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Olive pomace as a valuable source of bioactive compounds: A study regarding its lipid- and water-soluble components

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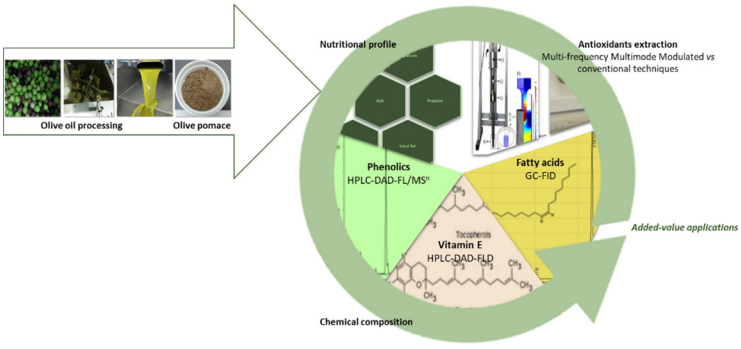
HIGHLIGHTS

- As olive oil production increases worldwide, huge amounts of wastes are generated.
- Olive pomace (the major waste produced) is an environmental burden.
- The chemical composition of the olive pomace was assessed.
- Olive pomace is a source of bioactive compounds with human health benefits.
- A sustainable method for antioxidants extraction is presented.

GRAPHICAL ABSTRACT



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ABSTRACT

Olive pomace is a major output of olive oil processing. This by-product is a valuable source of bioactive compounds with well-recognized benefits for human health and well-being.
In this work, the proximate composition and the profiles of vitamin E (HPLC-DAD-FLD), fatty acids (GC-FID) and phenolics (HPLC-DAD-FL/MS^n) were determined. Additionally, a sustainable process for antioxidants extraction - Multi-frequency Multimode Modulated (MMM) ultrasonic technique – was compared to a conventional solid-liquid extraction. The total phenolics content and antioxidant activity (ferric reducing antioxidant power and DPPH• scavenging ability) of the extracts were analysed to assess the efficacy of both extraction methodologies.
The vitamin E profile of the olive pomace comprised the vitamers α-tocopherol, β-tocopherol, α-tocotrienol and γ-tocopherol. α-Tocopherol was the major form (2.63 mg/100 g), while the other vitamers were present in amounts lower than 0.1 mg/100 g. The lipid fraction was especially rich in oleic acid (75%), followed by palmitic (10%), linoleic (9%), and stearic (3%) acids. Hydroxytyrosol and comsegoloside represented ≈79% of the total phenolics present in olive pomace. Hydroxytyrosol content was 83.6 mg/100 g, while tyrosol was present in lower amounts (3.4 mg/100 g).
Concerning the antioxidants extraction, the MMM technique allowed a faster and higher recovery ($p < 0.05$) of the compounds, compared to the conventional solid-liquid extraction. By this way, it seems to be a very promising eco-friendly and effective methodology to extract antioxidants from this and other matrices.
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1. Introduction

In 2050, it is expected that the world population (7.6 billion) will reach 9.1 billion (United Nations, 2017). Globally, more 30% of food will be necessary compared to the current days and, as a consequence, several major challenges are coming, especially related to food security and agricultural practices (Wezel et al., 2014). Indeed, over the last decades, the demand for larger amounts of food production has been increasing, which has been raising a key challenge to agriculture: it must be sustainable.

Sustainable agriculture is an interconnected system that involves environment protection, social fairness, technologies application and economic profitability (DeLonge et al., 2016). In addition to the world food supply, other issues emerge that compromises the Earth natural resources, such as water quality; higher demand for water, wood, and energy; increase of the land area for urbanization; soil degradation; loss of biodiversity; climate changes; among others (Lichtfouse et al., 2009). Overall, agriculture sustainability must enhance the food production without compromising the future generations (Wezel et al., 2014).

The olive oil industry is becoming one of the larger agro-food business sectors. In fact, the organoleptic characteristics together with recent advances on health properties of the olive oil have led to an increase of its consumption and production (Banias et al., 2017). Two different techniques can be used for olive oil production: the two-phase method or the three-phase method. In the first one, no water is added and a humid semi-solid pomace is obtained as by-product (wet olive pomace). Olive pomace is one of the major outputs of olive oil production and despite resulting from an eco-friendly system, it is considered phytotoxic due to its high phenolics content (Malapert et al., 2018). Simultaneously, those bioactive compounds can have health and well-being applications (*e.g.* for food and cosmetic industries) (Nunes et al., 2016; Rodrigues et al., 2017). More and more, the study of eco-friendly technologies that maximize the yield of extraction of such bioactive compounds, at low-cost, in a minimum period of time, and using environmental friendly solvents are mandatory (Galanakis, 2012). By this way, it is possible to obtain natural ingredients to develop new food products, adding economic value to the olive oil processing segment, and simultaneously reducing the environmental burden of wastes, contributing, therefore, to a sustainable agriculture system.

In this work, the nutritional profile and chemical composition (regarding fatty acids, vitamin E and phenolic compounds) of olive pomace was assessed. Moreover, the efficiency of a new sustainable process of antioxidants extraction (Multi-frequency Multimode Modulated Ultrasonic technique) was compared with that of a conventional extraction method.

2. Material and methods

2.1. Sample preparation

Olive pomace (about 5 kg) was collected in Alfândega da Fé (Trás-Os-Montes, Portugal), in 2015, from a two-phase extraction olive oil unit. The sample was homogenized, divided in different batches, frozen at −80 °C, and freeze-dried (Telstar Cryodos-80 Terrassa, Barcelona) for preservation till analysis.

2.2. Chemical characterization

2.2.1. Proximate analysis

Sample moisture was determined using an infrared balance (Scaltec model SMO01, Scaltec Instruments, Heiligenstadt, Germany). Total fat (AOAC 991.36) and total protein (AOAC 928.08) were determined by Soxhlet and Kjeldahl methods, respectively (AOAC, 2012). The total protein content was calculated using 6.25 as the nitrogen conversion factor (Tontisirin, 2003). Ash content was determined by incineration at 500 °C (AOAC 920.153) (AOAC, 2012). Total carbohydrates were calculated by difference (Tontisirin, 2003).

2.2.2. Vitamin E profile by HPLC-DAD-FLD

An aliquot (≈20 mg) of the lipid fraction, obtained according to Pimentel et al. (2014), was mixed with 50 μL of internal standard (tocol, 100 μg/mL) and 1 mL of *n*-hexane.

The chromatographic analysis was carried out in a HPLC system (Jasco, Tokyo, Japan) equipped with a multiwavelength diode array detector (MD-2015) coupled to a FP-2020 fluorescence detector (Jasco, Tokyo, Japan), programmed for excitation at 290 and emission at 330 nm. The chromatographic separation of the compounds was achieved using a normal phase Supelcosil™ LC-SI column (75 mm × 3.0 mm, 3.0 μm) (Supelco, Bellefonte, USA) according to Alves et al. (2009).

Vitamin E vitamers were identified based on their UV spectra and by comparing their retention times with those of standards. Quantification was performed based on the fluorescence signals.

2.2.3. Fatty acids profile by GC-FID

The lipid fraction of olive pomace was obtained using the Soxhlet method (4 h). The fatty acid profile was, then, determined according to ISO 12966-2:2011 (ISO 12966-2:2011, 2011). Briefly, about 15 mg of oil were vigorously mixed with 3 mL of *n*-hexane. Then, 200 μL of 2 M KOH in methanol were added. After addition of anhydrous sodium sulfate, the mixture was centrifuged (850 ×*g*, 5 min) and the supernatant was transferred to an injection vial.

The compounds separation was achieved in a Gas Chromatograph (Shimadzu GC-2010 *Plus*, Tokyo, Japan) coupled to a split/splitless AOC-20i auto-injector (Shimadzu, Tokyo, Japan) and a Flame Ionization Detector (Shimadzu, Tokyo, Japan). Helium was the carrier gas. A CP-Sil 88 silica capillary column (50 m × 0.25 mm i.d., 0.20 μm film thickness) from Varian (Middelburg, Netherlands) was used. The temperature program was as follows: 80 °C for 5 min; increase to 160 °C at 5 °C/min; 160 °C for 5 min; increase to 170 °C at 5 °C/min; 170 °C for 1 min; increase to 220 °C at 5 °C/min; 220 °C for 15 min. The injector and detector temperatures were 250 and 270 °C, respectively. A split ratio of 1:25 was used and the injection volume was 1.0 μL.

The fatty acid methyl esters were identified by comparison with a standard mixture (FAME 37, Supelco, Bellefonte, PA, USA). Data were analysed based on relative peak areas. The results were expressed in relative percentage of each fatty acid.

2.2.4. Phenolics profile by HPLC-DAD-FLD and HPLC-DAD-MS^n

Aqueous extracts were prepared according to Costa et al. (2014) and freeze-dried. The obtained residue was dissolved in 1 mL of acetic acid (1% v/v). The solutions were centrifuged and filtered with syringe filter (0.22 μm) prior injection in the HPLC-DAD-FLD system (Jasco, Tokyo, Japan). This system was composed of an AS-2057 automated injector, a PU-2089 pump, a MD-2018 multi-wavelength diode array detector (DAD) and a fluorescence detector (FLD) FP-2020 (Jasco, Japan), programmed for excitation at 280 and emission at 330 nm. The gradient elution program used was the following: 0 min, 10% B; 20 min, 30% B; 50 min, 75% B; 63 min, 100% B; 65 min, 10% B; 70 min, 10% B, being solvent A) 1% acetic acid and B) 100% methanol. The chromatographic column used was a Zorbax-SB-C18 (5 μm; 25 × 4.6 mm; Agilent Technologies, USA), at 20 °C, with a flow rate of 1 mL/min. The DAD recorded from 200 to 600 nm, being each run monitored at 280, 320 and 335 nm. To further identify the compounds, the extract was also analysed in an HPLC-DAD-ESI-MS^n system (Thermo Finnigan, LCQ Deca XP Max, San Jose, USA). The same elution conditions were applied, being the connection to the ion trap mass spectrometer made with split of half of the initial flow to 0.5 mL/min. An electrospray interface in the negative mode was used in the following conditions: capillary voltage of 15 V, tube lens voltage of 30 V, capillary temperature of 325 °C, sheath gas flow rate of 60 (arbitrary units) and aux/sweep gas flow rate of 23

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