UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

MED &FOOD Control and Quality Systems

Politecnico di Bari

GENP 2018

Green Extraction of Natural Products

November 12-13

University of Bari - Italy
Palazzo Ateneo
Piazza Umberto 1 - Bari

Congress Topics

- Alternative solvents for green extraction
- Valorisation of by products and biorefinery
- Sustainable and clean extraction technologies
- New tools for green extraction education and operator training
- Industrial and case study applications
- Innovative extraction process design

INTERNATIONAL ORGANIZING COMMITTEE: Farid Chemat (France); Giancarlo Cravotto (Italy); Jochen Strube (Germany); Francisco Barba (Spain) Patrizia Perego (Italy); Maria L. Clodoveo (Italy); Filomena Corbo (Italy); Riccardo Amirante (Italy) Ivana Radojčić Redovnikovic (Croatia); Carlo Franchini (Italy)

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COMPETITIVE Claims for Olive oil

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BIONAP Bioactive Natural Products

ATLAS FILTRI improving water

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RI-LAVO ULTRASOUND TECHNOLOGIES

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GENP 2018

GREEN EXTRACTION OF NATURAL PRODUCTS

III Edition

Book of Abstracts

Bari, Italy, 12th 13th November 2018

Questa opera ha beneficiato del sostegno del Progetto di ricerca COMPETiTiVE - Claims of Olive oil to iMProvE The markeT ValuE of the product, finanziato da AGER per migliorare la competitività dell'olio extravergine di oliva italiano valorizzando le sue proprietà salutistiche e nutrizionali, trasferendo alla filiera le innovazioni tecnologiche frutto della ricerca e applicando nuove tecniche di marketing. Ager - Agroalimentare e Ricerca - è un progetto di collaborazione tra Fondazioni per il sostegno alla ricerca scientifica in campo agroalimentare

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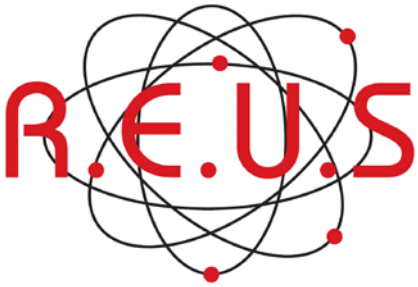

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SCIENTIFIC COMMITTEE

Program

November 12st

9.00 Registration and Welcome

10.00 **Opening Ceremony and meeting presentation**

Antonio Felice Uricchio -Rector of University Aldo Moro Bari (UNIBA)

Eugenio Di Sciascio- Rector of Polytechnic of Bari

Angela Agostiano- President SCI (Italian Chemical Society)

Gianluca Farinola-President of EuCheMs Organic Chemistry Division

Francesco Leonetti-Director of Department of Pharmacy-Drug Science, UNIBA

Carlo Sabbà-Director of Interdisciplinary Department of Medicine UNIBA

Topic Innovative extraction process design

CHAIR: **Cravotto G. and Chemat F.**

10.30 **PL1** Jochen Strube (University of Clausthal-Zellerfeld, Germany) *Natural Products Extraction of the future – Solutions for Sustainable Manufacturing*

11.00 **PL2** Giancarlo Cravotto (University of Turin Italy) *Toward a transdisciplinary model in green extraction*

Coffe Break

12.00 **K1** Lukas Uhlenbrock (Clausthal University of Technology) *Quality by Design focused process development and water based extraction techniques for the isolation of valuable components from naturally variable raw material.*

12.20 **O1** Daniele de Freitas Ferreira (University of Santa Maria Brasil) *Simultaneous solvent-free extraction of volatile and non-volatile antioxidant compounds from Rosemary using microwave hydrodiffusion and gravity.*

12.35 **O2** Giorgio Grillo (University of Turin) *Mass transfer kinetic design of multi-stage ultrasound-assisted extraction of tea leaves*

12.50 **SC1** Giada Maranaldi, Antonio Rosato (INDENA SpA) *Antibiofilm effect of plant extracts*

13.00 **SC2** Marco Fiore (Leanfa srl) *Innovative Microwave Generators for Process Optimization*

LUNCH

CHAIR: **Strube J., Perego P.**

Topic Alternative solvents for green extraction

14.30 **K2** Anne Rossignol-Castera (Oléos-Hallstar, Mauguio, France) *A new biomimetic green solvent coupled to co-intensified oleo-eco-extraction for performant skin actives: OSMOS^{TM} concept*

14.50 **K3** Ivana Radojčić Redovniković (Univeristy of Zagabria) *Green solvents for green technologies*

15.10 **K4** Vito Capriati (University of Bari) *Natural Deep Eutectisc Solvents: The perfect springboard toward a sustainable future*

15.30 **SC3** Luca Piemontese (University of Bari) *Deep Eutectic Solvents as effective media for the extraction of small molecules from natural sources*

Topic Sustainable and clean extraction technologies

15.40 **O3** Chiara Samori (University of Bologna) *Milking of the microalga Haematococcus pluvialis for a non destructive extraction of astaxanthin*

15.55 **O4** Ivo Rodrigues (Polytechnic Institute of Coimbra-Portugal) *Exploring the effects of carbohydrases-assisted pulp extraction from strawberry tree fruit*

Coffe break

Topic Valorisation of by products and biorefinery

16.40 **K5** Silvia Tabasso (University of Turin-Italy) *Enabling technologies and green solvents for lignin extraction and valorization*

17.00 **O5** Visnja Gaurina Srcek (University of Zagreb) *Biological potential of proteins and peptides obtained from hempseed cake*

17.15 **O6** A. S. Fabiano-Tixier (Avignon University, INRA, France) *Green Sono extraction: study of the physical impacts on the leaves of Rosmarinus officinalis*

17.30 **O7** Angela Cardinali (Institute of Sciences of Food Production (ISPA-CNR Bari Italy)*Phytochemicals from artichoke by-product and their applications as natural ingredients for cosmetic industry.*

18.00 1 st POSTER SESSION

20.00 Social Dinner

November 13st

CHAIR: **Franchini C., Amirante R.**

Topic: Industrial and case study applications

9.15 **PL3** Gerhard Schories (ttz Bremerhaven, Germany) *European Research Opportunities for Green Extraction Technologies*

9.45 **PL4** Farid Chemat (INRA, Université d'Avignon et des Pays de Vaucluse) *Alternative solvents for green extraction, purification and formulation for cosmetic, food and nutraceutical products*

10.15 **O8** Distaso Elia (Polytechnic of Bari, Italy) *Numerical Simulation of Unsteady Multiphase Non-Newtonian Fluid Flow for the Design of an Innovative Ultrasound Device Able to Improve Olive Oil Extractability and Quality*

10.30 **O9** Lea Vernes (Avignon University- France) *Bioactive compounds extraction from Arthrospira platensis through sonoporation induced by ultrasounds.*

10.45 **SC4** Alice ANGOY (IFTS, Foulayronnes, France) *Green extraction of natural products using microwaves and centrifugal force thanks to a semi-industrial pilote*.

10.55 **SC5** Roberto di Paolo: (REUS srl) *How to improve technology and usability of REUS ultrasounds machines: ULTRASONS REUS 2.0" Simple but effective.."*

Coffe break

11.30 **O10** Ramli, S.S. Siti Suhara (University Putra Malaysia) *Optimisation of the Oil Extracted from Sardinella lemuru Waste with Supercritical Fluid Extraction (SCCO2) using Response Surface Methodology (RSM)*

11.45 **O11** Meneguzzo Francesco (Institute of Biometeorology, CNR Italy) *Enhanced extraction from malt and hops in an innovative beer-brewing technology based on hydrodynamic cavitation processes*

12.00 **O12** Arrigucci Fredrik (ARCAROMA, Lund Sweden) *oliveCEPT® - A commercially available product for improving olive oil extraction yield and quality*

12.15 **SC6** Maria Grazia Roselli (Sergio Fontana srl) *Innovative Mixture With Antioxidant Action, With Positive Effect On The Intestinal Microbiota*

12.25 **SC7** Faraone Immacolata (University of Basilicata-Italy) *Green extractions of bioactives from Vitis vinifera L. (cv. Aglianico) leaves: phenolic profile, antioxidant and anti-cholinesterase activity of extracts.*

Topic New tools for green extraction education and operator training

12.35 **O13** Antonio Felice Uricchio (University of Bari) *Food taxes between ability-to-pay principle and extra-fiscal purposes*

LUNCH

Special Session "COMPETiTVE- Claims for Olive oil to iMProvE iTs markeT ValuE- Ager Project

CHAIR: **Sacchi R., Clodoveo M.L, Corbo F.**

14.00 **O1** Raffaele Sacchi **(**University Federico II Naples Italy) *Virgin olive oil biophenols: evolution during the shelf-life and influence on aroma release and sensory quality*

14.15 **O2** Luigi Roselli **(**University of Bari Italy) *Consumer acceptance of innovations in extra virgin olive oil production process: socio-demographic and psychographic determinants*

14.30 **O3** Nadia Mulinacci (University of Florence) *New analytical approaches to determine the EVOO quality: a study on the volatiles and phenolic fraction of Italian oils from 2017*

14.45 **O4** Lorenzo Guerrini (University of Florence Italy) *Studies on extra-virgin olive oil degradation during shelf-life.*

15.00 **O5** Carmen Lammi (University of Milan Italy) *Development and validation of versatile cellular models for investigating the absorption and bioactivity of natural extracts*

15.15 **O6** Giancarlo Cravotto (University of Turin) *Pilot scale cavitational treatments in oil extraction processing*

15.30 **O7** Clodoveo Maria Lisa –Filomena Corbo (University of Bari)*The Fast Track to Innovation: the right tool to face the innovation challenge and the valley of death.*

15.45 **O8** Gianluca Picariello **(**CNR Avellino Italy) *Exploring alternative analytical strategies to quantify polyphenols in virgin olive oil.*

16.00 **O9** Marco Pichierri – Gainluigi Guido (University of Bologna-University of Salento) *Communicating the health value of olive oil: An analysis of consumers'emotional and attitudinal responses to label health claims*

16.15 **O10** Nicola Marrano (University of Bari Italy) *Effects of Free Fatty Acids and Phenolic Compounds of Extra Virgin Olive Oil on Beta-cells Survival and Function*

16.30-17.30 2th POSTER SESSION

Awards Cerimony

17.30 FINAL REMARKS AND CLOSING

Index of papers

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Natural Products Extraction of the future – Perspectives for Sustainable Manufacturing

Strube Jochen [(1)*], Uhlenbrock Lukas [(1)]

[(1)] *Institute for Separation and Process Technology, Clausthal University of Technology*

**Speaker, Corresponding author: Strube Jochen*

Keywords: Natural Products, Phytomedicines, Extraction, Manufacturing, Regulatory

Presentation: Plenary

Objectives

Assessing small molecule pharmaceuticals approved between 1950 and 2010 shows, that approximately one third are either natural products or natural product derivatives [1]. In combination with increasing demands of regulatory agencies regarding quality control, new solutions for sustainable manufacturing from natural resources are necessary. These solutions need to be economically viable and to improve technical possibilities for quality control.

Methods

Current business models and regulatory frameworks are analysed to demonstrate the requirements for sustainable manufacturing solutions. Based on this analysis approaches composed of standardized laboratory equipment combined with physico-chemical predictive process modelling and innovative modular, flexible batch or continuous manufacturing technologies being fully automatized by advanced process control methods are purposed for discussion.

Results

The value chain of extraction processes consists of cultivation, extraction, purification, formulation and sales, with increasing added value towards costumer sales. At the same time, regulatory requirements regarding product quality are still steadily growing, resulting in an increased economic pressure for agricultural businesses. Efficient and green, water based extraction processes based on clear and well-defined quality attributes allow for cost efficient products.
The results will be exemplified and proposed for a mutual discussion on future perspectives and needs.

References

[1]. Cragg, G.M.; Newman, D.J. Natural products: a continuing source of novel drug leads. *Biochimica et biophysica acta* **2013**, *1830*, 3670–3695, doi:10.1016/j.bbagen.2013.02.008.

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Toward a transdisciplinary model in green extraction

Giancarlo Cravotto[1,2], Arianna Binello[1] Luisa Boffa[1], Valery V. Veselov[2], Alexander E. Nosyrev[2].

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(2) *First Moscow State Medical University (Sechenov), Inst. of Translational Medicine and Biotechnology, Center of Bioanalytical Research and Molecular Design, 8 Trubetskaya ul, Moskow, Russia.*

Corresponding author: Giancarlo Cravotto

Keywords: transdisciplinary research, green extraction, technology platform, business driven model, holistic perspectives.

Presentation: Plenary

Objectives

Disciplinary spaces must become connected if we are to inspire the new ideas and products that will respond to the dynamic issues that the world now faces. The common adjectives for the multidisciplinary, interdisciplinary and transdisciplinary models are additive, interactive and holistic, respectively. Each has its own specific meaning and these terms should not be used interchangeably. Innovative solutions and strategies can be more easily proposed within transdisciplinary domains and new answers can be provided for problems that conventional disciplinary perspectives cannot fully address. A transdisciplinary model of research has a wider horizon, and steadily moves onwards and upwards. The agreement between the authors' two scientific institutions aims to cover several research domains. The objectives are: training activities that will enable young chemists, pharmacists and food technologists to design experiments with engineering perspectives and will allow engineers to become acquainted with biotechnological solutions, while everyone shares effective business plans for sustainability and scalability. Research cannot be kept away from stakeholders who are affected by innovation. For this reason, a new technological platform with leading companies, who provide technologies and tools for chemical, pharma and food processing, has been established at the University of Turin.

Methods

Highly efficient procedures that adhere to green extraction principles[1], require expert teamwork that, beyond classical expertise, may also embrace complementary disciplines such as botany, physics, computational prediction, as well as *Good Manufacturing Practice* (GMP) and regulatory affairs. Derived from deep disciplinary foundations, transdisciplinary approaches stimulate collective, novel and dynamic strategies to transcend the boundaries of traditional fields. After a preliminary technical and economical estimation, the design of a

[1] Chemat, F.; Abert-Vian, M.; Cravotto, G. *Int. J. Mol. Sci.* 2012, *13(7)*, 8615-8627.

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cascade process typically entails: pretreatment, selective extraction (possibly in flow mode), filtration, separation, purification and by-product valorisation[2].

Results

We aim to integrate knowledge and methods from a range of disciplines (mainly natural-product chemistry and biology, process technologies, engineering, analytical chemistry and product marketing) to create holistic perspectives. New green extraction processes and new products are expected to result from this transdisciplinary synergy between universities and industries.

[2] Grillo, G.; Boffa, L., Binello, B.; Mantegna, S.; Cravotto, G.; Chemat, F. et al. Food Res. Int. 2018, DOI:10.1016/j.foodres.2018.08.057

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Quality by Design focused process development and water based extraction techniques for the isolation of valuable components from naturally variable raw material.

Uhlenbrock Lukas [(1)]*, Strube Jochen [(1)]

[(1)] *Institute for Separation and Process Technology, Clausthal University of Technology*

**speaker, Corresponding author: Strube Jochen*

Keywords: Quality by Design, Phytomedicines, Extraction, PAT

Presentation: key note lecture

Objectives

Plant metabolites are an important group of compounds with applications in medicine, crop protection and focus of extensive research. The isolation of these compounds from plants with their natural variability is complex and requires robust, and economically feasibly processes. The objective is the development of extraction and purification processes with minimal use of organic solvents, which guarantee optimal product quality and include modern quality control strategies, while maintaining economic efficiency. Furthermore, green technologies like water-based process sequences are a key-enabling technology for sustainable manufacturing

Methods

The Quality-by-Design (QbD) focused approach to process development combines statistical design of experiments and rigorous process models in order to characterize the impact of raw material properties on product quality [1]. The significance of variance of plant material and process parameter is evaluated and implemented into predictive process models. Alternative solvents and process chains are evaluated as well to achieve the most resource efficient process. In the final step towards industrialization, PAT (process analytical technology) is employed to monitor quality attributes.

Results

The different steps of Quality-by-Design focused approach to process development are shown on the example of the isolation of value fraction from e.g. yew, mugworth, artemsia, hawthorn and fennel [2]. Additionally, studies utilizing rigorous process models show the optimization potential of the extraction processes and their advantage over either a purely experimental study or statistical models. The influence of different process parameters and raw material properties, such as solvent composition, temperature or particle size on the extraction performance is demonstrated including their variance [3]. Finally, different applications of process analytical technologies and other quality control strategies are shown, which allow real-time monitoring of relevant quality attributes.

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The talk will integrate an interactive plenary simulation tutorial for exemplification and own evaluation of those methods available.

References

[1]. Sixt, M.; Strube, J. Systematic Design and Evaluation of an Extraction Process for Traditionally Used Herbal Medicine on the Example of Hawthorn (Crataegus monogyna JACQ.). *Processes* **2018**, *6*, 73, doi:10.3390/pr6070073.
[2]. Uhlenbrock, L.; Sixt, M.; Strube, J. Quality-by-Design (QbD) process evaluation for phytopharmaceuticals on the example of 10-deacetylbaccatin III from yew. *Resource-Efficient Technologies* **2017**, *3*, 137–143, doi:10.1016/j.reffit.2017.03.001.
[3]. Sixt, M.; Uhlenbrock, L.; Strube, J. Toward a Distinct and Quantitative Validation Method for Predictive Process Modelling—On the Example of Solid-Liquid Extraction Processes of Complex Plant Extracts. *Processes* **2018**, *6*, 66, doi:10.3390/pr6060066.

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Simultaneous solvent-free extraction of volatile and non-volatile antioxidant compounds from Rosemary using microwave hydrodiffusion and gravity

Ferreira, D. F.[(1)], Lucas, B. N. [(1)], Oliveira, A. S. [(1)], Schlesner, S. K. [(1)], Voss, M. [(1)], Mello, P. A.[(2)], Wagner, R.[(1)], Barin, J. S. [(1)]

[(1)] *Department of Technology and Food Science,* [(2)] *Department of Chemistry, Federal University of Santa Maria-RS, 97105-900, Santa Maria, RS, Brazil*

Corresponding author: Barin, J. S.

Keywords: MHG, clean recovery, Rosmarinus officinalis, solvent-free extraction

Presentation: Oral

Objectives

Microwave hydrodiffusion and gravity (MHG) has been used for extraction of valuable compounds from plants without the use of any solvent. MHG extraction is based on the internal heating of in situ water from plant material caused by microwave heating, which results in an effective cell rupture and release the compounds. However, MHG has not yet been used to obtain more than one fraction in a unique extraction step. Therefore, MHG is proposed as a rapid method for simultaneous extraction of two antioxidant fractions (phenolic compounds and essential oil) from rosemary leaves.

Methods

MHG was performed using a NEOS-GR microwave (Milestone, Italy). Rosemary leaves (100 g) were used for extraction (400 W, 20 min). The experimental conditions were optimized and the mixture of essential oil and phenolic extract were collected, separated and stored at -18°C. Conventional extractions were performed using hydrodistillation (HD) during 3 h for essential oil and maceration by 2 h with water for phenolic compounds. Essential oils were characterized by GC-FID and GC/MS and the total phenolic content determined using Folin-Ciocalteu method. ORAC assay was used to measure the antioxidant activity in both fractions.

Results

MHG in only 20 min of extraction. MHG presented essential oil with higher amount of oxygenated compounds (75.8%) than extracted by HD (72.8%), and higher antioxidant acitity (211 µmol ET/g by HD and 430 µmol ET/g by MHG). Additionally, MHG was also able to recovery 80% of phenolic content obtained by maceration (7.13 and 8.91 mg GAE/g of plant, respectively). Antioxidant activity of phenolic fraction obtained by MHG was higher than obtained by maceration (75,511 µmol ET/ mL and 29,052 µmol ET/mL,

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respectively). As observed, two fractions with higher antioxidant activity than conventional methods could be obtained simultaneously using MHG. This process integration could be a suitable alternative for reduction of the energy consumption and the improvement of the industrial productivity.

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Mass transfer kinetic design of multi-stage ultrasound-assisted extraction of tea leaves

Giorgio Grillo[(1)], Arianna Binello[(1)], Roberto Solarino[(1)], Luisa Boffa[(1)], Giancarlo Cravotto[(1)], Samir Bensaid[(2)], Giuliano Cavaglià[(3)]

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[(2)] *Dip.to di Scienza Applicata e Tecnologia, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino;*
[(3)] *K&E Srl, Via Ippolito Nievo 25, 10153 Torino.*

Corresponding author: Giorgio Grillo

Keywords: Ultrasound, Kinetic, Multi-step process, Extraction rate optimization

Presentation: Oral

Objectives

Tea (*Camellia Sinensis*), with a yearly production of 5.45 mln tons, is the most consumed beverage in the world, with related huge amount of production wastes. In the lasts decades, tea's healthy effects gained huge attention (antioxidant and chemopreventive properties), thanks to the bioactive polyphenolic fraction, mainly composed by catechins and phenolic acids. This attention has been merged with the industrial hot topic of new green processes design, keeping at the same time high production levels. Ultrasound-assisted extraction (UAE) is recognized as the technique of choice for a fast and efficient recovery of polyphenols at room temperature.

Methods

The broken leaf residues resulting from tea production, have been extracted in pure water using a cup horn system (24 kHz, 200 W). Total Phenolics Compounds (TPC, expressed in gallic acid equivalent) by the Folin-Ciocalteau essay have been measured on dry extracts, aiming to understand the process extraction rate. The Peleg hyperbolic model has been applied to describe the kinetic profile, verifying the fitting via parametrical linearization.

Results

The big data collection enables the design of a cross-flow UAE protocol, leading to an enriched polyphenolic fraction and an exhausted tea matrix. The final product has been characterized by DPPH essay, HPLC and LC-MS analysis and compared to a conventional benchmark.

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Antibiofilm effect of plant extracts

Maramaldi Giada[(1)] , Rosato Antonio[(2)]

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[(2)] *Department of Pharmacy-Drug Science, University of Bari Aldo Moro, Italy*

Corresponding author: Maramaldi Giada, giada.maramaldi@indena.com

Keywords: biofilm, XTT, natural extracts

Presentation: Short communication

Objectives

Microbial infections can cause serious human pathologies. S. aureus infections, for instance, are serious health care issues that are complicated by the virulence and the production of toxins and enzymes . S. aureus represent an emerging problem both in human and in veterinary Medicine. When S. aureus overcomes the host defenses, it can cause various different infections, both skin-localized as well as to invasive ones; this bacterial specie shows the specific capability to develop resistances against every antibiotic used in clinics. Furthermore, S. aureus biofilms are widely involved in chronic and on medical devices infections, including infections of cardiac valves, central venous catheters, urinary catheters, orthopedic prosthesis, contact lenses, and may cause endocarditis, otitis, osteomyelitis and sinusitis. These biofilm infections are difficult to treat and many in vitro susceptibility tests demonstrated that they are significantly more resistant to antibiotics than plankton cells. The standardized botanical extracts studied in this experimental research, known for their medicinal properties have demonstrated in our tests an excellent antibiofilm activity.

Method

Seven different extracts obtained from Indena spa (Milano, Italy) were tested against diverse S. aureus biofilm producing strains. The starting herbal material has been identified against a crude drug standard or an authoritative literature source by botanical a quality control analyst. The extracts were from Vitis vinifera seed (extract 1, 17, 9) Quercus robur wood (12), Camellia sinensis leaves (4), Olea europea fruit (7), Coffea arabica (16), grounded and generally extracted with a mixture of ethanol and water, evaporated, and dried. In order to evaluate their anti-biofilm activity, each extract has then been dissolved in media for antibacterial biofilm test. The evaluation of inhibitory effect was determined against seven S. aureus strains that caused serious infectious diseases. Four strains from ATCC international collection and three strains derived from clinic isolation (obtained from the Department of Biomedical Science and Human Oncology, University of Bari, Italy). The extracts were tested for their capability to reduce the bacterial biofilm with XTT method; the results were expressed as percentage of reduction of biofilm mass measured as DO with a spectrophotometer.

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Results

The outcomes of this research highlighted the anti-biofilm action of the tested botanical extracts with a significant reduction in vitro of the biofilm mass from 85 to 90%. Consequently, the plant extracts taken into consideration in this study were able to inhibit growth biofilm and its dynamic phases of formation.
The results obtained led us to conclude that standardized extracts with a common content of polyphenolic compounds could determine an efficacious resolution of biofilm growth: this research as a long-period perspective could be a new approach to the infective disease based on biofilm pathologies.

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Innovative Microwave Generators for Process Optimization

Fiore Marco[(1)],

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Corresponding author: Marco Fiore, marco.fiore@leanfa.com

Keywords: Microwave, Solid-state, Sustainable, Clean, Efficient, Accurate, Safe, Digital, Adaptive

Presentation: Short communication

Objectives

The presentation will emphasize the various advantages of innovative solid-state microwave generators against the standard magnetron sources for clean and sustainable agro food-related processing.

Methods

An innovative approach based on a modular generation platform with distributed intelligence allows very accurate handling of microwave energy for precise thermal profile generation. A real-time sensor network, managed by a self-learning software engine, gives birth to auto-adaptive workflow during batch or continuous processes.

Results

The new approach has demonstrated full optimization capability: minimization of energy consumption, maximum safety of operation, longest equipment life cycle, minimum maintenance and top processing accuracy. Additionally, the distributed software platform allows the highest flexibility of operation and future-proof investment in processing equipment.

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A new biomimetic green solvent coupled to co-intensified oleo-eco-extraction for performant skin actives: OSMOS™ concept.

Anne Rossignol-Castera[(1)], Noémie Lemoine [(1)], Anabelle L'Hermitte[(1)], Clément Celhay[(1)]

[(1)] *Oléos-Hallstar, 50, rue du Rajol, 34 130 Mauguio, France*

Corresponding author: Anne Rossignol-Castera

Keywords: Oléo-Eco-Extraction (OEE)

Presentation: keynote lecture

Objectives

Oléo-Eco-Extraction (OEE) is an eco-process intensified by high energy microwaves (MW) and low frequency ultrasound (US) using vegetable oils as green, safe and bioactive extractants. To reduce production time and energy, a new co-intensified [MW+US] process has been applied with an innovative biomimetic solvent inspired both from plant membranes and skin cells. This concept called OSMOS™ is able to increase the extraction yield of phyto-molecules and in the same time, their bioavailability as a skin delivery system.

Methods

Based on the oleo-supra molecular organization of non-polar and more polar molecules in a continuous oily phase, we decided to create a system of two biosourced compounds naturally present in plant and skin cells. This system must be a couple of an hydrogen-bond donor and acceptor to maximize extraction capacity and stabilization.

Results

Oleic acid (OA) is a natural hydrogen-bond donor present in all cells and able to modify the trans-epidermal flow by increase the intercellular disorder. OA acts as an enhancer of cutaneous penetration and contributes also to skin hydration. Phosphatidyl-Choline (PC) rich lecithin is an hydrogen-bond acceptor with a high affinity for cells membranes, acting as a good co-extractant in oily systems and giving a good vectorization of actives through the stratum corneum. We have demonstrated that a ratio 95/5 OA: PC (m/m) gives a better yield extraction of anti free-radical polar and non polar molecules (phenolic acids, polyphenols, carotenoids, triterpens) from leaves, flowers and fruits with co-intensified [MW+US] system, versus classical OEE with a vegetable oil without or with co-extractant (examples will be given). OSMOS™ concept gives a new opportunity of a plant and skin biomimetic system for green intensified eco-extraction of cosmetic actives, that are safe, non-oxidized, stable, food-grade, organic certified and highly bioavailable.

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Green solvents for green technologies

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Keywords: Green solvents, polyphenols compounds, grape pomace

Presentation: keynote lecture

Objectives

Over the past decade, among neoteric solvents, natural deep eutectic solvents (NADES) have become promising alternatives to traditional organic solvents from both environmental and technological perspectives and have been dramatically increasing in popularity. Herein, a brief overview of the up to date knowledge regarding these solvents, with special emphasis on to possible application in extraction of phenolic compounds from grape pomace are presented.

Methods

Application of NADES in polyphenols compounds' extraction from grape pomace was studied. The valorisation of obtained extracts was also performed by complete analysis of phenolic compounds. Recovery of polyphenols and recycling of the NADES were also accomplished. Green extraction method for polyphenols from grape pomace was performed on a bigger scale.

Results

An environmentally friendly extraction method for polyphenols from grape pomace with NADES coupled with alternative energy sources – ultrasound and microwave irradiation used simultaneously were developed on a bigger scale. Screening of 10 different NADES was performed and choline chloride-based NADES containing citric acid as a hydrogen bond donor was selected as the most promising one. Then, optimization of the process parameters of the extraction was carried out considering the anthocyanins extraction yield. Recovery of anthocyanins and efficient recyclation of NADES were accomplished on resin Amberlite XAD-7HP.

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Natural deep eutectic solvents: the perfect springboard towards a sustainable future

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Keywords: Deep Eutectic Solvents; Water; Catalysis; Organometallics; Extraction

Presentation: keynote lecture

The environmental impact associated with chemical synthesis has recently posed severe and compelling demands for sustainable chemistry, and the development of cost- effective and environmentally benign reaction systems in place of harsh and volatile organic compounds (VOCs) represents an active field of research. In this context, "*Deep Eutectic Solvents*" (*DESs*) are growingly recognized as superior green and bio- renewable reaction media. *DESs* are today generally referred to as combinations of two or three safe, and nature-inspired components able to engage in reciprocal hydrogen bond interactions to form an eutectic mixture with a melting point much lower than either of the individual components, due to self-association. Polar organometallic chemistry has become a cornerstone of modern organic synthesis. One of most momentous challenges in organic synthesis is to perfect the use of polar organometallics under air and at room temperature, also replacing VOCs by more environmentally benign solvents [1]. In this communication, we discuss the regioselective functionalization of a wide range of organic substrates using polar organometallic reagents, pioneering their application in nature-inspired DESs, and more challenging also in water [2]. These findings cross the great divide beween traditionally allowed and textbook-prohibited reactions by delivering beyond state-of-the-art strategies for organic chemistry performed in DESs and water. Unexpected and unprecedented performance offered by DESs in metal- catalysis[3][4], organocatalysis[5][6], biocatalysis [7], pharmaceutical science[8], extraction of food contaminants[9], and solar technology[10], will be discussed as well.

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Deep Eutectic Solvents as effective media for the extraction of small molecules from natural sources

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Keywords: Deep Eutectic Solvents (DESs), Food Matrices, Ochratoxin A

Presentation: short communication

Objectives

Deep Eutectic Solvents (DESs) represent an emerging class of biorenewable solvents usually prepared by mixing two or three safe and inexpensive components able to form an eutectic mixture with a melting point further below that of the individual components. Due to their low toxicity and biodegradability they are progressively replacing conventional volatile organic compounds (VOCs) in several fields of science. An increasing number of studies, in particular, have reported the feasibility of extracting bioactive compounds from natural sources (Ruesgas-Ramón et al., *JAFC*, **2017**, *65*, 3591). Our efforts of late have focused on the use of these media in the extraction of contaminants, such as mycotoxins, from food matrices.

Methods

An analytical method for the determination of the mycotoxin Ochratoxin A (OTA) in wheat and some processing products has been developed and validated using choline chloride (ChCl)-based solvent as privileged and biodegradable extraction media (Piemontese et al. *Molecules*, **2017**, *22*, 121). We have then implemented the method to include a larger screening on many solid food matrices using several DESs with different physical-chemical characteristics.

Results

Using the ChCl/urea (1:2)/water (+ 40% w/w) mixture, the analytical performances, in term of recovery and repeatability for durum wheat, bread crumbs, and biscuits, proved to be comparable to those obtained with conventional and hazardous VOCs, which are typically employed according to the standard and official methods. The tunability of DESs is promising to extend the use of the validated method to other food commodities. Preliminary results obtained with the ChCl/lactic acid (1:2)/water (+25% w/w) mixture have revealed good extraction performances on a wide number of solid matrices.

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Milking of the microalga *Haematococcus pluvialis* for a non-destructive extraction of astaxanthin

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Keywords: *Microalgae, Haematococcus pluvialis, astaxanthin, sustainable solvents, milking extraction*

Presentation: Oral

Objectives

The extraction of bioactive compounds from microalgae by exploiting the concept of "milking" is based on the recovery of secondary metabolites not essential for the growth and metabolism while maintaining microalgal vitality for the continuous production of these chemicals, like milking cows. Here, we propose the milking of the microalga Haematococcus pluvialis for a non-destructive extraction of astaxanthin (3,3′- dihydroxy-β,β′-carotene-4,4′-dione), a secondary carotenoid with a strong antioxidant potential accumulated outside the chloroplast in lipid vacuoles.

Methods

H. pluvialis was optimally grown and then stressed to induce its shift to a red phase where cells accumulate astaxanthin. Milking was performed by extracting directly algal culture with various sustainable solvents for 5, 10 and 30 min and then measuring the photosynthetic efficiency of the cells. Solvents performances were evaluated in terms of astaxanthin extraction, toxicity towards algae and general safety.

Results

Among the tested solvents, 2-methyltetrahydrofuran and dimethyl carbonate provided the best extraction performance but without preserving the integrity of the cells, even after 5 min. Contrarily, cyclohexane allowed to maintain healthy cells even after 30 minutes, but low extraction efficiencies were observed. Vegetable oil and isoamyl acetate showed the best milking potential giving a good compromise in terms of algal vitality and extraction efficiency.

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Exploring the effects of carbohydrases-assisted pulp extraction from strawberry tree fruit

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Keywords: *Arbutus unedo, strawberry tree fruit, pulp extraction, Viscozyme® L, Pectinex®, viscosity*

Presentation: Oral

Objectives

Arbutus unedo's fruits have a high content of sugars (40%), antioxidant vitamins such as vitamin C, beta-carotene, niacin, tocopherols, and organic acids. However, pulp extraction is very poor accounting only with 42%. This study aims to evaluate the potential use of two commercial carbohydrases, Viscozyme® L and Pectinex® for pulp extraction from strawberry tree fruit.

Methods

Carbohydrases were tested under different temperatures (20/25 °C and 45 °C), enzyme concentration (1, 2 and 4 µL/g of macered fruit) and reaction times (30 and 60 min). The same conditions were used for controls without enzyme addition. Pulp extraction yields (g extracted pulp /100 g macerated fruit), apparent viscosity (at 20±0.1 ºC), color, titratable acidity, pH, soluble solids (ºBrix) and total solids were accessed.

Results

Viscozyme® L allowed the production of pulps with lower apparent viscosity, while Pectinex® stimulated the pulp extraction yield accompanied by the increase of ºBrix. For the remaining physicochemical characteristics no significant differences were found. Carbohydrases-assisted extraction methodology provided promising results to increase the portfolio of added value products from strawberry tree fruit with high potential for the food industry (fruit beverages, jams, ice-creams, among others).

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Enabling technologies and green solvents for lignin extraction and valorisation

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Keywords: wheat straw, microwaves, ultrasound, lignin

Presentation: keynote lecture

Objectives

Lignocellulosic (LC) biomass has been extensively investigated as an alternative source of energy and chemicals, owing to its abundance and affordability. The main components of lignocellulosic biomass are cellulose, hemicellulose and lignin. As lignin contributes 15−30 % to the total mass of lignocelluloses, its extraction still remains a challenge in the pretreatment of biomass for the production of biofuels. However, lignin has no longer been considered as a residue to burn, but as a source of valuable bioaromatics. In this presentation, milder and more sustainable processes for the extraction and the further valorisation of lignin will be presented, focusing on green extraction and mass transport enhancing methods such as microwave-assisted extraction (MAE) and ultrasound- assisted extraction (UAE). To increase the efficiency and the sustainability of the extraction protocols, alternative solvents were used in combination with these enabling technologies.[3]

Methods

MAE was performed by a multimode MW reactor in a closed vessel system SynthWAVE (Milestone Srl), while for UAE, a high-power ultrasonic bath (Weber Ultrasonics AG, Germany), composed of an inox cell (5 L) with three probes screwed into the bottom of the bath was used. Different solvents and conditions were tested, evaluating both the delignification efficiency and the properties of the extracted lignin. In particular, a variety of NaDES and of GVL/H_2O mixtures were compared. The extracts were analyzed for their antioxidant properties by the DPPH test. The lignin was then isolated by precipitation for further conversion into bioaromatics through a microwave-assisted oxidative depolymerization.

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Results

The highest extraction yield for lignin (45%) was obtained under MW irradiation at 120 °C in only 30 min in the presence of a three-component NaDES., while the GVL/water mixture proved more efficient under US mild pretreatment (50 °C for 60 min). In terms of sustainability, the solvents can be recycled and reused. A microwave-assisted cascade protocol for the regeneration of GVL after the extraction was also reported.
The extracts obtained using NaDES as solvents showed the highest antioxidant activity. Furthermore, the extracted lignin can be converted into vanillin and syringaldehyde through a microwave-assisted catalyst-free aerobic oxidation.

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Biological potential of proteins and peptides obtained from hempseed cake

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Keywords: antioxidant activity, anticancer activity, hempseed cake, peptides

Presentation: Oral

Objectives

Oil cakes/meals, by-products obtained after oil extraction, are important source of proteins and other compounds. Peptides obtained by enzymatic hydrolysis of oil cakes proteins showed anti-hypertension, antioxidant, antimicrobial and immunomodulatory effects. In this study, we present results after the screening of biological potential of hydrolysed proteins derived from hempseed cake as well as the hydrolysate's biological properties (antioxidant and in vitro anticancer activity).

Methods

Hempseed protein isolate (HPI) was obtained from defatted hempseed cake by alkali precipitation and subjected to hydrolysis by commercial enzyme Alcalase 2.4 L during 150 min at optimum pH and temperature. Obtained hydrolysates were fractionated by ultrafiltration and their antioxidant activity was assessed by ORAC method. Additionally, they were tested on human tumour cell lines to assess their effects on cell viability by MTS assay and induced oxidative stress.

Results

This study has shown that prepared hempseed protein hydrolysates and its ultrafiltration fractions possess antioxidative activity and at some extent a protective effect against induced oxidative stress leading to increased cell survival. Based on our results, hempseed proteins and hydrolysates have potential of becoming a substance for production of peptide ingredients used in functional food formulations and nutraceuticals with bioactive properties.

Acknowledge

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Green Sono extraction: study of the physical impacts on the leaves of Rosmarinus officinalis

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Keywords: Ultrasound, Extraction, Mechanism, Diffusion, Microscopic observations.

Presentation: Oral

Objectives

There are more than 1300 articles in scientific literature dealing with positive impacts of Ultrasound-Assisted Extraction (UAE) such as reduction of extraction time, diminution of solvent and energy used, enhancement in yield and even selectivity, intensification of diffusion, and eliminating wastes. This present study aims to understand what are the mechanism(s) behind these positive impacts which will help to design a decision tool for UAE of natural products. Different microscopic observations (Scanning Electron Microscopy (SEM), Environmental Scanning Electron Microscopy (e-SEM), Cyto-histochemistry) have been used for spacial and temporal localization of metabolites in rosemary leaves, which is one of the most studied and most important plant for its antioxidant metabolites used in food industry, during conventional and ultrasound extraction. The study permits to highlight that ultrasound impacted rosemary leaves not by a single or different mechanism in function of ultrasound power, as described by previous studies, but by a chain detexturation mechanism in a special order: local erosion, shear forces, sonoporation, fragmentation, capillary effect, and detexturation. These detexturation impacts followed a special-order during ultrasound treatment leading at the end to the total detexturation of rosemary leaves. These mechanisms and detexturation impacts were identified in glandular trichomes, non-glandular- trichomes and the layer adaxial and abaxial cuticle. Modelling metabolites diffusion phenomenon during conventional and ultrasound extraction with the second Fick's law allowed the estimation of diffusivities and solvent penetration into the inner tissues and in meantime to accelerate the release of valuable metabolites.

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Phytochemicals from artichoke by-product and their applications as natural ingredients for cosmetic industry.

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Keywords: artichoke; polyphenols; vascular aging; cosmetic ingredient; by- products

Presentation: Oral

Objectives

Artichoke is a characteristic crop of the Mediterranean area recognized for its nutritional value and therapeutic properties due to the presence of bioactive components such as polyphenols, inulin, vitamins and minerals. The objective of this paper is related to the evaluation of the potential role of an artichoke ethanolic extract (AEE), recovered from undersized heads, in promoting the endothelial cell functions and stimulating the gene expression of some youth associated markers, with the main goal of characterizing it as cosmetic anti-age ingredient.

Methods

The methodology used was addressed to the characterization of polyphenols composition of AEE, to evaluate the antioxidant and anti-inflammatory activities in endothelial cells, macrophages and lymphatic vessels, and to assess the improvement of gene expression of some youth markers.

Results

The total polyphenol concentration of AEE obtained by HPLC analysis was 657.8 mg/100g FW, highlighting the presence of nine peaks with chlorogenic, 3,5-O-dicaffeoylquinic, and 1,5-O-dicaffeoylquinic as the most abundant compounds (87%); in addition, one flavonoid, apigenin-7-O-glucoside, was also recognized in the extract. Artichoke ethanolic extract enhanced important molecular markers responsible for the microcirculation and vasodilatation of endothelial cells, acted as a potential anti-inflammatory agent, protected the lymphatic vessels from oxidative damage by ROS formation, and improved the cellular cohesion by reinforcing the tight junction complex. In addition, the artichoke extract, through the modulation of molecular pathways, improved the expression of genes involved in anti-ageing mechanisms. *In vivo* studies on human subjects with sagging face reported an improvement in roughness and elasticity with application of the AEE cosmetic formulation

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compared to the placebo cream. The here reported results provide useful information on how the polyphenolic components of AEE act as a protective ingredient for both endothelial and lymphatic cells, supporting the possibility to reuse a vegetable by-products, such as undersized artichoke heads, for cosmetic and nutraceutical applications.

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European Research Opportunities for Green Extraction Technologies

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Presentation: Plenary

Keywords: Green technologies, European research projects

Objectives

Green technologies experience an increasing application in many industrial sectors. Such green technological concepts aim at minimisation of environmental impacts resulting from generation of (unwanted) by-products and solid or liquid waste. GHG emissions should be reduced by using renewable feedstocks as well as innovative and resources efficient technologies. Nevertheless, such technologies have to be economically feasible to be competitive. In order to develop such technologies scientific and technological expertise and financial resources are required. Especially small or medium sized enterprises (SMEs) are drivers for technological innovations, sometimes do not have required cooperation contacts, but many times lack the required financial resources.

Methods

The European research infrastructure offers several funding opportunities to be exploited for the development of green technologies in general and green extraction processes in particular. Pre-condition is, apart from a sound technological concept, an excellently composed European consortium of high complementarity. The next step is to develop a successful proposal in the context of a high competition.

Results

In the frame of a case study of the currently ongoing EU/BBI Research and Innovation Action Project US4GREENCHEM[4] (the ambition US4GREENCHEM is to combine only green technologies for the conversion of lignocellulosic biomass into a sustainable biorefinery.) at first the concept, the strategy, the approach and the exploitation potentials of this successful project will be demonstrated. In a second step the approach will be transferred to potential innovations in green extraction technologies.

[4] This project has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 669055

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Alternative solvents for green extraction, purification and formulation for cosmetic, food and nutraceutical products

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Keywords: Alternative solvents; green extraction, nutraceutical products

Presentation: Plenary

Objectives

A general definition of green chemistry is the invention, design and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances. In relation of green extraction of natural products, this definition can be modified as follows: "*Green Extraction is based on the discovery and design of extraction processes which will reduce energy consumption, allows use of alternative solvents and renewable natural products, and ensure a safe and high quality extract/product*". The listing of the "six principles of Green Extraction of Natural Products" should be viewed for industry and scientists as a direction to establish an innovative and green label, charter and standard, and as a reflection to innovate not only in process but in all aspects of solid-liquid extraction. The principles have been identified and described not as rules but more as innovative examples to follow discovered by scientist and successfully applied by industry.
Since solvents of petroleum origin are now strictly regulated worldwide, a growing demand of using greener, bio-based and renewable solvents for extraction, purification and formulation of natural and food products. The ideal alternative solvents are those non-volatile organic compounds (VOCs) that have high dissolving power and flash point with low toxicity and less environmental impact. They should be obtained from renewable and non-petrochemical resources at a reasonable price and are easy to recycle.

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Numerical simulation of unsteady multiphase non-Newtonian fluid flow for the design of an innovative ultrasound device able to improve olive oil extractability and quality

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Keywords: Numerical simulation-based design; cavitation; ultrasound treatment; extra virgin olive oil; green product extraction.

Presentation: Oral

Objectives

This work describes how 3D multiphase fluid dynamic simulations were employed in the design process of an innovative device, named Sono-Heat-Exchanger (SHE), that represents the first in the World continuous full-scale device combining the effects of a heat-exchanger with those of plate-shape ultrasonic transducers. The SHE is a revolutionary device able to drastically impact on the Extra Virgin Olive Oil (EVOO) extraction process, because it allows, for the first time, the simultaneous increase of the extraction yield and the phenols content. The numerical analysis was used for deeply studying the complex interaction between the non-Newtonian olive oil paste and the oscillating pressure waves generated by cavitation phenomena, which are induced by the ultrasound treatment. The calculations aimed at evaluating the flow parameters able to influence the process, thus avoiding expensive and time-consuming preliminary experimental tests for defining the optimal characteristics of the SHE. So that, the numerical analysis turned out to be essential in bridging the technology readiness "valley of death", advancing the device's TRL - Technology Readiness Level - beyond 6 and reaching level 9.

Methods

The SHE is composed of a couple of concentric annular sections. The olive paste flows into the external annular section, while water flows through the internal annular section to control the temperature of the olive paste. Outside of the external annular flow section, a plate-shape transducer is placed on each side of an octagon-shaped surface. The SHE allows to condition the olive oil paste with an ultrasound treatment, keeping, at the same time, its temperature under control and within the most favourable range for the EVOO extraction. As a result, it is possible to increase the extraction yield without the need of increasing the process temperature and affecting the product quality.
The fluid dynamic analysis was performed by means of the commercial software Ansys Fluent 17,1. The flow was modelled as laminar, due to the high viscosity of the olive paste. The olive oil paste was modelled as non-Newtonian compressible-liquid, following the Tait equation. In order to simulate the unsteady cavitating flow, the Schnerr-Sauer model was adopted. The oscillating frequency of the transducers was set to 23 kHz and a time step equal

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to a tenth of a single oscillation period was adopted. A whole structured dynamic grid was used to mesh a quarter of the geometry, for reducing the computational time. The best compromise suggested by a grid convergence analysis consisted of about one million of elements.

Results

In the case of the olive paste, ultrasound-induced cavitation promotes the disruption of tissue structures, enhancing the release of nutraceutical compounds. This means that every thing happens at very small scales and in a very short time. The numerical approach allowed to take a closer look at the phenomena occurring within the device, providing an analysis that would have resulted prohibitive to perform by means of a solely experimental-based approach.
Initially, the vapour appears in regions located extremely close to the transducer surfaces. Once formed, the vapour bubbles start to increase, as could be inferred from the fact that the vapour fraction increased accordingly in the simulations. An interesting result is that, in a given point, cavitation occurs with a periodicity that does not necessarily match the transducers oscillating frequency. This behavior is due to the fact that the pressure waves produced by a single transducer are not free to propagate in the liquid medium, but they experience many complex interactions with other negative or positive pressure waves generated both by wall reflections and other adjacent transducers. The study of such interactions allowed the definition of the optimal cross-section height for the SHE, as the best compromise between the need of maximize the ultrasound effects and that of avoiding undesired interference effects between adjacent transducers.
Another relevant results that the numerical simulation revealed is that, besides the cavitation phenomena, the ultrasound treatment produces a secondary effect that favors the extractability. The fluid near the transducer is forced to move radially, generating a stirring effect, comparable to that imposed to the olive paste during the malaxation step. This effect enhances the coalescence kinetics(the smaller oil droplets are forced to collide each other to form larger droplets), leading to an increase of oil recovery. The proposed staggered disposition of the transducers was thought for enhancing this beneficial stirring motion and reducing any possible interference effect. For these reasons, the SHE represents a great step towards the transformation of the discontinuous malaxation step into a fully continuous phase.
Experimental tests, performed on real scale mill plants, demonstrated the simultaneous increase in the extraction yield and the phenols content in the obtained olive oil achievable by means of the designed machine.

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Bioactive compounds extraction from *Arthrospira platensis* through sonoporation induced by ultrasounds.

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Keywords: Ultrasounds, sonoporation, Arthorpira platensis, extraction, microscopy

Presentation: Oral

Objectives

Arthrospira platensis, also called spirulina, is a cyanobacteria with a wide range of interesting biomolecules. Particularly, spirulina is interesting for its protein content (50 -70 %) with high digestibility. In the present study, ultrasounds were selected to extract bioactive compounds from spirulina biomass. Ultrasound-Assisted Extraction (UAE) is widely used for its positive contribution to extraction efficiency such as reduction of extraction time, yields enhancement, diminution of solvent quantity.

Methods

UAE (reactor, 25 kHz) was compared to conventional process performed in the same conditions without ultrasounds. Extraction parameters were optimized using a factorial design.
Microscopic investigations were carried out to allow a better understanding of ultrasound physical effects on spirulina filament over time. Crude extract and sections stained with toluidine blue were analyzed with optical and scanning electron microscopy.

Results

According to the experimental results, ultrasounds enabled to get 229.23 % more proteins and 200.88 % more phycocyanin than conventional process (maceration). With 52.50 gram of proteins per gram of dry spirulina biomass in the extract, a protein recovery rate of 87.50 % was achieved.
Microscopic observations proved that acoustic cavitation impacted spirulina filaments by different mechanisms such as fragmentation, sonoporation, detexturation. These various phenomena make easier extraction, release and solubilization of spirulina bioactive compounds.

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Green extraction of natural products using microwaves and centrifugal force thanks to a semi-industrial pilote.

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Keywords: Green extraction, microwaves, centrifugal force, pilote, polyphenols, essential oils.

Presentation: Short communication

Objectives

The objectives of this study is to extract natural products using a coupling of thermal and mechanical effects thanks to a pilote: heating of microwaves and rotational speed in the same time during the process. The goal is to determine the influence of rotational speed on microwaves extraction.

Methods

A semi-industrial pilote which combines MicroWaves and Centrifugal force (MW/C) with a wave guide without contact to avoid vibration on magnetron was used. For each vegetable matrix, a reference extraction with organic solvent is carried out to evaluate the extraction power of the pilote. Lettuce polyphenol extract was analysed by Liquid chromatography-Diode Array Detector and Uv-Vis spectroscopy. Analyses of essentials oils from lavender and orange peels were performed by Gas chromatography. The extract yield and quality of our pilote were compared with the reference.

Results

First results obtained for lettuce study showed the efficiency of rotational speed during microwaves extractions. Indeed, two extractions in the same conditions were carried out, only centrifugal force changed: in the first case 30g and the second one 2700g. In concern of the yield of total phenolic compounds, we obtained 4 mg equivalent chlorogenic acid (EQ CA) per 100 g of fresh matrix (FM) against 24 mg EQ CA per 100g of FM respectively. Rotational speed allows to intensify yield of total polyphenols.

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Results about lavender flowers and orange peels are in progress but some first analyses were carried out concerning lavender. Essential oils were evaluated on their quality. It can be given by the percentage of lynalyl acetate. It is a good indicator of degradation of compounds presents in essential oils. Our process gives an essential oil with high quality (52% of lynalyl acetate) in comparison with the conventional method: Hydrodistillation (HD) (23.7% of lynalyl acetate). However, the yield is lower for MW/C: 1% vs 2.6% for HD.

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How to improve technology and usability of REUS ultrasounds machines: ULTRASONS REUS 2.0 "Simple but effective".

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Keywords: *Ultrasounds, Cavitation, Production, Yield increase*

Presentation: Short communication

Objectives

When the working spaces are reduced and the production needs increase, it is necessary to study performance solutions that improve the work of the operators and increase the overall quality.

Methods

We start from the idea just to the project through risk assessment, up to EN and ATEX certification, assisted by a team of electronic, chemical and environmental engineers.
We use stainless steel 316L with high quality welds.
We can assure production of absolutely handcrafted ultrasound generators in our French workshop, where also transducers are produced with totally European components.
Continuous study of new electronic solutions to overcome the past and move towards digital and remote controls will allow us to certify the good functioning of our systems.

Results

Production of simple units to use, and with important results in terms of time and performance since the early 80s
Possibility to transpose the results on pilot and industrial devices.

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Optimisation of the Oil Extracted from Sardinella lemuru Waste with Supercritical Fluid Extraction (SCCO2) using Response Surface Methodology (RSM)

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Keywords: Supercritical fluid extraction (SC-CO_2), soaking technique, optimisation, EPA, DHA

Presentation: Oral

Objectives

The application of RSM for the experimental study design was an effective statistical tool to optimise the extraction conditions and valorise *S. lemuru* waste with higher contents of DHA and EPA. This research aimed to determine the optimum total oil yield extracted from sardine (*Sardinella lemuru*) waste using Supercritical fluid extraction ($SCCO_2$) with soaking technique prior to waste extraction. The utilisation of fish waste could be valorised as a cheap raw material for the generation of omega three (ω3) concentrates.

Methods

Supercritical fluid extraction ($SCCO_2$) of *S. lemuru* oil was performed to optimise various parameters, such as soaking (X_1) and extraction time (X_2) to obtain the highest yield of extracted oil with the highest amount of EPA and DHA recovery. The soaking and extraction time ranged from 1 to 4 h and 3 to 5 h respectively while maintaining constant conditions of 350 bars, 60°C and a 5 ml/min flow rate.

Results

$SCCO_2$ with soaking technique was successfully optimised at 1.7-h (X_1) and 3.75-h (X_2) soaking and extraction time, respectively, with a pressure of 350 bars, a temperature of 60 °C and a 5 ml/min flow rate remaining constant. The optimum condition of $SCCO_2$ with soaking technique reported with higher total oil yield, 3.3% and higher PUFAs recovery as compared to $SCCO_2$ continuous technique, 3.0%. Furthermore, soaking step prior to the extraction had a significant effect ($p<0.05$) on a yield and total oil can be increased as the as solubility of CO_2 increased. This study verify that the combination of soaking and extraction time (X_1X_2) time were highly significant to the oil yield as per quadratic term. This is showed by the experimental results fitted the predicted values with a coefficient value of R^2 = 98.47%.

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Enhanced extraction from malt and hops in an innovative beer-brewing technology based on hydrodynamic cavitation processes

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Keywords: Beer; Brewing; Extraction; Hops; Hydrodynamic cavitation; Malt.

Presentation: Oral

Objectives

Controlled hydrodynamic cavitation (HC) technologies are arising as new standards in a growing number of industrial applications, often synergically with conventional processes. Extraction of bioproducts, water remediation, waste and biomass processing, creation of ultra-stable nanoemulsions, represent few of the fields benefitting from HC technologies in terms of effectiveness, efficiency and process yields.
In the field of production and processing of beverages and other liquid foods, sometimes HC-based technologies can completely replace conventional ones with distinct advantages. For example, HC processes alone can achieve food-grade sterilization, pasteurization and homogenization, as well as enhance the extraction of valuable bound bioactive compounds.
In the case of beer-brewing, the main objectives of the use of HC processes in the mashing and hopping steps were the removal of long-established production stages such as dry milling of grains and wort boiling. Moreover, other objectives were the enhancement of the extraction of major constituents of the beer wort, such as starch and, ultimately, fermentable and unfermentable sugars, as well as of valuable bioactive compounds such as polyphenols and flavonoids, from malt and hops.
Finally, an important objective concerned the proof of the scalability up to the industrial level, as well as the integration of the HC-based brewing plant into an operational craft brewery.

Methods

The innovative HC-based brewing equipment is based on a closed-loop circulation, where centrifugal pumps are the only drivers of circulation and heating, while HC is triggered and sustained by reactors in the form of suitably sized Venturi tubes. The device comprises a main tank equipped with a thermostatic unit, a filtering device, and a cooling unit before adduction to fermenters. Temperature, pressure and pH gauges, as well as an electronic control panel allow a flexible process management. The only power source is electricity feeding the centrifugal pumps.
The HC-based brewing equipment was initially constructed as a pilot plant with capacity around 250 L, later upsized to an industrial-level system, with a net capacity of over 1,200 L.

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The brewing method consists of simultaneously circulating, cavitating, and heating, a mixture of pH-corrected water and non-pre-milled malt, until all the starch has been extracted and converted by the enzymes into fermentable and unfermentable sugars. Once the enzymes' inactivation temperature is reached, separation of the clear wort from residual pulverized solids takes place, this being a critical and sometimes overlooked step, due to the fineness of the spent grain particles.
Last, hops are pitched into the clear wort, which is further heated until the hops' utilization, based on the extraction and isomerization rate of hops' α-acids, achieves levels comparable with conventional boiling steps.

Results

Dry milling of grains was proved useless, due to the respective HC-driven pulverization, leading to considerable savings in operation time and equipment cost.
Starch extraction was accelerated and enhanced, as well as the respective saccharification, *i.e.* the conversion into fermentable and unfermentable sugars, occurred at substantially lower temperatures, leading to further considerable savings in operation time and energy consumption, as well as to savings in malt quantity for the same recipe. While the savings in operation time and energy consumption totaled about 50% with the use of malt as main ingredient, the same figures increased further when using up to 100% raw unmalted grains and exogenous enzymes.
The extraction of important prenylflavonoids from hops, such as xanthohumol, desmethylxanthohumol, and 6-geranylnaringenin, was increased, thereby extending the beer's shelf life due to the respective antioxidant activity.
All the above advantages were achieved by means of a compact installation including far fewer components than conventional technologies, while retaining safety, operation reliability, virtually universal application to any brewing recipe, and beer quality.
Moreover, the scalability of the HC-based brewing system was fully proven, since the capacity of the core of the brewing system, comprising the HC reactors, was upsized by five times, while retaining all the advantages of the pilot plant. As well, all the challenges posed by the integration of additional, industrial quality components, necessary to allow a regular operation of the brewery that houses the industrial-scale plant, were successfully overcome.
The technology and the related method were patented (patent No. WO/2018/029715, published on February 15th, 2018), as well as protected under the trademark CAVIBEER (application No. 017894648, registered on August 30th, 2018).

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oliveCEPT® - A commercially available product for improving olive oil extraction yield and quality

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Keywords: Olive Oil Extraction, Cold Extraction, Pulsating Electric field, Emerging Technologies

Presentatio: Oral

Objectives

The oliveCEPT® applies a Pulsating Electric Field (PEF) to the crushed olives through the patented CEPT® (Closed Environment PEF Technology) platform consisting of a generator unit and a treatment chamber. The oliveCEPT® is a commercially available product.

Methods

The generator unit creates high voltage pulses which are accurately controlled and applied to the substrate in the treatment chamber. The square high voltage pulses are repeated 1-2 thousand times per second, allowing for 2-10 m^3/h of substrate to be treated, which allows industrial scale applications. The pulses cause pore formation in the cell walls and membranes which can be either regenerative or critical. Regenerative pores, or re-sealing pores, will not kill the target cells and is thus, not useful for extraction purposes. The critical pores, on the other hand, are caused by higher energy pulses and will cause cell lysis, and are consequently destroying the cells. Energy consumption for the treatment is very low, ranging between 2-8 kW, rendering the treatment a very cost-effective alternative for boosting production values.

Results

The production volume of cold pressed olive oil is increased by at least 5%, naturally varying with malaxation time and temperatures as well as with the olive variety. This level of increased yield has been confirmed both internally by ARCAROMA and in literature. Additionally, the quality driving substances like polyphenols and vitamins are increased in the extracted olive oil after CEPT® treatment. ARCAROMA has successfully verified the results both in laboratory scale production plants and in full scale industrial plants. At the present, the return of investment for this application is around 2 years.

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Innovative mixture with antioxidant action, with positive effect on the intestinal microbiota

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Keywords: resveratrol, quercetin, proanthocyanidins, antioxidant, microbiota, chronic intestinal inflammation

Presentation: Short communication

Objectives

A study was carried out aimed at the identification of specific classes of biocomposites with a positive effect on the intestinal microbiota, able to delay the symptoms of chronic intestinal inflammation. The product developed is a mixture of: quercetin (65%), resveratrol (23%) and proanthocyanidins (12%) for which there is scientific evidence able to demonstrate the beneficial effect in mouse models and on humans.

Methods

The mixture was used for the evaluation of the anti-inflammatory effect on a model of intestinal inflammation. Murine dendritic cells were cultured in vitro and exposed to quercetin or mixture on day 5 and 7 of in vitro culture. On the 8th day, LPS was administered and mRNA was extracted to evaluate the gene expression of key molecules in the regulation of inflammatory checkpoints (SLPI: secretory leukoprotease inhibitor) in addition to TNF□ □and interleukin 6.

Results

The effect of the three classes of polyphenols that make up the mixture was synergistic: the sum of the three compounds (16 µM quercetin, 6 µM resveratrol, 3 µM anthocyanins) showed a synergistic effect higher than that of the single compounds (quercetin 25 µM) . Thanks to the presence of more polyphenols, the mixture is able to significantly inhibit the secretion of inflammatory cytokines of dendritic cells

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Green extractions of bioactives from Vitis vinifera L. (cv. Aglianico) leaves: phenolic profile, antioxidant and anti-cholinesterase activity of extracts.

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Keywords: green extraction, natural products, biological activity

Presentation: Short communication

Objectives

Preceding researches shown that grape polyphenol compounds defend against oxidative stress, responsible for neurodegenerative diseases, cancer and diabetes [1, 2]. The aim of this research is to analyze the effects of several green extraction methods on the quali-quantitative phenolic profile, on the antioxidant and cholinesterase inhibition activity of Vitis vinifera L. (cv. Aglianico) leaf extracts.

Methods

Aglianico leaves were collected in Basilicata Region and subjected to different green extraction methods: Accelerated Solvent Extraction at 40°C (ASE 40) and 50°C (ASE 50), Soxhlet extraction (SOX) and Ultrasound Assisted Extraction (UAE). The extracts were analysed by LC-DAD to determine their phenolic profiles. The extracts were also subjected to different in vitro assays to evaluate their polyphenols content and their antioxidant activity [3, 4]. Moreover, the Relative Antioxidant Capacity Index (RACI) was calculated to compare the data obtained by different chemical assays. Then, the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition assays were performed to test their enzymatic inhibitory activity. In fact, the cholinergic deficit is characteristic of the Alzheimer's disease (AD), the most common human neurodegenerative disorder [5].

Results

The HPLC studies led to the identification and quantification of 12 phenolic compounds. The Soxhlet method was the best extraction technique in terms of yield. Moreover, the SOX extract showed the highest RACI value, followed by ASE 50
extract. Again, the ASE 50 and SOX extracts exhibited the best AChE and BChE inhibitory activities, respectively. In conclusion, this research demonstrated as different green extractions of Vitis vinifera L. (cv. Aglianico) leaves were able to affect differently phenolic profiles, and antioxidant and anti-cholinesterase activity. In particular, it was demonstrated for the first time that Aglianico leaves are important sources of phenolics that could be used to prevent oxidative stress and potentially be helpful in Alzheimer's disease treatment.

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Food taxes between ability-to-pay principle and extra-fiscal purposes

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Keywords: taxation, food, extrafiscal, health, ability to pay

Presentation: Oral

Objectives

As emerges from more or less recent past experiences, food taxes have been largely adopted to increase revenue. Different taxes have been enforced over time to discourage food consumption, food production and food processing. Only recently, the need to promote healthy eating lifestyles has induced the legislator to act through fiscal instruments based on their capacity to condition individual and social group behaviour. It is in fact clearly evident how tax norms can "orient" eating habits by both taxing unhealthy food and by reducing taxes on healthy foods thus promoting and incentivizing the consumption of the latter. The introduction of a fat tax or, in general, tax increases on non-meritorious consumption, could produce a dual effect: on the one hand, the State would have financial funding to be allocated for information campaigns on the risks of being overweight, on the other it would discourage the consumption of products potentially harmful to human health, achieving savings in health care costs.

Methods

From a wider angle we can highlight that the taxes in question, although potentially having a transitory and fortuitous duration, can become permanent only by their conformity to the criteria of reasonableness and fairness of distribution [30] and social acceptance. These values, while composing the ethological humus from which our Constitution, draws its strength, must, in fact, find concrete expression in the tax forms even if novel and oriented towards manifestations of wealth, such as the consumption and the production of food harmful to health, which until now have not been given much consideration.

Results

The philosopher of Swedish origin A.J. Carlson defined obesity in 1942 as an "offensive luxury" that the State must limit and counteract by any means. Presently, in times of a public finance crisis, the fight against overweight is relevant especially for the substantial savings on health care costs. The introduction of a fat tax or, in general, tax increases on non-meritorious consumption, could produce a dual effect: on the one hand, the State would have financial funding to be allocated for information campaigns on the risks of being overweight, on the other it would discourage the consumption of products potentially harmful to human health, achieving savings in health care costs. It is, however, evident that a serious policy to

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combat obesity should not only be present at local or national level, but must expand at European Union level and in all developed countries in order to promote responsible choices and informed decisions.

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Virgin olive oil biophenols: evolution during the shelf-life and influence on aroma release and sensory quality

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Keywords: virgin olive oil, EFSA claims, phenol compounds (biophenols), shelf-life, off-flavour, olive fruity

Presentation: Oral

Objectives

The role of biophenols in the sensory and nutritional quality of extra-virgin olive oil (EVOO) has been carefully defined by assessing the sensory profile and phenolic composition, according to UE official methods and 'EFSA claims', respectively. New approaches have ben also developed ('artificial mouth' coupled with SPMEGC/MS analysis of volatiles, 'electronic nose', NMR 'electronic tongue') to follow the evolution of biophenols and volatiles during EVOO shelf-life and to verify 'EFSA claims' validity up to the end of shelf-life in bottled EVOO. The role of biophenols during EVOO shelf-life on aroma release in mouth and on the sensory evaluation of positive attributes and off-flavours has been also studied.

Methods

Several shelf-life test were made on bottled EVOO samples at 20° in the darkness and different light contitions /packaging materials. Biophenols were monitored by HPLC and LC/MS, volatiles were measured by SPME-GC/MS, sensory assessment (panel test) was made by two UE official panels. Interaction between saliva, biophenols and volatile compounds were studied by using an 'artificial mouth' (Genovese et al., 2015, 2018).

Results

EVOO biophenolic compounds showed a slight decrease during the shelf-life of filtered/unfiltered EVOOs with the validity of EFSA claims maintained for 6-18 months depending on shelf-life conditions and EVOO initial composition. Based on these data, a prevision model has been implemented (validation in progress). Interactions among human saliva, biophenolic and volatile compounds may significantly change the sensory perception of both 'olive fruity' and off-flavour intensity.

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Consumer acceptance of innovations in extra virgin olive oil production process: socio-demographic and psychographic determinants.

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Keywords: Extra virgin olive oil, innovation, consumers' acceptance

Presentation: Short communication

Objectives

Extra Virgin Olive Oil (EVOO) is one of the most popular Traditional Food Products (TFPs) (Vanhonacker et al., 2010) of Mediterranean countries. Despite the widely recognized advantages of innovation for the competitiveness of firms operating in global food markets, the EVOO industrial process has changed very little over the last few decades (Clodoveo et al., 2017). This seems mostly due to the fact that introduction of innovation in TFPs is more problematical compared to other conventional foods (Almli et al., 2011). In general, when innovations are applied to TFPs, their degree of acceptance is strongly dependent on the type of product and the type of innovation (Guerrero et al., 2009). In this study, we have tested acceptance and consumers' willingness to pay for a set of emerging innovations that may be introduced in EVOO production process: i) extraction process assisted with ultrasound application, ii) extraction process assisted with micro-wave application, iii) EVOO packed in bag-in-box, and iv) EVOO nitrogen packed. These innovations have been already tested for their technical feasibility but there is uncertainty about their possible market success.

Methods

A choice experiment has been conducted based on a consumer survey carried out in Italy, the largest olive oil consumer country in the world, accounting for about 20% of the global consumption (IOC, 2016). The survey was conducted in November-December 2017 through a web-questionnaire administered by an agency specialised in market surveys. The inclusion criteria for the target population were: i) being household responsible for food purchasing, and ii) have bought olive oil at least once in the last year.

Results

A total of 1,003 participants were recruited with a stratified quota sampling based on geographical area, municipal size, age, gender, and education, in order to ensure the representativeness of the sample at national level. In the choice experiment, we compared the four types of innovations jointly with the most reliable EVOO attributes (e.g. country of

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origin, taste, and price, etc.). Results show different degrees of acceptance among innovations and between different groups of consumers. In particular, those innovations that provide consumers with tangible and relevant benefits seem to be well accepted, provided that these innovations do not damage the traditional character of the product.

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New analytical approaches to determine the EVOO quality: a study on the volatiles and phenolic fraction of Italian oils from 2017

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Keywords: secoiridoids, OH-tyrosol, HS-SPME-GC-MS, HPLC-DAD

Presentation: Oral

Objectives

Aim of this work was to improve the analytical procedures to define the quality of the EVOOs focusing on the phenolic component and the volatile organic compounds (VOCs). Revised analytical procedures and new methods were applied to a selection of EVOOs produced during the 2017 crop season from Tuscany and Apulia, as part of the activities of the COMPETiTiVE project founds by the AGER Project.

Methods

The phenolic fraction of seventy EVOOs was extracted in agreement with the COI method [1]. The HPLC-DAD analysis were performed using three different columns. The phenolic extracts were also analyzed after acidic hydrolysis according to a revised method, validated within this project. The VOCs were determined by HS-SPME-GC- MS according to a recently validated method [2].

Results

The first step was focused to test new reverse phase columns able to overcame a well- known criticality: the chromatographic resolution of secoiridoids and lignans. The validated acidic hydrolysis procedure was confirmed as the best method to determine in a simply way the total OH-tyrosol and tyrosol in different EVOOs, as requested for EFSA claim. The analysis of VOCs using a set of structurally different internal standards, allowed to obtain an accurate characterization of monovarietal oils and of oils obtained by the application of ultrasounds. The obtained findings allowed to build two blends, one richer in secoiridoids and the other one richer in lignans. These oils were used to obtain phenolic extracts intended for the biological test on Caco2 cells and hepatic HepG2cells.

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Studies on extra-virgin olive oil degradation during shelf-life.

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Keywords: colloids, quality, shelf-life, stabilization, turbidity

Presentation: Oral

Objectives

The quality of extra-virgin olive oil decreases during the storage. The oxidative indexes usually increase the phenol composition changes as well as the volatile profile, leading to the appearance of sensorial defects and to the decrease of olive oil nutritional value. Colloidal suspensions contribute to these changes and they are usually removed from the olive oil with filtrations. However, a part of consumers require a veiled extra-virgin olive oil. In fact, they consider veiled olive oil "less processed" and with superior sensory characteristics. Hence, the development of strategies to slow olive oil degradation phenomena and the understanding of the mechanisms responsible for these changes are key tasks of the extra-virgin olive oil industry.

Methods

The effect of suspended solids, water, and microorganisms on olive oil quality was assessed with 2 different tests. In the first test the olive oil was treated with high hydrostatic pressure (HPP) to evaluate the changes of oil without microorganisms and with water and solids. In the second test, the solids and the water fractions were selectively removed from olive oil in order to evaluate the specific effect of these fractions.

Results

Several degradation pathways on the volatile fraction were prevented with the sterilization of the oil with HPP. On the contrary, the degradative reactions on phenols were not prevented by the HPP. The selective removal of water and solids from an olive oil produced several changes in term of volatile and phenolic compositions. The tests highlight the importance of the 3 olive oil turbidity components (i.e. solids, water, and microorganism) for the olive oil quality degradative phenomena during the shelf-life.

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Development and validation of versatile cellular models for investigating the absorption and bioactivity of natural extracts

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Keywords: antioxidant activity, Caco-2 cells, cholesterol metabolism, EVOO polyphenols, HepG2 cells

Presentation: Oral

Objectives

In order to explain the health benefits provided by high quality extra virgin olive oil (EVOO), the objectives of the work are the following: **a)** Assessing whether EVOO polyphenols are absorbed at intestinal level and their antioxidant capacity on human intestinal Caco-2 and hepatic HepG2 cells. **b)** Evaluating their effects on cholesterol metabolism in Caco2 and HepG2 cells and on ox-LDLs and lipid peroxidation in HepG2 cells.

Methods

For the absorption experiments, differentiated human intestinal Caco-2 cells are incubated in the apical side with the tested samples and absorbed species are detected and quantified by HPLC-chip/ESI/MS/MS. The antioxidant effect is evaluated *in vitro* and at cellular level by DPPH assay and analysing the reactive oxygen species (ROS) production. The effects on cholesterol metabolism are assessed by measuring the OxLDL production and lipid peroxidation.

Results

All methodologies were optimized working on plant protein hydrolysates as testing samples. Using the HPLC-chip/ESI/MS/MS approach, absorbable lupin peptides were identified and strategies developed to analyse further their biological activities. Methods to evaluate the antioxidant activity at *in vitro* and cellular level were optimized on soybean protein hydrolysates. Their ability to reduce the ROS and nitric oxide (NO) production after induced oxidative stressed on HepG2 cells were assessed. The same approaches are currently applied to study the EVO polyphenols bioactivity.

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Pilot scale cavitational treatments in oil extraction processing

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Keywords: oleaginous plants, flow ultrasound, hydrodynamic cavitation, high shear homogenizers.

Presentation: Oral

Objectives

Cavitational treatments in oil extraction showed several advantages over the conventional processes with organic solvents. Besides yields, organoleptic and physicochemical profiles comparison, the oxidative stability of the oils need to be investigated. Non-conventional techniques such as flow ultrasound, hydrodynamic cavitation, high shear homogenizers were object of this study. Aiming to design a green and efficient extraction process to be applied on olive, a process optimization was carried out on high oleic sunflower seeds.

Methods

The oils obtained under flow ultrasound, hydrodynamic cavitation (rotor/stator unit) and high shear homogenizers using water as dispersing medium, have been compared to those extracted by cold pressing and solvent extraction. The samples were analyzed in terms of fatty acids profile, antioxidant activity, and content in polyphenols, tocopherols and phytosterols.

Results

Oil yields, antioxidant activity and polyphenols, tocopherols and phytosterols content, were strictly related to operative conditions. A fast sequential treatment with high shear homogenizer and either flow ultrasound or hydrodynamic cavitation offered the best results with the possibility of scaling up.

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The Fast Track to Innovation: the right tool to face the innovation challenge and the valley of death.

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Keywords: innovation, valley of death, Fast Track to Innovation, extra virgin olive oil, ultrasound.

Objectives

Taking an idea from basic concept to commercially available product is highly rewarding, but can be a very long, complex and difficult journey. Recognising and understanding the stages of the process, and using the right support to help you navigate through it, can mean all the difference between success and failure. The road from concept to market is marred with obstacles, and many businesses fail to pass beyond the development stage.
A better understanding of the innovation process is essential from the outset if the pioneers of innovation are to overcome the dangers that they are likely to face along the way, and maximise their opportunities for success.
The main challenge among the innovation road is the very well publicised phenomenon in the innovation industry is known as the 'valley of death'. This is the point of no return from which many new ideas going through the innovation process fail to progress, and even those ideas that do make it out of the valley can spend anywhere between 5 and 10 years trying to escape from it.
To understand what the valley of death is and when innovators can expect to arrive there, it's useful to consider the concept in terms of Technology Readiness Levels (TRLs). The TRLS are the terminology used by NASA to demonstrate the innovation process.
An invention is creating something new that the market has not seen before. An innovation is taking an existing concept or idea and improving it.
The 'valley of death', also known as the innovation gap, occurs between TRL levels 4 and 7. The valley tends to appear at the point where a conceptual idea needs to be turned into a working prototype in order to demonstrate that it works, assess production costs, and to outline the equipment and processes needed for manufacture.
It is at this stage that the European Programs, such as Horizon 2020 in general, and the program Fast Track to Innovation, can support companies in assessing the potential feasibility and value of their idea and provide information and advice on the best way forward and the needed financial support.

Methods

The Fast Track to Innovation (FTI) is a fully-bottom-up innovation support programme promoting close-to-the-market innovation activities open to industry-driven consortia that can be composed of all types of participants. It can help partners to co-create and test

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breakthrough products, services or business processes that have the potential to revolutionise existing or create entirely new markets, under the helm of the new European Innovation Council (EIC) pilot.

Results

The Fast Track to Innovation (FTI) has financed the project OLIVE-SOUND. OLIVE-SOUND is an Ultrasonic (US) reactor for the treatment of olive paste which enhances the working capacity of olive mills. It reduces malaxation time, the current bottleneck in olive oil extraction, by 70% and hence reduces energy usage. At the same time, it increases extraction yield, improves olive oil quality and reduces investment costs compared to a traditional malaxation system.

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Exploring alternative analytical strategies to quantify polyphenols in virgin olive oil

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Presentation: Oral

Keywords: alternative analytical strategies, polyphenols, virgin olive oil.

Objectives

The regulation EU n.432/2012 allows to label commercial extra virgin olive oil (EVOO) containing more than 250 mg/kg of polyphenols ("hydroxytyrosol, tyrosol and their derivatives, e.g. oleuropein complex and ligstrosides") with the health claim: "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress". Olive oil polyphenols constitute a heterogeneous class of compounds, in general structurally not correlated. Although several spectrophotometric, enzymatic and chromatographic methods have been proposed to determine EVOO polyphenols, at present the international olive council (IOC) has approved only the method based on the HPLC-UV (λ=280 nm) analysis of hydroalcoholic EVOO extracts.

Methods

Several methods alternative to HPLC are in course of evaluation to determine EVOO polyphenols. The "ideal" method should exhibit robustness comparable to HPLC, while improving both cost- and time-effectiveness as well as rapidity and easiness for real time and on-site (at the oil mill) classification of EVOO. Among the alternative methods, we are evaluating, on set of 22 EVOO samples: i) the antioxidant (radical scavenging) classic DPPH assay; ii) a novel colorimetric strategy based on the covalent pairing of activated phenols to a diazonium salt dye (Medina, 2011); iii) attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR); iv) total antioxidant activity (TAC) determined by microamperometry, using electrogenerated bromine as the active species.

Results

ATR-FTIR of unfractionated EVOO might be a valuable method, but its sensitivity still appears inadequate. The combined use of ATR-FTIR and chemometry remains to be evaluated.
The DPPH assay exhibited an unsatisfactory correlation with the classical Folin-Ciocalteau colorimetric method and both were scarcely correlated to the HPLC-UV determinations.

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Similarly, TAC measurements did not mirror the HPLC figures, while showing comparable trends with DPPH assay, most likely due to the nature of the methods, both substantially detecting electron transfer events. Upon optimization, TAC has potential to be an easy, robust, cheap and rapid test and might be a candidate method for an alternative classification of EVOO, independent on the HPLC-determined content of polyphenols.

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Communicating the health value of olive oil: An analysis of consumers' emotional and attitudinal responses to label health claims

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Keywords: *olive oil, health value, label health claims, consumer emotions, sensory features, slogan persuasiveness.*

Presentation: Oral

Objectives

The aim of this research is to identify the best marketing strategies to communicate the health value of the olive oil product. Specifically, the research investigates the effects of different label health claims authorised by the EU Register on Nutrition and Health Claims on consumers' attitudinal (i.e. attitude toward the product) and behavioral responses (i.e. emotional reactions; willingness to pay for the product) toward olive oil. Furthermore, the research examines whether using slogans that associate olive oil health properties with the sensory aspects of the product (e.g. color, flavour, taste) elicits more favourable consumer responses (i.e. slogan memorability, consumer engagement, willingness to buy the product) compared to health-based slogans that do not refer to these features.

Methods

Two different studies will be designed in order to address the research objectives. Following a between-subjects experimental design, in Study 1 participants will be randomly exposed to product labels containing one of the health claims authorised by the EU Register on Nutrition and Health Claims. Then, their attitudinal responses toward the olive oil product (i.e. attitude toward the product) will be assessed through an online questionnaire. Additionally, participants' emotional reactions (e.g. whether the label claim elicited a positive or negative emotion) will be monitored through the Noldus FaceReader™ software. Again, in Study 2, a between-subject experimental design will be used to assess consumer response to two sets of advertising slogans related to olive oil health properties (with or without reference to olive oil sensory aspects – e.g. color, flavour, taste). Particularly, the study will assess the persuasiveness of these messages (i.e. memorability, willingness to buy the product) through

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an online- based questionnaire, while, consumer engagement in terms of emotional reactions (i.e. arousal, elicited emotions) will be evaluated through the Noldus FaceReader™ software.

Results

The results will highlight the different impact of the authorised health claims on consumers' intention to buy olive oil. Additionally, the analysis of the different effects of the authorised olive oil claims on consumers' attitudes and emotional reactions toward the product will help marketers design effective communication strategies aimed at changing consumer perceptions of the product. Finally, determining whether the inclusion of sensory aspects in advertising slogans that promote olive oil wholesomeness could increase the persuasiveness of such messages, will provide marketers with useful suggestions to increase olive oil consumption and expand the for this product.

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Effects of Free Fatty Acids and Phenolic Compounds of Extra Virgin Olive Oil on Beta-cells Survival and Function.

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Keywords: Diabetes, beta-cell, extra virgin olive oil, free fatty acids, phenolic compounds

Presentation: Oral

Objectives

Beta-cell dysfunction is a key event in the onset and progression of type 2 diabetes (T2DM). Extra virgin olive oil (EVOO) is one of the major components of the Mediterranean diet and is appreciated worldwide because of its nutritional benefits in metabolic diseases, including T2DM. EVOO is composed mainly of the mixed triglyceride esters of oleic and palmitic acid and of other fatty acids. In smaller amount, it contains micronutrients such as phenolic compounds (PC) that may positively influence the metabolic status. In this study we have evaluated the effects of palmitic and oleic acid, as well as of the main PC of EVOO, on pancreatic beta-cell survival and function.

Methods

Rat insulinoma INS-1E cells, human pancreatic 1.1B4 cells, murine and human pancreatic islets were incubated with 0.5 mM palmitic or oleic acid, followed or not by stimulation with 10 nM or 50 nM exendin-4. INS-1E cells were also incubated with the main PC of EVOO at the dose of 10 μM, for up to 24 h. Apoptosis was detected by cytosolic release of oligosomes. Insulin content and secretion were measured by ELISA. Gene expression was evaluated by quantitative RT-PCR. Protein expression and phosphorylation were visualized by immunoblotting.

Results

Both palmitic and oleic acid induced beta-cells apoptosis through a mechanism dependent on the increased expression and function of the $p66^{Shc}$ redox adaptor protein.
Palmitic acid was able to activate also the stress kinases JNK and p38 MAPK, known to be involved in the apoptotic process. However, palmitic acid, but not oleic acid, reduced the beneficial effects of exendin-4, an analog of the incretin hormone glucose-like peptide 1 (GLP-1), on beta-cell signalling and function. Among the PC of EVOO, Apigenin was able to improve insulin biosynthesis and secretion without inducing apoptosis.

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Effects of ultrasound on biogas production from olive pomace

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Keywords: Ultrasound pre-treatment, Biogas, Olive pomace.

Presentation: Poster session

Objectives

A precious economic and social resource for Mediterranean Countries is certainly represented by olive trees, especially for Apulia, a region in Southern Italy. A great research effort is focused on the valorization of such resource, included the by-products of the olive oil production (olive pomace). New technologies and new process for high-quality virgin oil production are developing fast, as well as new technologies are proposed for an energetic valorization of residues deriving from olive oil industry.
Among these residues, olive pomace has recently attracted increasing attention since it can be used for producing biogas via anaerobic digestion processes, other than heat and power via classical thermochemical conversion processes.
Recent findings have pointed out that a pre-conditioning of olive pomace with low-frequency (20-60 kHz) ultrasound techniques (US), can enhance the biogas production yield. Consolidated evidences about the beneficial effects of an ultrasound treatment can be found in extra virgin olive oil extraction process, as well as in sludge treatments.
Ultrasound (US) is a promising emerging technology that has already found application in the food industry. The main reason relies on the mechanical effects generating during an ultrasound treatment and which are due to cavitation phenomena.
In this work experimental laboratory tests were performed with the purpose of assessing the effects that an ultrasound preconditioning of three-phase olive pomace (3-POP) can produce on the biogas yield in batch process.

Methods

With the aim of increasing the biogas production from olive pomace, an ultrasound pre-treatment was applied to 3-POP. Several samples were prepared starting from 3-POP provided by an Apulian oil mill. Part of these samples were pre-treated with ultrasound, while the remaining part followed a traditional procedure. Specifically, low-frequency and high-power ultrasounds (at a frequency of 37 kHz and with an installed power of 256 W) were generated by means of the Elmasonic P30H ultrasonic bath. Rumen liquid was added to all the samples (20 g of rumen liquid and 5 g of olive pomace), in order to promote the anaerobic digestion of the 3-POP. The samples were randomly placed in a thermally-controlled water

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bath to ensure mesophilic conditions during the digestion phase. The biogas production was constantly monitored for 43 days. The volume of the gas produced by the sonicated samples was compared to that obtained with the standard procedure.

Results

After the first ten days, the non-sonicated olive pomace biogas production resulted to be almost saturated, while in the case of the sonicated samples the biogas production kept increasing in the subsequent days. For the entire duration of the experiment, the production rate of the sonicated olive pomace samples resulted constantly higher than that of the non-sonicated ones. In addition, at the end of the 43th day, the production rate of the 3-POP sonicated samples was still slightly higher than zero.
This result highlighted that the olive pomace produces more biogas in a faster way when preconditioned by an ultrasound pre-treatment.

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Dry powder formulations from olive oil wastewater with high nutraceutical properties

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Keywords: olive oil wastewater, solid formulations, nutraceuticals.

Presentation: Poster session

Objectives

The olive oil extraction process leads to a high amount of olive oil waste water (OW), a rich source of polyphenolic compounds that can exert a wide range of beneficial effects on human health, most of them due to polyphenolic compounds antioxidant properties [1, 2]. In order to minimize the impact to the environment and to valorise the sustainable development of several local products, a concentrated solution from OW was obtained and, starting from this solution, we developed a stable dry powder formulation which maintains its high nutraceutical properties.

Methods

On the concentrated olive oil wastewater, here called HP30, a preformulation study was performed in order to select the appropriate excipients in terms of solubility, viscosity and dispersibility of the systems. In particular, we tested four different excipients, here called E1 (polysaccharide), E2 (polysaccharide), E3 (oligosaccharide) and E4 (desiccant) and after the above mentioned studies we selected the three giving better results, i.e. E1 E3 and E4.
Different amounts of the selected excipient were used to obtain the formulations from HP30, called F1 (5% E1, 10% E3), F3 (5% E1, 30% E3), F1b (5% E1, 10% E3, 2% E4) and F3b (5% E1, 30% E3, 2% E4), respectively.
DSC studies were carried out to evaluate the thermal properties of the prepared formulations and to optimize the freeze-drying process. All obtained formulations were characterized by the Folin assay to analyze the polyphenols content with respect to the HP30 and by stability studies.

Results

The HP30, a concentrated solution from the OW was treated to obtain stable solid formulations with high nutraceutical properties.

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After preliminary studies we selected three excipients and we prepared four different formulation called F1, F3, F1b and F3b. All formulations were obtained by an optimized freeze-drying process. From a macroscopic point of view all powders showed a light brown colour, they had good flow properties with respect to the sticking HP30 powder and they resulted stable up to five months storage under normal conditions of humidity and temperature.
The Folin assay, carried out on the formulations, did not show significant differences in term of polyphenolic amount with respect to the HP30 for the formulations F1 and F1b, while a reduction of 20 % w/w was evidenced on F3 and F3b powders.
So, we can assume that the freeze-dryied process is an useful method to obtain solid formulations from the OW not significantly modifying the composition of the principal components and that F1 and F1b are promising nutraceutical product obtained from olive oil wastewater.

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Green paths for the valorization of Chichoriumintybusand Ocimum-basilicum: microwave/ultrasound-assisted extraction of polyphenols

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Keywords: Microwaves-Ultrasound-Polyphenols-Biomass-Green chemistry

Presentation: Poster session

Objectives

Polyphenol compounds have attracted significant interest due to their distinctive antioxidant capacity. Indeed, these biomass-derived components are largely exploited in the food, cosmetic and pharmaceutical industries. However, conventional methods for the extraction of polyphenols are generally associated with long extraction times and large organic solvent volume, with significant environmental impact. Herein, aiming to design greener methods for the extraction of polyphenols from chicory and basil, ultrasound (US) and microwave (MW)alone or combined have been explored. Under these conditionsbesides adramatic reduction of extraction times and solvent consumption, higher yields have been obtained. Lastly, the new protocols allowed the shifting from unsafe organic to less risky or non-toxic solvents.

Methods

Different procedures in H_2O and EtOH/H_2O mixtureshave been optimizedunder ultrasound and microwave irradiation. Polyphenol profile was determined using Folin-Ciocalteu assay and HPLC analysis.

Results

Comparison among US, MW, and US/MW-assisted extraction, efficiency increased in the following order US<US/MW< MW.
Extraction conditions that gave the highest polyphenols content were: sub-critical water under MW (400 W, 75 °C, 15 min), while the highest extraction yield was observed in EtOH/H_2O 6:4 under combined US (20.3 kHz, 30 Wcm^{-2}) and MW (400 W, 75 °C, 15 min).

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Comparative evaluation of the chemical composition and bioactivity of oils obtained by supercritical CO_2 extraction from cured mullet roes (bottarga) and waste salt from salting process.

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Keywords: Bottarga production; waste salt; supercritical CO_2 extraction; oil.

Presentation: Poster session

Objectives

The food delicacy "bottarga" is the final product of a number of treatments (salting and drying) on the ovaries of mullet (*Mugil cephalus*) and represents an important natural source of n-3 polyunsaturated fatty acids (n-3 PUFA) with nutraceutical properties.
During the salting process, huge amounts of waste salt are generated, rich in residual ovary material. We compared the lipid composition (main lipid components and fatty acids) and bioactivity of oil obtained by extraction of the residual marine salt from the mullet roes salting process (salt oil) with that obtained by the direct extraction of mullet bottarga. Oils were obtained by supercritical fluid extraction (SFE) with CO_2, an alternative, environmentally benign, separation technique amply used to obtain oils for nutraceutical supplements and functional foods. Salt and bottarga oils were assessed and compared for cytotoxicity in normal and cancer cells. The attractant effect of waste salt and salt oil to insects (*Ceratitis capitata)* was also tested to gain preliminary information about their potential application for animal supplementation.

Methods

Oils were obtained from waste salt and a grated bottarga sample by SFE-CO_2 extraction in a laboratory apparatus at 300 bar and 40 °C. Oils were subjected to a mild saponification and main lipid classes and fatty acid compositions were characterized by HPLC-DAD/ELSD technique. Oil cytotoxicity was evaluated in murine B16F10 melanoma cells and 3T3 fibroblasts after 24 h of incubation by MTT assay. Behavioral experiments on insects were performed by two-choice tests.

Results

Oil extraction yields were 6% and 28% for waste salt and bottarga, respectively. Lipid and fatty acid composition of salt oil was similar to that of bottarga oil, with a great content of wax esters and high amounts of 16:1 n-7, 22:6 n-3, 18:1 n-9, 16:0, and 20:5 n-3, in particular

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n-3 PUFA averaged 122 mg/g of oil (178 mg/g in bottarga oil). Oils showed similar toxic effect in cancer cells and attractiveness to insects. Contrary to bottarga oil, salt oil showed a slight toxicity in 3T3 cells. Waste salt showed a greater attractant effect on insects than oils. Our results qualified waste salt from bottarga production as an important source of bioproducts with potential industrial application.

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Citrus Water-Extracts Obtained by Microwave-Assisted and Conventional Methods: Antimicrobial and Antibiofilm Activity Evaluation.

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Keywords: MAE, yield, green solvent

Presentation: Poster session

Objectives

Citrus pomace is a huge agro-food industrial waste mostly composed of peels. Owing to its high content of compounds beneficial to humans (e.g., flavonoids, phenol-like acids, and terpenoids), citrus waste is increasingly used to produce valuable supplements, fragrance, or antimicrobials. Sustainable and efficient extraction strategies by solvent-free techniques for environmentally-friendly good practices should be desirable.

Methods

In this work, both conventional extraction methods using hot water (HWE) and microwave-assisted extraction (MAE) were used to obtain water extracts. The antimicrobial and antibiofilm activity of water extracts of three citrus peels (orange, lemon, and citron) against ten different sanitary relevant bacteria were evaluated.

Results

Even though no extract fully inhibited the growth of the target bacteria, these latter (mostly pseudomonads) showed a significant reduction in biofilm biomass. The most active extracts were obtained from orange and lemon peel by using MAE at 100 °C for 8 min. These results showed that citrus peel water infusions by MAE may reduce biofilm formation possibly enhancing the susceptibility of sanitary-related bacteria to disinfection procedures.

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The impact of ultrasounds in the EVOOs quality: a study on the volatile and phenolic fractions of Apulian oils

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Keywords: olive oils quality; extraction yields; hydroxytyrosol

Presentation: Poster session

Objectives

The virgin olive oil extraction process has the malaxation process as one of the critical step. Recently, the advantages to apply the ultrasound (US) technology, that induces acoustic cavitation phenomenon using low frequency, resulted a promising method to overcome this critical step. Objective of this work was to compare the quality of several couples of Extra Virgin Olive Oils (EVOOs) from Apulia obtained by the application of an innovative ultrasound system placed between the crusher and the decanter during the milling process. The phenolic fraction was determined by HPLC-DAD both before and after acidic hydrolysis, while the volatile fraction was analyzed by HS-SPME-GC-MS.

Methods

Different Apulian EVOOs from cv. Coratina, Peranzana, Cellina di Nardò and a blend of Coratina and Ogliarola were analysed. Phenolic compounds were extracted and analysed by the internal standard method, according to the IOC olcial method [1]. The acidic hydrolysis was also applied to evaluate the total content of free and bound tyrosol and hydroxytyrosol. The volatile fraction was analyzed by HS-SPME-GC-MS using a multiple internal standard method [2].

Results

Fourteen EVOOs were produced by the help of US working in three different mills, thanks to the versatility of the applied US device; blend and monocultivar oils were analysed. The preliminary comparison with the EVOOs obtained by a traditional milling process and applying US, highlighted very similar profiles for both the phenolic compounds and the volatile fraction. These results demonstrate that the new process produces EVOOs with high quality and not induces oxidative phenomena. Finally, the increment of the extraction yields obtained by the US improved the sustainability of the productive process.

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Green extraction protocols of Mitragynaspeciosa leaves leading to a possible large scale production

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Keywords: Mitragynaspeciosa, extraction, alkaloids, ultrasound, microwaves, hydrodynamic cavitation

Presentation: Poster session

Objectives

Mitragynaspeciosa (K.) H. (*Rubiaceae*), is atropical tree that is indigenous to Southeast Asia and Indochina. Also known as Kratom, it has been widelyused, for hundreds of years, for its stimulant and opioid-like analgesiceffects. The present study aims to design a green protocol for alkaloidsextraction, in particular mitragynine, from the leavesusing green techniques and solventsboth in pretreatment and in extraction steps. For this purpose, we compared several non-conventional techniques(ultrasound, microwave, hydrodynamic cavitation) with classic methods.

Methods

Dried *M. speciosa* leaves belonging to a red vein variety from Bali were in some cases pretreated with a phosphate buffer (pH = 7.5) and then extracted with ethanol, ethanol/water mixture oracidic water (pH = 3), using ultrasound-assistedextraction (UAE), microwave-assisted extraction (MAE). Moreover, hydrodynamic cavitation (HC) was also used for the scaling-up of the processes, using a pilot scale reactor (Rotocav®).
All the sampleshave been analyzed using HPLC-DAD for the quantification of the principal alkaloids presentbased on previous literature data.

Results

The extraction technique and solvent choice influenced both the raw product yield and the relative alkaloid contentof *M. speciosa* leaves. Of the several methods tested, UAE with an immersion horn (21.4 kHz, 150 W, 30 min at 25-50°C with acidic water or 15 min at rtwith ethanol/water 7:3 mixture) showed the best yield for mitragynine.HC demonstrated to be determinant both in the pretreatment and in the extraction step, increasing the final mitragynine content in the extract, paving the way toa future large scale production of *M. speciosa*extracts with different potential applications.

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Ultrasound-Assisted Extraction (UAE): induced physical impacts on Rosemary (*Rosmarinus officinalis* L.) and Artichoke (*Cynara scolymus* L.) leaves

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Keywords: Ultrasound, extraction, microscopic observations, e-SEM 3D, mechanisms.

Presentation: Poster session

Objectives

The ultrasound positive contribution on the extraction performances such as reduction of extraction time, diminution of required solvent quantity and enhancement in yields were widely proved [1]. This performances' gain is generally attributed to the cavitation phenomena [2]. However, ultrasound mechanisms were poorly investigated [3].
Our study aims at better understanding ultrasound-induced mechanical effects on complex plant matrices in the particular case of Rosemary and Artichoke leaves.

Methods

Effect of ultrasound has been assessed by submitting a single leaf, fixed in a perforated disk, to an ultrasonic field (US probe, 20 kHz) at different treatment durations. Extraction was performed in demineralized water. Conventional process was carried out at the same conditions without ultrasound.
The surfaces of untreated and treated leaves were examined by Scanning Electron Microscopy (SEM) and Environmental Scanning Electron Microscopy (e-SEM 3D). Cyto-Histochemical study was also conducted to analyze ultrasound-induced alterations on the inner structures. Mechanisms of action were then concluded on the basis of these different observations

Results

Our findings proved that conventional process preserved structural properties and the integrity of both rosemary and artichoke leaves compared to the initial leaves. In contrast, ultrasound induced noticeable alterations on the two studied leaves. It is crucial to note that ultrasound impacted Rosemary and Artichoke leaves by different mechanisms. In the case of rosemary leaf, ultrasound seemed to act through chain detexturation mechanism respecting a sequential steps: local erosion, shear forces, sonoporation, fragmentation, capillary effect and detexturation: local erosion, shear forces, sonoporation, fragmentation, and

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detexturation. As for Artichoke leaf, only four mechanisms were noticed: shear forces, sonoporation, erosion and fragmentation.

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DNA Isolation and Analysis: A Valuable Tool for Quality Control of Botanical Extracts

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Keywords: DNA Isolation, Quality Control, Botanical Extracts

Presentation: Poster session

Objectives

Introduction: The growing market demand for botanicals requires the availability of accurate and rapid tools to authenticate plants and their adulterants for a safe use of medicinal plants, adherence to regulatory compliance and prevention of economic loss to manufacturers. The DNA barcoding approach is a simple and cost-effective strategy [1] that has already been applied to the identification of several plant raw materials. DNA barcoding is a molecular and computational-based identification system that aims to assign biological specimens to a given species. This assignment is based on the comparison of the nucleotide sequence composition of a standard genome region/s (i.e. the barcode) of the specimen with those stored in reference public databases [2,3]. The aim of this study was to investigate different methods of DNA isolation from several botanical extracts and to define the best species identification process.

Methods

Materials and Methods: About 30 botanical extracts were collected and analysed. They differed in the species used as raw material, solvent, procedure and temperature of extraction. Three methods of DNA isolation were tested: a standard laboratory protocol, and two commercial extraction kits with protocol modifications. High quality DNA samples were used as template for the amplification and sequencing of standard marker regions with DNA barcoding and mini-barcoding approaches.

Results

Results: We selected the best DNA extraction method providing the highest yields in terms of DNA quantity and quality. Generally, aqueous and sonicated extracts provided better DNA extracts. We performed DNA amplifications and we observed that the best amplification rate was obtained with sonicated samples, supplied by Euphytos. The 90% of these amplicons returned a reliable DNA sequencing result.

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Through the comparison with the reference databases, all the analyzed amplicons allowed to identify the genera. In other cases, we were able to identify the species.

Conclusion

The DNA-based identification analysis is a valid quality control method for the production chain of botanical extracts, from the raw material to the finished product. The essential element for the success of the analysis is the preservation of nucleic acids. Industrial processes that use alcohol solvents and high temperatures can degrade the DNA and thus compromise the success of the analysis. Sonication, as a method of extraction, allows to preserve the DNA quality and consequently to perform successful DNA barcoding analysis.

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Green extraction optimization of sulphated polysaccharides from Nizamuddinia zanardinii with non-conventional technologies

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Keywords: Fucoidans, sulphated polysaccharides, seaweed, ultrasound-assisted extraction, microwave-assisted extraction, subcritical water.

Presentation: Poster session

Objectives

Fucoidans, a class of fucose rich bioactive sulphated polysaccharide, can be found in in high amount in different seaweed species (25/30% of the dry plant weight depending on specie and season). These compounds are usually extracted in acid or alkaline media, in hot water or solvents with long extraction times. In the present study, fucoidans have been isolated from *Nizamuddinia zanardinii* under ultrasound and microwaves as well as with subcritical water, aiming to optimize the extraction procedure. The technologies impact on extracts yield, biochemical characteristics and saccharides composition, along with their antibacterial antiviral and cytotoxic properties will be described.

Methods

Different enabling technologies were tested: ultrasound-assisted extraction (UAE, 200 W, 20 kHz, two runs of 20 mins each), microwave-assisted extraction (MAE, 90 C°, two runs of 20 mins each) and subcritical water extraction (SWE, 150 C°, two runs of 10 mins each). On the base of the excellent results achieved in particular in subcritical water a further optimization study was conducted. Analysis: monosaccharide composition (GC analysis), molecular weight determination and FTIR.

Results

The best technology in term of Fucoidans yield and activity was SWE. The optimization study could define the following best conditions: water/matrix ratio 21 ml/g, for 30 min at 150 °C, with a fucoidan yield around 26%.

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Fractional design to estimate the significant factors to recovery phytochemicals from spent coffee grounds

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Keywords: Spent Coffee Ground, Phytochemicals, Green Extraction, By-Products

Presentation: Poster session

Objectives

During the preparation of a coffee beverage, a solid residue known as spent coffee ground (SCG) is produced. The study aimed to develop a green extraction method for water- soluble compounds from SCG, and to test which physical and chemical variables influence the amounts of phytochemicals extracted.

Methods

Two types of SCG were produced from commercial coffee blends. One type was obtained from Espresso and the second recovered from a French Press. We used a Plackett-Burman design to estimate which factors have more influence on the amount of phytochemicals to be recovered. We have performed physical and chemicals measurements including the quantification of total dissolved solids, pH, caffeine and total phenolic compounds. The tested variables were: type of water, water temperature, extraction time, type of SCG, solid liquid ratio, SCG storage time, and SCG pre-treatment.

Results

The results obtained from the fractional design showed that the significant factors to recover phytochemicals were Temperature and type of SCG.
Temperature has a significant effect on the recovery of caffeine from the extracts. With temperature of extraction 115°C, the amount of caffeine recovered was 19.07±6.7 mg/g against the 14.84±5.7 mg/g of the lower temperature (100°C). A significant effect was revelated for the different type of SCG, both for caffeine and total phenolic compounds. The amounts of phytochemicals recovered from SCG was significantly higher in French Press than the Espresso.

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Chemical and biochemical characterization of *Opuntia ficus-indica* cladodes as a bio-product supplement.

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Keywords: Opuntia ficus-indica, polyphenols, bioaccessibility, minerals, antioxidant activity

Presentation: Poster session

Objectives

The prickly pear cactus, *Opuntia ficus-indica* (OFI) is a tropical or subtropical plant originally grown in South America; its cladodes represent a by-product that generally is used for animal feed. Moreover, they are rich in pectin, mucilage and minerals, vitamins and antioxidants and the use as food medicine and cosmetic ingredients, could be proposed. In the present study, the characterization of bioactive compounds (polyphenols, sugars and minerals) in dehydrated OFI cladodes were performed. Further, antioxidant activity and polyphenols/minerals stability after simulated gastro-intestinal digestion, were also evaluated.

Methods

Opuntia ficus-indica dehydrated cladodes harvested at 6-8 months age, were collected, washed, cut, mixed and dried to produce OFI flour as reported by Santos-Zea [1]. Extraction, characterization, and *in vitro* digestion process were carried out as described by D'Antuono [2]. Antioxidant activities were performed by following the methods reported by Re [3] for ABTS, and Schleisier [4] for DPPH. Samples were analysed for the cations and anions following the procedure of D'Imperio [5]. For the carbohydrates determination the Sluiter method [6] was followed.

Results

The HPLC analysis on OFI dehydrated cladodes showed that the most abundant flavonoids (392 mg/100 g DW) were the isorhamnetin glycosides and among them, the narcissin has been identified (75 mg/100 g DW). Other two flavonoids, kaempferol derivatives, together with piscidic, eucomic and caffeic acids, were recorded. In particular, piscidic acid was the most abundant (967 mg/100 g DW) as reported by other authors [7]. OFI polyphenols extract showed an high antioxidant activity reaching about 95% of inhibition at polyphenols concentration of 80 mg/mL. Some differences between the two tests have been recorded and

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probably related to the higher stability of $ABTS^+$ compared to the $DPPH^+$. The chemical analysis confirmed that cladodes are a good source of functional minerals as Ca (75.17 mg/g DW), Mg (13.79 mg/g DW), and K (16.83 mg/g DW) although high Na level (19.2 mg/g DW) was found. In addition, after *in vitro* digestion the higher bioaccessible element was Mg (93%) followed by K (52%) and Ca (32%). Also all the identified polyphenols were highly bioaccessible with a percentage ranging from 88% to 172%. This result could be attributable to hydrolysis processes on polymeric molecules not detected before digestion in the starting matrix. The carbohydrates analysis by three steps acid hydrolysis and polysaccharides estimation showed the presence of glucan (75.5 mg/g DW), arabinan (35.6 mg/g DW), galactan (27.2 mg/g DW), xylan (15.5 mg/g DW), mannan (3.7 mg/g DW). Our findings permit to consider cladodes extract as a good source of natural antioxidants, water-soluble fiber and minerals.

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Optimisation of Polyphenolic Extraction from Bamboo Shoot (Phyllostachys Pubescens) with Microwave Extractor (MCE) using design of experiments (DOE).

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Keywords: Microwave extraction, Bambooo Shoot, DOE, Folin-Ciocoltau Assay.

Presentation: Poster session

Objectives

The application of design of experiment was an effective statistical tool to optimise the extraction conditions. This research aimed to determine the optimum total polyphenolic content in Bamboo Shoot using Microwave Extraction associate at reduction extraction time of conventional extraction that require 24h.

Methods

Microwave extraction (MWE) of polyphenolic content in Bamboo Shoots was performed to optimise various parameters, such as volume and extraction time, to obtain the highest polyphenolic content using a Design of Experiment (DOE) than conventional extraction. The volume and temperature ranged from 2-9 mL of methanol and 58-110°C respectively while the extraction time resulted irrelevant in MWE. The polyphenolic content was determined using Folin-Ciocalteu assay.

Results

The first phase of this study was evaluated main factors that could influence the MW extraction process. It was possible using DOE, 11 experiment were made changing temperature, volume and time in a range included respectively to 2-4 mL, 58-68 °C, 5-10 min. In screening phase the best value obtained was at 68 °C, 10 min and 4 mL evaluated by Folin-Ciocoltau assay; high value of reproducibility and good R^2 and Q^2 were obtained. The model obtained showed that the direction to optimize polyphenols content would be to increase temperature and volume, while time was irrelevant. Therefore the ranges chose were 75-95 °C, 5-8 mL and 3-5 min and using a quadratic and cubic model the total number of experiment were 17. In condition of T, V and time respectively 95 °C, 10 mL and 5 min was obtained the best value polyphenolic content of 1.479 g/L. The value of R^2 and Q^2 were respectively of 0.943 and 0.88, with high reproducibility and good model validity. DOE prediction at 110 °C, in 9 mL and 4 min showed a polyphenolic content of 2.5 g/L compared to 3.5 g/L calculated by the mathematic model, but greater than, 0.413 g/L, of conventional extraction. In conclusion polyphenolic content of MWE is six times greater than conventional extraction.

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An analytical strategy for a more accurate determination of the phenolic compounds in EVOOs

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Keywords: hydrolysis, secoiridoids, tyrosol and OH-tyrosol, health claims

Presentation: Poster session

Objectives

The focus was the validation of an hydrolytic procedure previously applied, suitable to furnish a simple analytical method applicable on different samples to better investigate the composition and the antioxidant potency of the phenolic constituents in the EVOOs.

Methods

The acidic hydrolysis procedure previously proposed [1] and applied [2] was validated by the following steps: i) evaluation of the chemical stability of oleuropein, tyrosol, pinoresinol, luteolin as pure standards; ii) definition of the calibration curves at 280 nm for all the external standards; iii) application of the acidic hydrolysis at two representative blends of the phenolic compounds extracted from the EVOOs collected in 2017 from Tuscany and Apulia. All the analyses were performed by HPLC-DAD.

Results

This hydrolytic method was recently recognized as suitable for the purpose [3].
The results confirm the applicability of the acidic hydrolysis to the complex mixtures of minor phenols and secoiridoidic derivatives typical of EVOOs. This approach allowed to easy measure the total OH-tyrosol amount (free and linked forms) for all the samples produced in 2017 within the COMPETITIVE project. The total OH-tyrosol amount evaluation is needed to apply the EFSA health claims, but it can be also proposed as predictive index for the oils stability. A more accurate evaluation of the antioxidant potential of an EVOOs will help to forecasting the resistance of an oil against the aging and overall to control its quality over time.

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Comparison of the fatty acid composition and cytotoxic activity of oils obtained by supercritical CO2 extraction from Cynomorium coccineum L. growing in Italy and in Tunisia

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Keywords: Maltese mushroom; fixed oil; B16F10 cells; anticancer activity.

Presentation: Poster session

Objectives

Cynomorium coccineum L. is an edible, non-photosynthetic parasitic plant, spread over the Mediterranean countries, amply used in traditional medicine. Fixed oil extracted from *C. coccineum* exhibited nutraceutical properties. Melanoma is the most malignant skin cancer and several *in vitro* and *in vivo* studies have evidenced the potential antimelanoma activity of lipophilic natural extracts. The present study aimed to compare the fatty acid (FA) composition and cytotoxicity in melanoma cells of oils of plants taken from Tunisia and Sardinia, characterized by different climates. Oils were obtained from dried stems by supercritical fractioned extraction with CO_2, a more sustainable separation technique, alternative to classical solvent extraction processes.

Methods

Fixed oil was extracted from dried and ground aerial parts of *C. coccineum* with supercritical CO_2 in a laboratory apparatus, working at 250 bar and 40 °C for 4 h. Oils were subjected to a mild saponification and FA composition was characterized by GC- FID and HPLC-DAD techniques. Oil cytotoxicity was evaluated in murine B16F10 melanoma cells after 24 h of incubation by MTT assay.

Results

The Tunisian oil (TO) showed a concentration of approximately 23% saturated FA, mainly lauric acid 14:0, palmitic acid 16:0, and stearic acid 18:0, 48% monounsaturated FA, mainly oleic acid 18:1 n-9 (40%) and palmitoleic acid 16:1 n-7, and 24% of polyunsaturated FA,

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constituted by the essential linoleic (18:2 n-6, 18%) and α-linolenic (18:3 n-3, 6%) acids. The Sardinian oil (SO) showed a slight higher level of saturated FA (34%) and lower amount of monounsaturated FA (44%) than TO. The 24 h- treatment with oils induced a significant reduction in B16F10 cell viability, in comparison with control, from the concentration of 100 μg/mL and 250 □g/mL for SO and TO, respectively. The results qualify C. coccineum as a resource of oil, with potential benefits in melanoma prevention, for nutraceutical and pharmaceutical applications.

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Extraction of bioactive compounds from Cannabis sativa L. and their use as food preservatives and active pharmaceutical ingredients

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Keywords: Cannabis sativa L., bioactive compounds, RSLDE, antioxidant activity

Presentation: Poster session

Objectives

In the last decades, more and more agricultural farms, research organizations and institutions have shown a new interest in the cultivation of Cannabis sativa L. with low content of THC (industrial hemp), because of its innovative applications in different fields as foods, cosmetics and pharmaceuticals.
The purpose of this work is to extract bioactive compounds from industrial hemp local crops, for their use in the food and pharmaceutical fields. For the extraction, different techniques will be taken into consideration: beside conventional methods as maceration, ultrasound and Soxhlet, will be exploited one of the most innovative strategies that is the RSLDE - Rapid Solid-Liquid Dynamic Extraction, performed by "Naviglio Extractor®". The different extracts will be evaluated for their antioxidant activity, volatile fraction composition and cannabinoids content.

Methods

Dried inflorescence of Cannabis sativa L. var. Futura 75 were homogenized, grindered and sieved. Extracts obtained by the different techniques were dried after under vacuum solvent removing. Antioxidant activity was evaluated through the FRAP, ABTS, DPPH in vitro assays, and total phenolic content by Folin- Ciocâlteu method. The volatile fraction composition analysis was performed by SPME-GC-MS. Cannabinoids content was determined by LC-MS/MS analysis.

Results

The results obtained from this preliminary study, show a good antioxidant activity of the hemp extracts, justified by the presence of high biological value components as terpenoids and cannabinoids. Moreover, the RSLDE performed by Naviglio Extractor® proves to be a good extraction technique to obtain extracts with high biological potential, interesting candidates for industrial application as food preservatives.

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Lactic acid production from undetoxified cardoon hydrolysate by different *Lactobacillus* species.

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Keywords: fermentation; cardoon hydrolysates; lactic acid; Lactobacillus species.

Presentation: Poster session

Objectives

Lactic acid is a promising building-block for the production of PLA (polylactic acid), a most important bio-based polyester (Okano et al. 2010). The specific objective of this study is the evaluation of the fermentative performances of different Lactobacillus species for lactic acid production from a non-food lignocellulosic feedstock Cynara cardunculus L. (Ciancolini et al., 2013).

Methods

The *Lactobacillus* strains used were *L. brevis* ATCC 14869, *L. reuteri* ATCC 23272 and *L. plantarum* ATCC 14917. Cardoon biomass pretreatment was carried out by using the acid catalyzed steam explosion batch technology followed by enzymatic hydrolysis with Cellic™ Ctec2 (Novozymes). A microplate lactic bacteria screening, batch fermentation tests and metabolites HPLC analysis were carried out.

Results

Cardoon hydrolysate obtained by acid steam pretreatment and enzymatic hydrolysis contained 90.1 gL^{-1} and 20.8 gL^{-1} glucose and xylose, respectively. The microplate screening showed optimal growth condition with a 70/30 ratio, in terms of cardoon hydrolysate and MRS content, respectively, ensuring the 50% of the maximum growth rate of the *Lactobacillus* species tested. In terms of metabolic yield, *L. plantarum* showed the highest value, 78%, identical to the value obtained on MRS medium, followed by *L. reuteri*, 73%, significantly higher than that obtain on MRS (57%), and *L. brevis* with 67%, which showed a significantly lower value than that obtained on MRS.

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Nematicidal potential of aqueous extracts of strawberry tree leaves and flowers

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Keywords: Strawberry tree, nematicidal activity, extraction

Presentation: Poster session

Objectives

The main objective of the present study was to evaluate the nematicidal activity of aqueous extracts of strawberry tree leaves and flowers of two different geographical origins in Portugal.

Methods

Strawberry tree leaves and flowers of Serra de Monchique and Serra do Caldeirão, both located in southern Portugal, were subjected to conventional aqueous extractions at 60 ºC. The amount of phenolic and flavonoid compounds in the extracts was quantified, as well as their antioxidant activity (DPPH free radical). Their acetylcholinesterase inhibitory activity, indicative of their nematicidal potential was also quantified.

Results

Extraction yields were between 27.2 and 44.0% (dry basis), being higher for the flowers, while total phenols contents were higher for the leaf extracts (28.7 – 33% GAE), when compared to the flower ones (20 – 23.5%). Values of IC50 for the DPPH assay were around 0.026 mg/mL for all the obtained extracts. Finally, values of IC50 for the acetylcholinesterase inhibitory activity assay were lower for the leaf extracts of both geographical origins (0.28 mg/mL – 1.43 mg/mL) when compared to the flower extracts (□6 mg/mL). Values of IC50 of the strawberry tree leaf aqueous extracts obtained in this work were smaller than commercial insecticide ones (4.50 –34.68 mg/mL). Therefore, both leaf extracts revealed a strong nematicidal potential which have brought a new insight on the development of a promising natural, cheap and environmentally-friendly alternative to chemical synthetic pesticides for nematodes management.

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Valorisation by microwaves of food co-products through bio refinery and eco-extraction of plants

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Keywords: Eco-extraction, microwaves, co-products.

Presentation: Poster session

Objectives

Increasing consumer demand for natural and genuine products has led in recent years to a strong demand for plant extracts for the cosmetics, pharmaceutical, food and perfume industries. These plant extracts are generally derived from plants, fruits and vegetables or more and more of their waste. They are found in many manufactured products in the form of aromas, essential oils, food antioxidants, polyphenols, dyes and other active ingredients. This is a fast-growing market.
To produce these plant extracts, companies, including hydro distillation and solvent extraction, now use various extraction processes. However, these processes are heavy energy consumers and raise a number of questions regarding the environmental impact and their use in BIO certified productions.
Also, at the beginning of the 21st century, the field of extraction has enter edits "green" revolution by changing to "eco-extraction", to develop and offer industrialists a sustainable chemistry using less solvents, less energy, reducing releases, and encouraging the creation of co-products instead of waste to integrate the path of bio or agro-refinery, while ensuring the quality of finished products.
Through this presentation, GREEN's expertise will be presented to you on the study of different co-products touching different fields of application: food[1], cosmetics and nutraceuticals.

References

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Optimization of grape marc polyphenols extraction assisted by simultaneous ultrasound and microwave irradiation

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Keywords: Simultaneous ultrasound/microwaves; natural deep eutectic solvents; polyphenols; optimization

Presentation: Poster session

Objectives

Polyphenols from wine industry by-products and wastes may be active components of interest for food, cosmetics and pharmaceutical production. The recovery of these compounds is commonly performed by solvent-extraction procedures with conventional techniques, that may show drawbacks in term of environmental impact. Aiming to design a green extraction procedure for grape marc polyphenols, simultaneous ultrasound and microwave (US/MW) assisted extractions were optimized by response surface methodology (RSM). As a green alternative to conventional solvents, a natural deep eutectic solvent (NADES) was used.

Methods

Based on the preliminary experiments, the NADES choline chloride:citric acid (Ch:Cit) with 30 % of water (w/w) was selected. The extractions were performed under simultaneous US/MW irradiation. The parameters studied were: time of extraction (5 to 15 min), MW power (200 to 400 W) and temperature (50 to 90 °C). Total polyphenolic content (TPC) was determined using Folin-Ciocalteu assay and HPLC analysis. The optimization of aforementioned parameters was carried out by RSM using Design Expert 7.0.0 software based on experimental data derived by implementing Box-Behnken experimental plan. Detailed statistical analysis of the obtained model, analysis of variance (ANOVA), was also provided using Design Expert 7.0.0.

Results

Optimal extraction conditions for achieving the maximum TPC are: time 10 min, MW power 300 W and temperature 80 °C. By applying the listed conditions, TPC yield was 59.53 mg_{GAE} $g_{DW^{-1}}$. RSM using experimental data, could provide the extraction model. By ANOVA statistical analysis, it has been proven that the approximation of the experimental TPC yields with the modelled TPC yields is statistically significant, which means that the model application is justified for the investigated area. The two variables with the greatest impact on the TPC yield are extraction time and temperature. This study presents a fast and environmentally friendly extraction process of valuable biologically active compounds from grape marc, paving the way for further industrial development.

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Supercritical fluid extraction of Bioactive compounds from apple peel

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Keywords: bioactive compounds, vitamin E, apple peel, supercritical fluid extraction, modifier

Presentation: Poster session

Objectives

A simple green and inexpensive procedure for the supercritical fluid extraction (SFE) of bioactive molecules from apple peels of common Italian cultivars has been developed. Apple peels from organic (Kanzi, Royal Gala, Red Murgina, Yellow Murgina and Stark) and non-organic farming (Gala, Golden delicious, Red Delicious, Annurca, Fuji, Cripps Pink, Granny Smith) were analysed. Each extract was characterized in terms of total phenolic, flavonoid, anthocyanin, vitamin C and E and antioxidant activity to establish the potential of apple peals as bioactive compounds.

Methods

One gram of each freeze-dried peel sample was subjected to the extraction with supercritical CO_2, using ethanol as co-solvent, and the following parameters: 60°C oven temperature, 250 bar pressure, 70°C micrometric valve temperature. A static period of 3 minutes was applied before the extraction start by activating the co-solvent pump. In the so-called dynamic procedure, the extraction continued with regular collection of extracts in an amber vial for 15 minutes using a co-solvent flow rate of 0.4 mL/min and SC-CO_2 flow rate of 1 LPM. Each extract sample was filtered and then subjected to the analytical determination.

Results

Supercritical CO_2 conditions were optimized for the simultaneous extraction of the target bioactive compounds, i.e. total phenolic, flavonoid, anthocyanin and vitamin C and E. In particular, vitamin E was determined here for the first time in apple peel.
Even if a great number of factors could affect the content of bioactive substances, the highest concentrations of total phenolic compounds were found in Stark, Golden Delicious, Cripps Pink, and Kanzi cultivars while Red Delicious, Red and Yellow Murgina, Stark, and Royal Gala were characterized by the highest flavonoids content. On the contrary, Golden Delicious, Fuji and Cripps Pink showed the highest values in anthocyanins, Annurca, Stark, and Royal Gala in vitamin C content, Golden Delicious, Red Murgina, and Kanzi were found to contain the most abundant vitamin E levels. The highest Antioxidant activity was shown from Royal Gala and Stark. Optimized ethanol assisted SC-CO_2 extraction proved to be a fast and very useful tool for the recovery of

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bioactive compounds from freeze-dried apple peel, demonstrating that apple peels could be considered a food commodity of high nutraceutical value.

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Green Procedure for One-Pot synthesis of Azelaic Acid Derivatives using Metal Catalysis

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Keywords: removable resource, rare earth, molybdenum complex, sustainability, azelaic acid, green chemistry

Presentation: Poster session

Objectives

A green one-pot synthesis of oleic acid derivatives is promoted by Rare Earth Metal (REM) triflates and commercial Molybdenum dioxo dichloride ($MoCl_2O_2$) at the presence hydrogen peroxide as a green oxidant. The protocol permits to govern the oxidation selectivity by simply choosing the proper combination of Mo and Sc catalysts. Methyl oleate epoxide and azelaic acid thus obtained are valuable industrial intermediates for synthesizing bio-compostable plastics, plasticizers of PVC, lubricating oils, cosmetics and pharmaceuticals (bactericides, anti-inflammatories, etc.).

Methods

To a stirred solution of HCOOH 85% (19.2 µL, 4.22·10-4 mol), H_2O_2 50% (188 µL, 33.08 10^{-4} mol), and catalyst (14.7 10^{-6} mol, 3.6 mol% of namely $MoCl_2O_2$, $Sc(OTf)^3$, $Yb(OTf)_3$, or $La(OTf)_3{\cdot}H_2O$ amounting at 2.92, 7.23, 9.12, and 8.87 mg, respectively) methyl oleate (140 µL, $4.12{\cdot}10^{-4}$ mol) was added. Then, the solution was heated at 70 °C and left to react for the appropriate reaction time. The progress of reaction was monitored by GC-MS setted in scan mode. After completion of reaction, mixture was diluted with distilled water and extracted with 2 mL of CH_2Cl_2 to remove catalysts. The organic phase was evaporated under vacuum and the residue submitted to the silylation procedure to evaluate both conversions and product ratio.

Results

A flexible method for governing the selectivity in the oxidation of methyl oleate has been developed by using $MoCl_2O_2$ and $Sc(OTf)_3$ as the catalysts. The protocol possesses several advantages: i) the possibility of obtaining in good yields methyl oleate epoxide or azelaic acid, a precious industrial intermediate, by simply choosing the combination of Mo and Sc

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catalysts, ii) the replacement of non-eco-friendly and unrecyclable HBF_4 or H_3PO_4 commonly used in these processes and iii) the use of simple procedures and less toxic Molybdenum as catalyst.

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Pomegranate husk as source of polyphenols and its extraction using ultrasound-microwave extraction

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Keywords: microwave, ethanol, pomegranate husk, ultrasound.

Presentation: Poster session

Objectives

1) To identify the most appropriate conditions for obtaining extracts from pomegranate husk using combined ultrasound-microwave technology. 2) To determine the poplyphenolic profile of the pomegranate extracts. 3) To characterize the organic compounds in the extracts of pomegranate husk by means of FT-IR and NMR.

Methods

Three mass/volume ratios (1: 8, 1:12 and 1:16) and aqueous ethanol (0, 30 and 70%) were considered, using a fractional factorial experimental design. Treatments were irradiated for 20 min in an ultrasound bath and then subjected to a microwave treatment for 5 min at 70 ° C. The color, presence of alkaloids and triterpenes, hydrolysable and condensed polyphenols content were determined. Polyphenolic fraction was obtained after Amberlite XAD16N column chromatography and then characterized using FT-IR spectrometry (ATR), HPLC / ESI / MS, ^{1}H and ^{13}C Nuclear Magnetic Resonance. Solubility and relative pH were also evaluated.

Results

The extracts with the highest amount of polyphenols were obtained using 70% aqueous ethanol and 1: 16 ratio (w/v). The presence of alkaloids was recorded in one of the five extracts of pomegranate husk; however, extraction of triterpenes was not achieved in any of the extracts. The extraction of sixteen compounds, belonging to the families of ellagitannins, catkins, alkylphenols, hydroxybenzoic acid and their dimers was achieved. Presence of punicalagin was also confirmed by NMR.

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Green extraction of polyphenols from cocoa beans

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Keywords: Green extraction polyphenols, cocoa beans

Presentation: Poster session

Objectives

Conventional extraction techniques for plant phenolics are usually associated with high organic solvent consumption and long extraction times. In order to establish an environmentally friendly extraction method for cocoa beans phenolics, natural deep eutectic solvents (NADES) as a green alternative to conventional solvents coupled with highly efficient microwave-assisted and ultrasound-assisted extraction methods (MAE and UAE, respectively), used simultaneously or separated, have been considered.

Methods

Screening of three different NADES for proposed extraction was performed. In order to optimize extraction methods, after the selection of optimal NADES, alternative methods ultrasound- or microwave-assisted extraction (UAE and MAE, respectively) was applied. The identification and quantification of phenolic compounds was performed by HPLC.

Results

NADES capacity to extract phenolic compounds varied considerably according to the target phenolic compounds and the NADES itself. In general, it was found that extraction efficiency strongly to depend on hydrogen bond donor (HBD). In the case of total polyphenols (sum of all quantified polyphenols), the best extraction efficiency was obtained with ChGly, followed by ChGlc>ChCit>aqueous ethanol (60%, v/v). Comparison among MAE, UAE, MAE/UAE, extraction efficiency increased in the following order UAE < MAE/UAE < MAE. The use of NADES from natural sources in the extraction processes coupled with alternative energy sources could lead to highly efficient and truly eco-friendly extraction methods.

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Isolation of fixed oils using supercritical CO2 extraction

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Presentation: Poster session

Keywords: *Supercritical extraction, Fixed oil, biological activity.*

Objectives

Vegetable oils are becoming increasingly important as suppliers of vitamins and essential unsaturated fatty acids. The objective of this work is to explore the potential application of supercritical CO2 to the extraction of oils from raw materials and to investigate their chemical composition and their biological activity. Several matrices are used such as seeds (Apium graveolens, Myristica fragrans and Nigella sativa) and fruits (Laurus nobilis, Lycium europaeum, Pistacia terebinthus and Sarcopoterium spinosum). Methods
Supercritical CO2 extraction experiments were carried out at pressures of 250-300 bar and temperature of 40 °C in a laboratory apparatus. The obtained fixed oils were subjected to a mild saponification and fatty acid composition was characterized by GC- FID and HPLC-DAD techniques. On the supercritical extracts some biological activities were evaluated (antimicrobial, antioxidant, radical scavenging and tyrosinase inhibitory activities and cytotoxicity).

Results

The investigated samples showed variations in oil composition. The most interesting among them for the nutritional value of the oil were N. sativa, L. europaeum and S. spinosum since their fatty acids were highly unsaturated (58÷71 % of polyunsaturated fatty acids), rich in essential fatty acids (18:2 n-6 and 18:3 n-3). The oils showed radical scavenging activities, antifungal activity against C. albicans, C. tropicalis, and C. krusei and inhibited cancer Caco-2 cell growth.

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Polyphenols extraction from coffee pulp using ultrasound, microwave and green solvents

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Presentation: Poster session

Keywords: Water, Ethanol, HPLC mass, caffeine, FT-IR

Objectives

To determine the best conditions for polyphenols extraction from coffee pulp using ultrasound, microwave and as solvents water and ethanol.
To determine the most abundant polyphenols extracted from coffee pulp using HPLC-mass spectrometry analysis.
Chemical characterization of extracts from coffee pulp by FT-IR and NMR

Methods

Polyphenols extraction was performed using three combinations of mass/volume (1:8, 1:12 and 1:16) and three aqueous ethanol (0%, 30% and 70%) having a total of nine treatments. These treatments were placed for 20 minutes in an ultrasound bath, after, samples were exposed to microwave for 5 minutes. The obtained phytochemical compounds were characterized by FT-IR, HPLC/ESI/MS, NMR of ^{1}H and ^{13}C.

Results

FT-IR analyses of the polyphenols from coffee pulp showed signals within the phenol group (methyl group, single and double bonds with oxygen), as well as possible carboxylic groups. While, with the HPLC/ESI/MS analyses were detected 34 compounds among them: (+)-Gallocatechin; 2,3-Dihydroxybenzoic acid; (-)-Epicatechin-(2a-7)(4a-8)-epicatechin 3-O-galactoside; 2,3-Dihydidroxybenzoic acid; 3-Feruloylquinic acid; 1,3-Dicaffeoylquinic acid; 3,7-Dimetil quercetin and Esculin. NMR analyses showed that the predominant chemical compound in the extracts from coffee pulp was caffeine.

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