Biovalorization and Sustainability of Agrofood Products

Valorization of Natural Products and Food Wastes

Sustainable Extraction Processes

KEYWORDS: Natural Products/ Sustainability/ Green Extraction / Cosmetic and Food Applications

Supercritical extraction using carbon dioxide (SFE-CO2) is an alternative green technology for the extraction of aromas and bioactives from natural matrices.

Humans often respond to sensory impulses provided by aromas, and current trends have generated interest in natural sources of fragrances rather than the commonly used synthetic additives. Here, the resulting aroma of a selected culture of Thymus *mastichina* L. was studied as a potential food ingredient.

Moringa *oleifera* L. tree (Mo) has emerged as a rich alternative source of bioactive compounds to design cosmetic formulations. Supercritical carbon dioxide fluid extraction (SFE-CO_2) was successfully applied to the screening of Mo seed, leaf, and root extracts.

Introduction

Project Valor Natural, a consortium of R&D institutions and companies in the Trás-os-Montes and Alto Douro region, aimed at identifying natural matrices highly rich in added-value molecules and enabling its industrial production by developing solutions that solve identified weaknesses of the production chain. Within this projetc, the LSRE-LCM team led a line aiming at the development of aromas and aroma models for the bread industry. Extraction tests were carried out using supercritical extraction using carbon dioxide (SFE-CO2) with dried fruits (*Corylus avellana, Prunus dulcis* and *Juglans regia*) and aromatic plants (Rosmarinus *officinalis*, *Thymus mastichina* and *Origanum vulgare*).

Thyme's dual activities highlight this herb for edible applications. First, the plant is widely used as a natural additive due to its expressive antimicrobial property against common food pathogens (e.g. *Staphylococcus aureus*). On the other hand, the terpenoids compounds (e.g., thymol, carvacrol, p-cymene) demonstrate desirable improvements in foods as flavouring agent. Its pleasant aromatic properties play an interesting role in replacing synthetic ingredients generally introduced in industrial processes. In fact, through the organoleptic characteristics of food products, the deepest human sensations and feelings can be revealed. Thus, exploring these perceptions constitutes a potential strategy for marketing purposes.

The properties of *Moringa oleifera L.* tree (Mo) as a traditional medicine treatment option for skin injuries, malnutrition, and other diseases have been well-known for centuries. Recognised
as a GRAS (Generally Recognised as Safe) process by the United States FDA (Food and Drug Administration) and European Union statements, SFE-CO_2 has been applied to obtain Mo seed and leaf and root extracts. A response surface methodology (RSM) was applied to define the best extraction conditions regarding the extraction yield and phytochemical composition in view of their potential use as skincare ingredients.

Current Development

Thymus mastichina

Terpinene-rich extracts were isolated from dried (DR) and fresh (FR) samples of T. *mastichina* using SFE-CO2**.** Extraction conditions were set at 80 bar and 50ºC for 2 h in a stationary system with CO_2 recycling. Hydrodistillation HD was also used for comparision purposes and both the essentoial oil (EO) and hydrolate (HY), lipophilic and hydrophilic phases, respectively, were evaluated. The extracts obtained were characterized by GC-MS, cytotoxic potential, and sensory analysis of odours.

The most abundant aromas were quantified, and the analysis performed by GC-MS revealed an abundance of terpenoids such as thymol chemotype, followed by the precursors α-terpinene and *p*-cymene. DR and FR extracts (EX) obtained from SFE-CO2 show the highest content of thymol, achieving 52.7% and 72.5% of the isolated volatile fraction.

The DR essential oil (EO) contained the highest amount of terpenoids, but it was also the most cytotoxic extract. In contrast, SFE-CO2 products showed the lowest cytotoxic potential.

The sensory profile described the extracts as having green, fresh and floral notes, with no significant statistical differences (α=0.05) regarding the odour detection threshold (ODT) values (Fig.1).

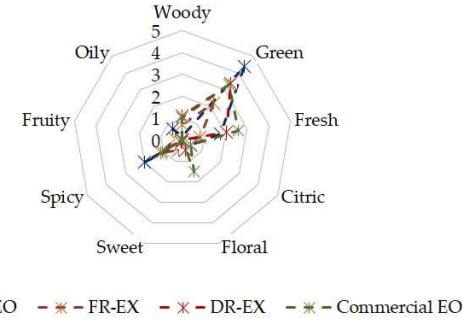

Fig 1. Sensory description and intensity of selected T. mastichina samples.

Moringa oleifera L.

Extracts obtained from the leaves, seeds, and roots of *Moringa oleifera* L. tree, using SFE-CO2, were studied as potential cosmetic ingredients. Leaf, seed and root extraction resulted in yields between 0.12–3.38%, 0.22–1.83%,and 0.02–0.18%, respectively. Seed extracts showed the presence of oleic acid (>80%), while the leaves presented a rich composition in linolenic acid (~20%), nonacosane (~22%), and α-tocopherol (>20%). Mo root resulted in higher relative composition for sterol molecules, despite its very low affinity with CO_2.

The extraction yield was evaluated by response surface methodology (RSM), for pressure and temperature ranges of 117–273 bar and 41–60 ◦C, respectively, using a design of experiments (DOE). The pressure significantly affected the results (α = 0.05), with the highest extraction efficiency obtained at conditions above 195 bar. The extracts' composition, evaluated by gas chromatography-mass spectrometer (GC-MS), revealed an increasing correlation between the pressure, total extract solubility, and mass of extract at a constant temperature, due to the higher extraction yield.

The RSM optimisation revealed 250 bar at 45 ºC and 195 bar at 55 ºC as the optimal extraction conditions to improve the richness of the target compounds in the respective extracts, that is oleic acid in seeds and α-tocopherol in leaves (Fig. 2).

Moreover, temperature increase reduced the solvation power of CO_2 upon the total seed extraction yield and the α-tocopherol content from the leaf extract. The results also suggested a low solubility of the recovered compounds regarding the supercritical CO_2, mainly represented by sterol molecules.

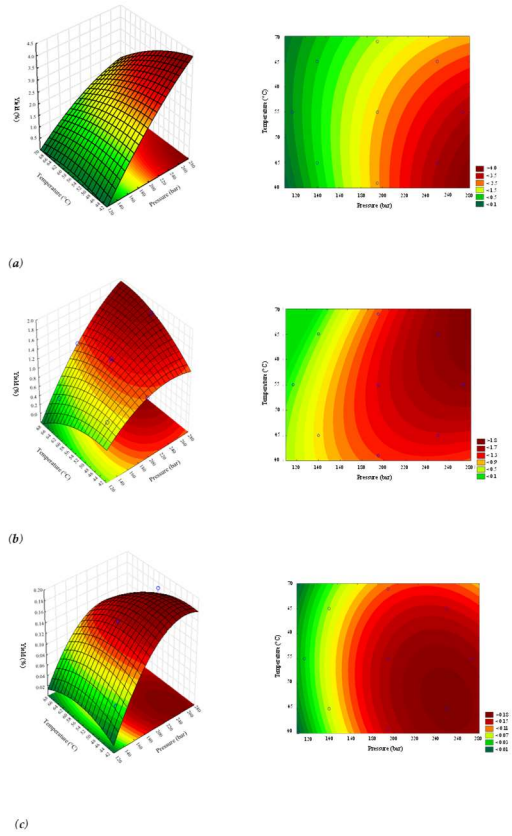

Fig 2. Response surface methodology (RSM) applied for (a) Mo seed, (b) leaf, and (c) root SFE-CO2 extracts, according to DOE analysis.

Future Perspectives

Thymus mastichina

The species T. *mastichina* was studied for the first time under supercritical conditions as well as regarding its sensory profile. The resulting research presented an unexplored point of view for one of the most studied aromatic plants in recent decades.

The analysed characteristics of the extracted products suggested the extracts obtained from fresh samples through SFE-CO2 as the most promising sample to use as a flavouring additive for food applications, namely in breads.

Moringa oleifera L.

The optimised Mo seed and leaf SFE-CO_2 extracts have been selected for further studies as ingredients for cosmetic purposes since they present a set of biocompounds useful to ensure photoprotective, moisturising, and skin-lightening properties.

The potential use of the two target compounds as cosmetic ingredients was studied by correlating the compounds and bioactivities. The online Scopus database was used to export the literature occurrences of the selected keywords: Mo, oleic acid, and α- tocopherol (Fig. 3a), as well as cosmetic, oleic acid, and α-tocopherol (Fig. 3b). In both cases, the analyses resulted in two different clusters, suggesting a higher correlation among Mo, oleic acid, and α-tocopherol, all connected to the antimicrobial, antioxidant, and antineoplastic activities. Cosmetic keyword has also evidenced the antioxidant capacity over the presence of the target compounds, increasing the prospect of using Mo extracts as ingredients for topic applications.

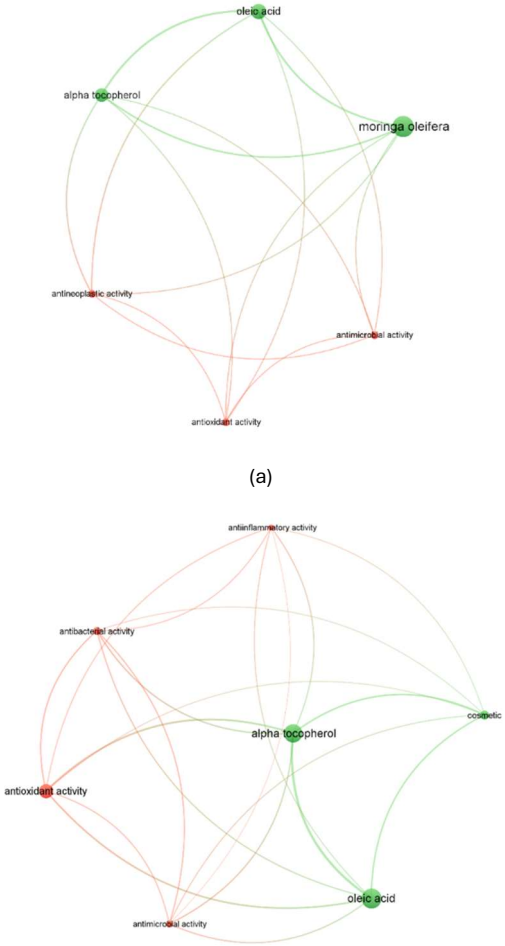

Fig 3. Correlation for oleic acid, α-tocopherol to use as cosmetic ingredients, and their bioactivities reported in scientific works during 2000 to 2022. Key-words: (a) Moringa oleifera, oleic acid, α-tocopherol, and (b) Cosmetic, oleic acid, α-tocopherol. Clusters are represented by different colours using selected keywords in, at least, 5 number of occurrences. Larger circles represent higher keyword occurrences. Thicker lines represent higher correlation among keywords.

Related Sustainable Development Goals

Outputs

PhD Theses

[1] Júlia Cristiê Kessler, Supercritical extraction and NETmix technology to produce green and stabilized M. *oleifera* functional ingredients having in view cosmeceutical formulations, PDEQB, FEUP (ongoing)

Selected Publications

[1] J. C. Kessler, V. A. Vieira, I. M. Martins, Y. A. Manrique, A. Afonso, P. Ferreira, F. Mandim, I.C.F.R. Ferreira, L. Barros, A.E. Rodrigues, M.M. Dias. Molecules, 27 (2022).

[2] J.C. Kessler, Y.A. Manrique, I.M Martins, A.E Rodrigues, M.F. Barreiro, M.M. Dias. Separations, 10 (2023).

Team

Madalena M. Dias, Professor; **Alírio E. Rodrigues**, Professor; **Isabel M. Martins**, Researcher; **Yaidelin A. Manrique**, Researcher; **Vanessa A. Vieira**, Posdoc; **Júlia C. Kessler,** PhD Student

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Valorisation of Natural Products and Food Wastes

Industrial Applications

KEYWORDS: Supercritical Fluid Extraction / Refining / CO2 Hydrates

Some natural products and food wastes were identified as natural matrices highly rich in added-value molecules. These were tested to enable the production of aromas and bioactive ingredients with functional properties. Aromas from dried fruits and aromatic plants were added to bread. Extracts rich in bioactive compounds were incorporated into fruit preparations to develop fortified juices.

To enable the industrial production of natural ingredients, it is crucial to develop solutions that overcome weaknesses in the production chain, namely versatile and efficient extraction/refining equipment. Two systems were designed for supercritical CO_2 extraction and refining of ingredients. Additionally, a new process for food conservation based on CO2 hydrates was developed.

Introduction

Extraction of Aromas and Bioactives

The identification of natural matrices with the potential to be a source of highly rich-in-added-value molecules and enable its industrial production is essential; for example, in the food sector, the use of additives is a common practice to increase shelf-life and/or to make the product, more appealing, being that the use of synthetic additives is increasingly restricted, and given the market trends, the ValorNatural Project aimed to mobilize a value chain based on the development of natural ingredients in the classes of preservatives, colourants, aromas and bioactive to use as an alternative to synthetic additives. The raw materials selected were dried fruits — almond (*Corylus avellana*), hazelnut (*Prunus dulcis*) and nut (*Juglans regia*) — and aromatic plants — rosemary (*Rosmarinus officinalis*), thyme, (*Thymus mastichina*) and oregano (*Origanum vulgare*).

The BIOMA project intended to move entities in the agri-food value chain to more competitive and sustainable levels, promoting strategies and an environment that enhances the adoption of integrated bioeconomics solutions. Five types of waste presented the most promising outlook for extracting bioactive compounds and formulating preservative/bioactive ingredients: olive leaf, olive pomace, kale, onion peel, and pineapple (peel and crown).

Extracting these target compounds commonly uses conventional methods such as hydrodistillation, steam distillation, or organic solvents (e.g., Soxhlet, maceration). However, those methods promote chemical alterations and should have a negative impact on thermosensitive compounds. In this work, supercritical fluid extraction using CO_2 (SFE-CO2), which is at the top of emerging green methodologies, was used to perform the extraction. SFE-CO2 is safe, non-flammable, non-toxic and non-carcinogenic. It is possible to obtain a higher selectivity of the target volatile substances by modifying the pressure and temperature of the carbon dioxide; also, it is possible to perform extraction at relatively low temperatures to achieve thermal unstable and oxidative compounds.

Development of Prototype Equipment

Within the Valor Naturat project, three prototype systems have been developed, designed, and assembled: (i) a state-of-the-art SFE-CO2 extractor; (ii) an innovative extration/refining system based on the NETmix technology; and (iii) a food conservation process based on CO2 hydrates. These systems were designed developed by a multidisciplinary team led by LSRE-LCM. The INEGI (inegi.pt) team designed all non-standard equipment and the company Paralab (paralab.pt) was in charge of the system assembly and construction.

Current Development

Extraction of Aromas and Bioactives

Aroma extracts from aromatic plants and dried fruits were obtained for incorporation in food products, specifically in bakery products.

All SFE-CO2 extractions were set up to 110 bar and up to 60 °C for 2 h in a stationary system. All extracts were characterized by GC-MS, sensory analysis of odours and cytotoxic potential. The extracts of dehydrated almond (*Prunus dulcis*) and rosemary (*Rosmarinus officinalis*) were considered the most promising for incorporation into bread. The R. *officinalis* extracts showed an abundance of α-pinene, eucalyptol, S-verbenone and camphor, contributing to the green, fresh, citric, and woody main sensory notes. The P. *dulcis* extracts are a rich source of terpenoids such as D-limonene, α-terpineol, and elemol. The results obtained in the Odor Threshold (ODT) and Vero cell proliferation inhibition (GI50) analyses established the range of extract concentrations to be incorporated into bread. The olfactory perception of bread samples incorporated with these extracts was evaluated according to a discriminative analysis applying a multiple comparison test (MCT) and following the sensory analysis practices established by ISO 8586:2012. The aromas of plain bread and bread with natural extracts were characterized and compared according to the profile of volatiles present in crust and crumb samples. In carrying out these tests, the evaluators were asked to establish an olfactory comparison of unknown samples against a standard according to a scale of five comparative levels. This study revealed that the incorporation of rosemary or almond extracts obtained by SFE-CO2 under optimized conditions resulted in an increasing perception of bread odour. This result could represent a stepping stone for the improvement of bread's sensorial properties using novel ingredients, namely natural aromas.

An onion peel extract, both in free and stabilized (solid dispersion) forms, was selected for incorporation into apple juice. Incorporating this extract improved the antioxidant capacity since the EC50 values decreased compared with the control, indicating an increase in functional ingredients, namely with antioxidant activity. The EC50 values were more promising for the juice with stabilized extract. This may indicate that the extract in this form allows better homogenization of the active compounds in the food matrix.

Development of Prototype Equipment

The CO2 supercritical fluid extraction system (shown in Fig. 1a) is an innovative equipment where CO_2 can be recirculated during the extraction phase and reused. This installation was designed to withstand temperatures up to 80 °C, pressures up to 300 bar in the extraction vessel, and a maximum flow rate of carbon dioxide in the range of 0.1-5 L/min. The system includes two separators with temperature

and pressure controls to fractionate the extract. The extractor, designed to comply with the European Pressure Equipment Directive 2014/68/EU, has a total volume of up to 5 L, allowing for a production of circa 10 g of extract, a significative advantage compared to bench-scale equipment. It will be a push-forward to the scale-up for industrial applications. Also, a clear innovation in the design of the extraction process allowed the use of raw material with no pre-treatment, that is, in its hydrated form, avoiding the usual dehydration process before the extraction stage.

Fig 1. a) Supercritical fluid extraction system. b) Extraction/refining system and c) CO2 Hydrates production and characterization systems.

The extraction/refining system (shown in Fig. 1b) integrates the NETmix technology to promote the mixture and consequently refine and extract. To guarantee the complete mixing of the phases, the optimal geometry was obtained for hydraulic diameter of 0.67 mm and chamber diameter of 6.75 mm. To minimize the wall-effect a minimum of 8 columns and 44 lines was defined, and the system must operate at Re > 300. Analysis of the CO2 phase diagram led to a minimum operating temperature of 15 °C to avoid the formation of hydrates. The operating temperature is up to 40 °C to maximize the solubility of the compounds in CO_2. The operating pressure can be set for a range of 60 to 100 bar for liquid or supercritical.

For the production of CO2 hydrates (Fig. 1c), a system with a production rate of 1 kg/h was designed. The minimum operating temperature was set between 0 °C, avoiding freezing of water, and 10 °C to ensure the formation of CO_2 hydrates. Concerning the pressure, the system will operate between 10 and 45 bar. Also, to complement this system, a system of analysis and characterization of carbon dioxide was developed.

Future Perspectives

The results obtained from incorporating natural products in food matrices are promising for developing innovative foods oriented to consumer demands, allowing the reuse of agri-food waste, the development of added-value foods and the integration of natural ingredients in food and/or cosmetic products with market potential.

The state-of-the-art SFE-CO2 extractor is currently located at Instituto Politécnio de Bragança and can be used within the scope of academic and industrial research projects, but also directly from local companies and producers interested in developing extracts towards valorization of their natural products.

The innovative extraction/refining system based on the NETmix technology is currently under proof of concept in the scope of a PhD Thesis (C. Almeida).

The food conservation process based on CO_2 hydrates has been successfully tested for the conservation of mushrooms. Other conservation applications and incorporation of CO2 hydrates (in lieu of formation) into bread are being considered.

Related Sustainable Development Goals

Outputs

PhD Theses

[1] Cláudia Almeida, Valorisation of natural products via supercritical fluid extraction and product refining by NETmix technology, PDEQB, FEUP (on-going).

Master Dissertations

[1] Rharyne de França, Extração de compostos bioativos a partir de desperdícios alimentares, MIEQ, FEUP, 2021

[2] Lorrayne Rocha, Extração de compostos bioativos a partir de desperdícios alimentares, MIEQ, FEUP, 2022

Selected Publications

[1] Kessler J.C., Vieira V., Martins I.M., Manrique Y.A., Ferreira P., Calhelha R.C., Afonso A., Barros L., Rodrigues A.E., Dias M.M. Food Chemistry, 384, 132514 (2022)

[2] Kessler J.C., Vieira V., Martins I.M., Manrique Y.A., Ferreira P., Calhelha R.C., Afonso A., Barros L., Rodrigues A.E., Dias M.M. Food Chemistry, 417, 135845 (2023)

Prototypes

[1] State-of-the-art SFE-CO2 extractor

[2] Innovative extraction/refining system based on NETmix

[3] Food conservation process based on CO2 hydrates

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