the recovery of bioactive compounds and nutrients from food and side streams. Different mushrooms (*A. bisporus*, *P. ostreatus*, *L.edodes*, *A.brunnescens*) were chosen in this study for their nutritional profile to recovery of high-added-value compounds under PEF optimal conditions (2.5 kV/cm, 50 kJ/kg and 6h of total extraction time) and the results of recovery of carbohydrates, proteins, total antioxidant capacity and minerals such as Mg and P were compared with a conventional aqueous extraction (6h in agitation). After the different extractions, the PEF pre-treatment showed an increase in the recovery of macronutrients higher than 300%, total antioxidant capacity and minerals reaching 49.1% and 61.63% for Mg and P, respectively. In addition, statistically significant differences were observed between the different mushrooms, highlighting *L. edodes* the highest recovery results of carbohydrates, and antioxidant capacity after PEF. Moreover, it covered a ration 63.34% of the recommended daily intake of P. Regarding minerals, *A. bisporus* stands out with the highest contents of P and Mg. These results suggest that PEF pre-treatment is effective for the extraction of beneficial compounds for health in a sustainable and innovative way that allows compliance with the Sustainable Development Goals related to climate change and health, making it very useful in matrices of high nutritional value such as mushrooms.

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Recovery of polyphenols, antioxidant compounds and minerals from spirulina: Influence of supercritical fluid extraction

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Keywords: microalgae, supercritical fluid extraction, polyphenols, minerals, heavy metals

Microalgae represent a new source of nutrients and bioactive compounds, with a more sustainable production. In particular, spirulina is the most widely known and consumed microalgae worldwide. On the other hand, conventional extraction of these compounds involves long extraction times, as well as the use of toxic solvents and a large amount of energy. In this sense, extraction with supercritical fluids represents a sustainable alternative, with high process efficiency and in an environmentally friendly way.

In the present work, a supercritical fluid extraction (SFE) is compared with a conventional stirred extraction (used as a control). For this purpose, 5 g of spirulina were used for each process. The supercritical fluid extraction (SFE) was carried out for 1h, using a temperature of 50°C, a pressure of 25 MPa and a 90:10 CO₂:ethanol ratio, with a flow rate of 16 mL/min. For conventional extraction, 5 g of microalgae were suspended in 50 mL of pure ethanol. The mixture was stirred at 500 rpm for 30 minutes at room temperature and protected from darkness. The process was done twice. Finally, the solvent was evaporated, and the dried residue of the SFE extract and the conventional extract were resuspended to a final concentration of 1 mg/mL. Antioxidant capacity was measured by TEAC and ORAC assays. The phenolic profile was analysed by Triple TOF-LC-MS-MS. Minerals and heavy metals were analysed by ICP-MS.

The results obtained for antioxidant capacity revealed that no differences were obtained between the two extracts when measuring antioxidant capacity by TEAC assay. However, in the ORAC assay, the extract obtained by SFE showed a higher antioxidant capacity. Regarding the phenolic profile, the extract obtained by SFE mainly detected 4-hydroxybenzaldehyde and benzoic acid. On the other hand, in the conventional extraction, these polyphenols were also detected in addition to glycitin. Concerning the minerals, the SFE extraction recovered 77% more Mg than the conventional extraction. However, the conventional extraction obtained a higher yield for the recovery of P and Ca. Finally, for heavy metals, a higher Pb extraction was observed for the SFE extract, while for Hg there are no significant differences between both extractions.



These results demonstrate that SFE technology can be a valuable tool to sustainably extract bioactive and health-relevant compounds. However, potential contaminants such as Pb, which would also be increased after the process, need to be controlled.

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Comparison of different acrylamide green extraction methods from food matrix

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Keywords: AA, ASE, SFC, SPE, green extraction

Acrylamide (AA) is a processing-based contaminant produced in food as a by-product of the Maillard reaction in starchy foods processed at high temperatures (>120 °C) such as bread, coffee, and cookies. It is classified by the IARC as a probably carcinogenic to humans (Group 2A) and presents others adverse health effects which are related to its neurotoxicity and reproductive toxicity. The aim of this work is to compare several acrylamide green extraction methods from food and subsequent quantification of AA using HPLC coupled to a UV detector. The green extraction techniques compared were: QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), accelerated solvent extraction (ASE), supercritical fluid extraction (SFE) and solid phase extraction (SPE). The food matrices studied were coffee and cookies spiked with concentrations ranging from 4 µg/L to 300 µg/L. The SFE was carried out using a supercritical extraction system (JASCO, Tokio, Japan) and ASE was performed by ASE-150 (Thermo Scientific) both located at the Faculty of Pharmacy (University of Valencia, Valencia, Spain). Detection was carried out using UV-1570 (Jasco). The best results were obtained using ASE combined with the use of MgO and ZnSO₄ with different recovery percentages that reached up to 90%. ASE uses low energy for its operation and allows extraction without the use of organic solvents. Instead of organic solvent water was used as extractant liquid due to AA is very polar. Similar recoveries were obtained by SPE, where different columns were studied, and carbon cartridges showed the best results. In conclusion, the main advantage of ASE as a green extraction process over SPE is the low cost and the automation of the process. In addition, more studies are needed about ASE as a technique for decontamination of AA in food.



Potential applications of avocado byproducts in various industries

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Keywords: avocado, byproducts, bioactive compounds, peel, seed

The avocado (*Persea americana*) is a fruit widely consumed throughout the world and is an important dietary source of valuable essential nutrients and phytochemicals. Due to the high consumption and industrial processing of avocado, large amounts of residues, peels and seeds are generated, which represent a major environmental problem due to the large production volumes and the inadequate and insufficient use of avocado residues. Currently, most of this waste is discarded and underutilised. The pulp of the avocado and its waste (peel, seeds, pulp after oil extraction) contain bioactive compounds such as ascorbic acid, vitamin E, phenolic compounds and carotenoids, high levels of proteins, carbohydrates (starch, fibre), monounsaturated fatty acids and minerals (especially potassium). Extracts from all parts of the avocado fruit exhibit antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, and antiatherogenic properties. Due to their numerous health benefits, the consumption and production of avocados has increased greatly in recent years. Therefore, the pulp and waste of avocado are a potential source of bioactive compounds suitable for use in foods or dietary supplements. In addition, avocado seed waste is the main source of biomaterials such as starch and can therefore be used as an alternative starch source in the textile industry. Avocado seeds are also rich in antioxidants, natural dyes, biodiesel, and a phenolic compound that can be used for various applications in the pharmaceutical and cosmetic industries. The processing of avocados produces large quantities of avocado peels, which contain large amounts of valuable compounds. Avocado peels are a remarkable source of biomolecules, including phenolic and flavonoid compounds, and can be used as a food preservative, antioxidant, or functional ingredient in novel food formulations. The use of avocado byproducts as a source of natural compounds is a way to extract value-added ingredients while having a significant impact on avocado industrial applications and the environment.

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Influence of convective drying and ultrasound-assisted extraction on the recovery of proteins and sugars from pumpkin pulp (*Cucurbita maxima* D.) and its fractions

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Keywords: Cucurbita maxima, dried pumpkin, pumpkin by-products, ultrasound assisted extraction, nutritional profile

Drying is an important method to prevent post-harvest losses of pumpkin fruit and to obtain a more stable pumpkin powder. Pumpkin powder has a longer shelf life, desirable sweet taste and yellow-orange color, and could be used as a functional ingredient and natural color additive due to its nutritional and health-promoting properties. The simultaneous separation of pumpkin pulp for drying purposes resulted in large amounts of the waste fractions, i.e., the peel and seeds. Considering the nutritional potential of all pumpkin (*Cucurbita maxima* Duchese) products, i.e. pulp, dried pulp and by-products, the aim of this work was to compare the amounts of proteins and sugars obtained from them. Drying was carried out at temperatures of 50, 60 and 70 °C and air velocity of 0.5 m/s. To maximize the recovery of proteins and sugars from the pumpkin samples, ultrasound-assisted extraction was performed at a frequency of 37 kHz and a temperature of 50 °C for 10, 20, and 40 min. The results showed that all dried pumpkin pulp samples contained significantly higher amounts of proteins (7.80-12.67%) than raw pulp (1.03-2.30%), making them a potential source of nutrients. As the results show, a drying temperature of 60 °C can be considered optimal for protein extraction during 20 minutes. The optimal time for sugar extraction with ultrasonic treatment was set at 40 minutes with mass fractions of 49.39%, 49.70%, and 49.44% at temperatures of 50, 60, and 70 °C, respectively. Obviously, the dried samples are a valuable source of sugar compared to the raw pumpkin, which contains 2.99-5.61% sugar depending on the extraction time. The protein and sugar content is also higher in the pumpkin by-products than in the raw pulp. The results show that the amounts of sugars are 1.10-1.79% (seeds) and 4.86-9.11% (peels) and proteins are 4.31-4.76% (seeds) and 1.87-7.54% (peels), depending on the extraction time. In conclusion, all pumpkin components studied in this work can be considered as a valuable source of nutrients. Considering that seeds and peels are usually discarded, both by-products are interesting from a nutritional point of view and could be reused in line with global sustainable trends in the use of food by-products.

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Content of Carotenoids and Other Pigments in the Transition from the Green to the Red Stage of *Haematococcus pluvialis* Microalgae

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Keywords: photobioreactor, microalgal culture, Haematococcus pluvialis, carotenoids, astaxanthin

Haematococcus pluvialis is a unicellular freshwater alga containing many bioactive compounds, especially carotenoids, which are the strongest antioxidants among the pigments. This study evaluates the composition and content of carotenoids and other pigments in both stages of algae life cycle, especially in the green vegetative stage, less studied in comparison to the red stage.

To determine the composition and content of carotenoids, a combination of HPLC-DAD and LC-QTOF-MS was used. The content of carotenoids in the green vegetative stage was significantly lower than in the red stage. In the green vegetative stage, 16 different carotenoids and other pigments were identified. Among the total 8.86 mg g⁻¹ DW of pigments, 5.24 mg g⁻¹ DW or 59% of them were chlorophyll a with its derivatives, and 3.62 mg g⁻¹ DW or 41% of them were free carotenoids. After the transition from the green to the red stage, the carotenoid composition was replaced by secondary carotenoids, astaxanthin and its esters, which predominated in the whole carotenoid composition. In addition to free astaxanthin, 12 astaxanthin monoesters, 6 diesters and 13 other carotenoids were determined. The majority of 37.86 mg g⁻¹ DW pigments were monoesters. They represented 82% of all pigments, and their content was about 5 times higher than both, diesters (5.91 mg g⁻¹ DW or 12% of all) and free carotenoids (2.4 mg g⁻¹ DW or 6% of all).

The results of the study contribute to the data on the overall pigment composition and content of *H. pluvialis* algae and provide the basis for further improvement of cultivation of the *H. pluvialis* alga.

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Use of orange peel waste as a source of active substances for incorporation in chitosan coatings applied on commercial polymeric film

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Keywords: food waste, green extraction, chitosan, functional coating

Multifunctional packaging systems have a high potential for commercial packaging applications. By applying the layer of the biopolymer coating on the polymer surface, it is possible to change its barrier properties and can serve as a transporter to deliver active substances having a special function to packed food. Life style and market demands pushes food industry towards the development of new materials that consequently leads to the increased product shelf life while minimising the impact on the nature and climate. The main aim of this study was to investigate the possibility of applying naturally friendly chitosan coating on the surface of commercially available oriented poly(ethylene-terephthalate)/polypropylene (OPET/PP). This bi-layer material is used as the cover film for PP buckets; in which bakery fruit fillings are stored. Moreover, orange peel essential oil was used as the active compound that was nanoencapsulated in the coating. The essential oil was prepared by microwave assisted extraction coupled with ETHOS. This method is environmentally friendly providing green production concept by minimising the use of naturally unfriendly solvents. Also, by reusing the orange peel as a food waste, this study contributes to a sustainable material production. Produced materials were tested for their gas and water vapour barrier performance, physico-chemical properties (thickness, UV transmission and colour), and antimicrobial properties. The results show the possibility of improving the commercial material properties. It is possible to couple the traditional with green extraction technology to reuse natural product considered as a food waste for food packaging and thus minimise the negative impact on the nature. In order to better check the functional character, further investigation is needed on real food products during their storage and impact on their shelf-life.

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Ultrasound-assisted extraction of proteins and sugars from the dried pulp of *Curcubita moschata* pumpkin

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Keywords: Curcubita moschata, pumpkin pulp, hot air drying, ultrasound-assisted extraction, protein and sugars

Pumpkin is one of the inexpensive and widely available fruits grown mainly in tropical and subtropical countries. After harvesting, pumpkins can be kept for one to three months. After that, they become susceptible to microbial spoilage, moisture loss and colour changes. To extend the shelf life of pumpkins, various drying methods such as convection and vacuum drying can be used. These methods allow the use of pumpkin powder as an excellent ingredient rich in colour and nutrients in the production of various food products.

In this context, the proposed work shows the results of protein and sugar evaluation of pumpkin powder obtained after drying the pulp with hot air at temperatures of 50, 60 and 70 °C and velocity of 1.5 and 0.5 m/s. The main objective of this work was to investigate whether the parameters used affect the extraction of proteins and sugars and whether the nutritional properties of pumpkin are maintained after drying.

Extraction of two analytes from dried samples was performed by ultrasound-assisted extraction (UAE) at a frequency of 37 kHz, a temperature of 50 °C, and times of 20 and 40 min for protein and sugars, respectively.

The results obtained by UV/Vis spectrophotometry showed that the dried samples had a high content of proteins (9.26-10.98%) and sugars (37.97-66.07%) depending on the drying temperature and air velocity. As the results show, there is not much difference among the dried samples in terms of protein content. However, the drying parameters had a stronger influence on the sugar content. The highest protein content (10.98%) was found in a pumpkin powder dried at 70 °C at an air velocity of 0.5 m/s, while the sugar content was highest in a sample dried at 60 °C at an air velocity of 0.5 m/s.

Overall, the results showed that dried pumpkin powder is rich in proteins and sugars and therefore can be considered as an acceptable and valued food ingredient due to its nutritional potential.

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Evaluation of fallen autumn leaves as a source of carotenoids for egg yolk pigmentation

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Keywords: carotenoids, fallen autumn leaves, egg yolk, pigmentation

Plant leaves are a rich source of carotenoids, which become visible in autumn due to the faster decay of chlorophylls. Carotenoids are a diverse family of yellow-orange pigments contributing to vitamin A, antioxidant and pigment status of animals. However, animals must obtain carotenoids from the diet and plant leaves could be used as a carotenoid source in laying hen diets. Leaves are usually left to decompose naturally, so their potential remains unused. Furthermore, the use of fallen autumn leaves as a dietary supplement for poultry pigmentation has been poorly studied. Therefore, this study was conducted to determine the carotenoid profile in leaves of plant species with intensive autumn leaves colour and to evaluate their potential as a carotenoid source for egg yolk pigmentation. Leaves of five species (*Acer saccharinum*, *Liquidambar styraciflua*, *Parrotia persica*, *Quercus robur*, and *Tilia cordata*) were collected (once in October and November 2021). Immediately after collection, leaves were dried at 40 °C, ground, and the content of individual (lutein, zeaxanthin, α - and β -cryptoxanthin, and α - and β -carotene) and total carotenoids (TC; expressed as β -carotene equivalents) was quantified using HPLC method. Potential daily carotenoid intake in hens was calculated based on their contents in leaves and the potential average daily diet intake of the hens (130 g). The addition of 1% or 3% of leaf meal in the diet was assumed based on previous studies. The plant species studied differed in leaf carotenoid profile, with higher contents in *Acer saccharinum* and *Tilia cordata* compared to the other species (250 vs. 112 $\mu\text{g/g DM of TC}$). Lutein and zeaxanthin, the main carotenoids in the natural hen diet, were the predominant leaf carotenoids in all collected species (79-95% of TC). Content of zeaxanthin was higher than of lutein only in *Liquidambar styraciflua* leaves. Higher supplementation of plant meals resulted in higher potential carotenoid intake in hens (on average, 931 μg of lutein, 880 μg of zeaxanthin and 2322 μg of TC), which were comparable to diets in previous studies with supplemented pigments. To achieve higher carotenoid intake, the diet could be supplemented with a higher proportion of leaf meal, but the effects of increasing fiber content on hen performance should be studied to avoid negative effects. In conclusion, meals prepared from fallen autumn leaves of some plant species could be used as a carotenoid source in hen diets after confirming their microbiological and hygienic suitability.

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Influence of type and power of ultrasonic treatment on the drying time of pumpkin (*Cucurbita moschata*)

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Keywords: pumpkin, ultrasound, waste, drying

High-intensity ultrasonics as novel food processing technology have increased use in the food industry. The cavitation effect of ultrasonic processing can influence a wide array of food processing operations such as mixing, emulsification, inactivation of microorganisms, and drying. Drying is one of the most energy-intensive operations in the industry, and as such could strongly benefit from using novel technologies to help before or during drying. High-intensity ultrasonics have a thermal and mechanical impact on processed food, due to the high energy and pressures obtained in an implosion of cavitation bubbles during the propagation of the ultrasonic wave in high liquid-content materials. Such impact in the vicinity of the food surface can have an impact on the cellular structure, specifically modifying the matrix and enlarging pores inside processed food.

As drying is a mass transfer process, it is heavily influenced by the pore sizes in the food. In this way, ultrasonic pre-treatment can significantly increase mass transfer and consequently shorten drying times. This can potentially lead to a decrease in energy consumption and ensure a more sustainable process. This study investigates the effect of the ultrasonic treatment of pumpkin on the drying time. For the experiment, square pumpkin pieces were processed using two different types of ultrasonic treatment. Ultrasound with the probe was used at 30, 60, and 90 % of maximal amplitude during 3, 6, and 9 min. For the second type of treatment, an ultrasonic bath was used at amplitudes of 30, 60, and 90 % during 30, 45, and 60 min. Drying was performed using a conventional convective tray dryer at 60 °C using an airflow of 0.5 ms⁻¹. Statistical analysis is performed using ANOVA and posthoc tests in Statistica software.

MANOVA analysis of obtained results shows a significant change in drying times which correlate to both types of ultrasonic pre-treatment. The drying time of control samples was 305 min, which was a baseline for further analysis. Samples treated using an ultrasonic bath show a statistically significant decrease in the drying time (down to the shortest drying time of 248 min, processed at 90 % of maximal amplitude) which correlates to the increase of the amplitude. This same property was confirmed using the ultrasonic probe, as drying time was shortened to 245 min for samples treated with 30 % of maximal amplitude for 9 min. Further increase of ultrasonic power seems to have no further influence on the drying time. The ultrasonic probe was proven to be more



effective in the sonication of pumpkin samples, with much shorter processing times and lower amplitudes needed for the same effect on the drying time.

In conclusion, while pre-treatment using an ultrasonic have a significant influence on the decreasing of drying time, such a process would be much more beneficial using high-powered ultrasound with an immersion probe, as it can process the surface of the material with the same amount of mechanical energy in a much shorter amount of time.

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