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Obtaining sugars and natural antioxidants from olive leaves by steam-explosion

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ABSTRACT

In this work, steam-explosion treatment was evaluated as a procedure to recover sugars and natural antioxidants from olive tree leaves. The treatment was carried out following a Box-Behnken experimental design, with three factors, temperature (180–220 °C), process time (2–10 min) and milling time (0–15 s). Response surface methodology showed that temperature was the most influential factor, followed by process time, while the best results were achieved with whole leaves. The operational conditions for simultaneously maximizing the sugars and natural antioxidants recoveries resulted to be 180 °C, 8.3 min and whole leaf; under these conditions 18.39 g and 1950 mg were obtained from 100 g dry olive leaves, respectively. This is equivalent to 70% recovery of the initial sugars present in olive leaves, with a very low formation of inhibitory compounds and an important amount of natural products with antioxidant capacity such as oleuropein, hydroxytyrosol and flavonoids.

1. Introduction

Olive leaves are found in large quantities in the olive oil and olive table industries, where they are separated from olives using pneumatic separation systems, creating a residue that only occasionally is removed for animal feed, but generally without application of industrial interest. The amount of olive leaves accumulated annually along these industries may exceed 1 million tonnes. Harvested olives are accompanied by approximately 6% of leaves and annual world production of olives exceeds 18 million tonnes (average 2006–2013) (FAOSTAT, 2015). Therefore, this olive industry residue can be of interest in a biorefinery context (Romero-García et al., 2014) and it is worth recovering high added-value compounds from this material which have a great interest in the pharmaceutical, food and cosmetic sectors (El & Karakaya, 2009; Erbay & Icier, 2010; Rodrigues, Pimentel, & Oliveira, 2015). As prominent examples, phenolic compounds (oleuropein and hydroxytyrosol), as well as flavonoids with antioxidant capacity, and sugars (mannitol, oligosaccharides, and so on) can be mentioned.

Olive leaves constitute a biomass of lignocellulosic composition, a complex mixture of cellulose, hemicelluloses and lignin. In addition to those biopolymers, they also contain a significant amount of soluble compounds(extractives) among which bioactive products such as secoiridoids (the major constituent of the secoiridoid family in olive leaves is oleuropein), flavonoids (apigenin, luteolin, together with their 7-O-glucosides) and phenolic compounds (mainly hydroxytyrosol) have been identified (Rodrigues et al., 2015). The concentration of these compounds in the leaves, which depends on several factors including olive variety and cultivation conditions, is available in the literature. For example, Guinda et al. (2015) reported that up to 14% of oleuropein (dry basis) was present in olive leaves, while hydroxytyrosol accounted for up to 0.94–1.12% dry weight.

The phenolic content makes the olive leaf extracts to show great potential as natural antioxidant. Oleuropein is the main compound responsible for antioxidant properties of hydroalcoholic extracts (produced with ethanol or methanol at lab scale but also commercial extracts), Hayes, Allen, Brunton, O'Grady, and Kerry (2011). Olive leaf extracts possess other significant biological properties such as antimicrobial activity and are well known for their effect on metabolism in particular as a traditional anti-diabetic and anti-hypertensive herbal drug (Sato et al., 2007; Sudjana et al., 2009; Susalit et al., 2011).

Olive leaves composition allows for the development of a multiproduct industry that utilizes various components in biomass and their intermediates thus maximizing the value derived from biomass feedstock (Romero-García et al., 2014), thus contributing to increase farmers rent.

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In the last few years, different extraction techniques have been established in order to extract oleuropein and other phenolic compounds from olive leaves (Rahmanian, Jafari, & Wani, 2015). In contrast to traditional laboratory extraction techniques based on solid-liquid extraction methodology (maceration and Soxhlet extraction), a steam-explosion (SE) technology appears as a potentially economic way of optimizing the exploitation of olive leaves on an industrial scale. A SE process offers several attractive features when compared to alternative processes involving modern technologies for polyphenol recovery such as supercritical fluid extraction, pressurized liquid extraction and microwave and ultrasound-assisted extraction, Luque de Castro and Priego Capote (2010). Its main advantages are the possibility of avoiding the use of hazardous chemicals and/or solvents (frequently methanol and hydroalcoholic solutions) for reducing the process time compared to that required by other extraction techniques such as maceration and Soxhlet extraction (typically lasting for 24 h, compared to several minutes in the case of steam explosion), and a more complete recovery of all biopolymers (cellulose, hemicelluloses and lignin) in a usable form (Fernández-Bolaños et al., 2008; Gong, Huang, & Zhang, 2012). The potential of SE as a refining process has been demonstrated in many raw materials, including forest and agricultural biomass such as wood chips, rice husk, sugarcane bagasse, and corn stalks (Chen & Liu, 2015).

Different parts of olive tree and solid wastes of the olive oil production (olive tree biomass from pruning, olive stones and olive pomace) have already been subjected successfully to SE treatment in order to obtain an effective recovery of high-value compounds such as antioxidants (e.g.: hydroxytyrosol) and fermentable sugars (Ballesteros et al., 2011; Cara et al., 2008; Castro et al., 2008; Conde et al., 2009; Fernández-Bolaños et al., 2004) which can be used for the production of a wide range of food and non-food compounds like feed, fuels, chemicals, and materials (Kamm, Schönicke, & Hille, 2015).

Considering that SE results in the solubilization of the hemicellulosic fraction of the raw material, together with alteration in the lignin, it is likely that this pretreatment improves the enzymatic hydrolysis of the cellulosic fraction, as well as the recovery of the above-mentioned compounds. In addition to antioxidants and fermentable sugars, a number of sugar degradation or lignin-derived compounds can also be obtained. Some of these are well known to be inhibitory to a different extent to the microorganisms employed for further processing (fermentation) so their occurrence in the pretreated material should also be controlled.

Olive leaves so far have not been subjected to SE treatment for recovering high added value compounds.

In this study, SE treatment using response surface methodology (RSM) was applied for the first time as a procedure to optimize the extraction of olive leaves, with focus on sugar solubilization and natural antioxidants recovery. RSM has already been used successfully to optimize the recovery of antioxidants and sugars from other olive by-products, such as olive stones (Lama-Muñoz, Romero-García, Cara, Moya, & Castro, 2014). In the present study, the effects of treatment temperature, process time and the particle size (milling time) are studied and the corresponding mathematical models are developed. Also the main characterization of antioxidant compounds was elucidated, and finally a quantitative process scheme, based on the mass balance, is proposed.

2. Materials and methods

2.1. Raw material

Olive tree leaves of Picual variety were picked randomly from an experimental olive grove located at the Instituto de la Grasa (CSIC) in Seville (Spain) in March 2014. The total solid content of the leaves was 49.1% at 105 °C (measured after 24 h).

After collection, olive leaves were stored at 4 °C in the dark. Before SE treatment, they were ground using a Thermomix®TM 31 (Corporate Group Vorwerk, Germany) set at speed 7, into elliptical particles whose average sizes were between 5.5 × 1.5 (whole leaf), 3 × 1.3 and 2.5 × 0.9 cm for milling times of 0, 7.5 and 15 s, respectively.

The equivalent particle diameter (epd) (assimilating the surface of the ellipse to the circle) is 2.9–1.5 cm for milling times of 0–15 s, respectively. Milling time and epd showed a linear relationship. All experiments were performed with fresh olive leaves but the results are expressed respect to dry olive leaves (DOL).

2.2. Experimental design and SE treatment

Three factors were studied: treatment temperature (180–220 °C), process time (2–10 min), and milling time (0–15 s, or epd 2.9–1.5 cm). The Box-Behnken experimental design included twelve points and five replicates at the centre of the domain for each factor (Supplementary Table 1). In addition, Response Surface Methodology (RSM) was applied for result assessment.

The SE treatment of olive leaves was performed in a custom-built batch pilot unit (Nusim, S. A., Madrid, Spain) based on Masonite technology, equipped with a 2 L reaction vessel designed to reach high operation pressures (up to 40 bar), as described in Fernández-Bolaños et al. (2002). The reactor, previously preheated, was charged with 200 g of fresh sample, and at once heated again with saturated steam. The process time (2, 6 and 10 min) was counted once the selected temperature (180, 220 and 220 °C) was reached (a few seconds only were required). The reactor incorporates an electronic computing device that controls the time and the temperature in a pre-programmed manner.

Experimental data were analyzed by the statistical software Design-Expert 8.0.7.1 (Stat-Ease Inc., Minneapolis, USA).

2.3. Analytical methods

2.3.1. Composition of raw material

The composition of raw material, e.g., total solid content, extractives, acid insoluble lignin, acid soluble lignin, ash, carbohydrates, and acetyl groups, was determined in triplicate according to NREL (National Renewable Energy Laboratory, 2013) analytical methods for biomass.

2.3.2. Determination of sugars and inhibitors

Monomeric and oligomeric sugars released to liquid fractions issued from SE treatment were determined by HPLC, as well as the content of potential inhibitory compounds (acetic acid, formic acid, levulinic acid, furfural and hydroxymethylfurfural (HMF)). Liquid fractions obtained from SE were centrifuged and filtered through 0.22 μm membranes (Gelman Sciences, Inc., Michigan, USA) and analyzed by HPLC for quantitative carbohydrate analysis. The HPLC system (Waters, Milford, USA) was equipped with a refractive index detector (model 2414). A 7.8 × 300 mm CARBOSep CHO-782 Pb (Transgenomic, Inc., Omaha, USA) carbohydrate analysis column operating at 70 °C with ultrapure water as a mobile-phase (0.6 mL/min) was used for the monomeric sugars (arabinose, galactose, glucose, mannose and xylose) and mannitol determinations. The volume of the injection was 20 μL. The totals oligomers are obtained as the difference between total free sugars in the liquor before and after post-hydrolysis (120 °C, 20 min, 3% w/v H_2SO_4).

Furfural, HMF, acetic acid, formic acid, levulinic acid and xylitol content were analyzed in duplicate by HPLC in a Hewlett-Packard 1100 system (Palo Alto, CA, USA) equipped with a refractive index