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Improvement of oleuropein extractability by optimising steam blanching process as pre-treatment of olive leaf extraction via response surface methodology

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ABSTRACT

Impact of steam, hot water blanching and UV-C irradiation as pre-treatments on extraction of oleuropein and related biophenols from olive leaves (OLs), was investigated. Moreover, particle size effect of olive leaves and steam blanching duration were selected as independent variables to optimise steam blanching process in terms of oleuropein content (OC) and antioxidant activity (AC) of ethanolic extracts, by using response surface methodology. Optimum conditions for OC and AC were 10 min steam blanching of 20–11 and 3–1 mm olive leaf fraction, respectively. Depending on the extraction procedure, at optimum conditions of steaming the results indicate that steam blanching of OL prior to extraction can significantly increase oleuropein yield from 25 to 35 times compared to non-steam blanched sample, whereas the antioxidant activity increased from 4 to 13 times. No significant UV-C effect was observed in OC and AC, while hot water blanched samples showed significantly higher oleuropein yields and antioxidant activity compared to untreated samples.

1. Introduction

The climate of the Mediterranean region favours the growth of the olive tree (*Olea europaea*) which has provided Mediterranean people with economic and dietary benefits since ancient years. The properties of olive biophenols (OBPs) are demonstrated by their biological functions in the olive tree (namely, defence system against pathogen attacks and response to insect injury). Several studies have shown that the healthy properties of virgin olive oil are mainly due to the presence of OBPs (Servili & Montedoro, 2002). Oleuropein and related phenolic compounds (e.g. hydroxytyrosol, apigenin-7-glucoside, luteolin-7-glucoside rutin) contributes positively in the prevention of chronic diseases such as cardiovascular diseases (Kim, Kim, Jin, & Yun, 2006; Omar, 2010), cancer (Bonoli, Bendini, Cerretani, Lercker, & Toschi, 2004) and Alzheimer's (Patil et al., 2003). These effects of olive leaf extracts have been attributed to synergistic phenomena among OBPs (Benavente-Garcia, Castillo, Lorente, Ortuno, & Del Rio, 2000). Rich sources of OBPs are considered to be olive leaves and oil mill waste water (OMW) coming from olive oil production. Among the different parts of the olive tree, olive leaves have the highest oleuropein content, which ranges from 1% to 14% compared to olive oil (0.005–0.12%) and OMW (0.87%) (Japón-Luján & Luque de Castro, 2006). As a further consequence, the raw materials containing OBPs are attractive sources to obtain these nutraceuticals. Methanol–water mixtures (Savournin, Elias, Dargouth-Kesraoui, Boukef, & Balansard, 2001) have been used in conventional solid–liquid extraction methods for the isolation of OBPs. Nevertheless, the use of non-toxic solvents can provide natural extracts for the development of functional foods with beneficial properties for human health.

Moreover, shorter extraction times than 24–48 h reported, have to be achieved for the isolation of OBPs at an industrial scale. Thus, several extraction-assisted techniques have been developed, like extraction with superheated liquids (Japón-Luján & Luque de Castro, 2006) ultrasound-assisted extraction (Japón-Luján, Luque-Rodríguez, & Luque de Castro, 2006a) and microwave-assisted extraction (Japón-Luján, Luque-Rodríguez, & Luque de Castro, 2006b). Alternatives to those methods have been reported, such as supercritical fluid extraction, with CO_2 as supercritical fluid and ethanol or methanol as modifier (Tabera et al., 2004).

Thermal treatment like steam and hot water blanching as well as cooking, has been reported to cause structural changes in plant tissues (Alzamora, Nieto, & Castro, 2003, part II, chap. 4). The extractability of phenolic compounds appears to increase in cooked, blanched or canned products (Chaovanalikit & Wrolstad, 2004; Turkmen, Sari, & Velioglu, 2005). Hot water and steam blanching treatment has been under investigation for its impact on drying process, antioxidant activity, vitamins and total phenol content of several agricultural products (Boudhrioua, Bahloul, Slimen, & Kechaou, 2009; Volden et al., 2008).

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Up to now, there is no study (to our knowledge) aiming to enhance the oleuropein extractability by applying thermal treatment as a pre-step of the ethanolic extraction from olive leaves. Thus and in what regards oleuropein content (OC) and antioxidant activity (AC) of ethanolic extracts, optimisation of steam blanching process has been performed by combining chromatographic analysis and response surface methodology. Additionally and prior to extraction, impact of hot water blanching and UV-C irradiation on OC and AC of olive leaves has been investigated as well.

2. Materials and methods

2.1. Chemicals

Methanol, acetic acid and acetonitrile were purchased from Merck (Darmstadt, Germany), tyrosol and caffeic acid were purchased from Sigma–Aldrich (Hohenbrunn, Germany). Oleuropein and hydroxytyrosol were purchased from Extrasyntese (Genay, France). Sodium acetate trihydrate from Carlo Ebra Reactifs – SDS (Val de Reuil Cedex). Rutin from Sigma (St. Louis, USA).

Olive leaves were milled and separated in three fractions by sieves. The fractions were treated (steam and hot water blanching as well as UV irradiation) and were subsequently extracted with ethanol. Then, the oleuropein content and the antioxidant activity of ethanolic extracts of all treated fractions were determined. The results were used for the optimisation of steam blanching as well as for the evaluation of impact of hot water blanching and UV irradiation on the extractability and the stability of oleuropein and related polyphenols in olive leaves.

2.2. Samples

In November 2010, olive leaves were collected, cut and separated by sieves in three fractions with particle sizes: 1 (>20 mm; non-cut), 2 (20–11 mm) and 3 (3–1 mm).

2.3. Treatment of olive leaves fractions

2.3.1. Steam blanching

Every fraction of olive leaves was separately exposed to steam for 0 (control), 5, 10 and 20 min, at atmospheric pressure, produced by a household steam cooker (total volume 10.5 l) consisted of 3 layers of removable grids. The proportion of olive leaves exposed to steam was 1/10 (w/v). Afterwards, they were cooled down by cold water at 17 °C. The excess water was removed by an absorbent paper and subsequently, the fractions were dried in an air oven dryer for 4 h at 60 °C and at air velocity of 2 m/s.

2.3.2. Hot water blanching

All the fractions were separately immersed in boiling water (olive leaves/water; 1/10 w/v) for 2 min and then, they were cooled down by cold water at 17 °C. Excess water was removed by an absorbent paper and all the fractions were dried as described in Section 2.3.1.

2.3.3. UV-light

All the fractions were treated with a 30 W UV-C lamp (peak at 253.7 nm) for 24 h. The distance between the samples and the lamp was 40 cm. After treatment, the leaves were dried as described in Section 2.3.1.

2.4. Extraction of olive leaves

2.4.1. Extraction of olive leaves for full factorial design experiments

Prior to extraction, all the treated and dried fractions were ground and passed through a 1 mm sieve. One gramme of each dried, milled and sieved fraction of olive leaves and 8 ml of extractant (70:30 v/v, ethanol–water mixture) were placed in a vial and they were stirred with vortex for 5 min. All ethanolic extracts were filtered through 0.45-μm syringe filters and they were analysed by reversed-phase HPLC-DAD.

2.4.2. Extraction of olive leaves at optimum conditions of steam blanching process

Fresh olive leaves were treated at the optimum conditions of steam blanching process (10 min steam blanching of 20–11 mm olive leaves fraction). Afterwards, steam blanched OL were dried as described in Section 2.3.1. Then, a quantity of 10 g of steamed and non-steamed, dried, milled and sieved olive leaves were separately placed in a beaker and were stirred at 800 rpm with 80 ml 70% (v/v) ethanol/water at 40 °C for 30 min. Subsequently, the samples were centrifuged (5000 rpm, 10 min) and the pellets were re-extracted at the same conditions. Then, the extracts of two step extraction were collected and passed through 0.45-μm syringe filters and they were analysed by reversed-phase HPLC-DAD. Moreover, antioxidant activity of extracts of steam blanched and non-steam blanched olive leaves were measured as described in Section 2.6.

2.5. Chromatographic conditions

The equipment utilised was a HITACHI coupled to an autosampler L-2200, pump L-2130, column oven L-2300 and diode array detector L-2455 and controlled by Agilent EZChrom Elite software. The column was a Pinnacle II RP C_{18}, 3 μm, 150 × 4.6 mm (Restek), protected by a Kromasil 100–5 C_{18} guard cartridge starter kit for 3.0/4.6 mm id. Column oven was set at 40 °C. Eluent (A) and (B) were 0.02 M sodium acetate adjusted at pH 3.2 with acetic acid and pure acetonitrile, respectively. The flow rate was 1 ml/min. The elution gradient profile was as follows: started (A) 90%; 2 min, 85%; 9 min, 75%; 12 min, 65%; 15 min, 55%; 18 min, 40%; 20 min, 90%. The elute was monitored at 280 nm for oleuropein, hydroxytyrosol and tyrosol and at 355 nm for flavonols.

2.6. Determination of antioxidant activity

Antiradical activity was performed by using 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) assay according to Braca et al. (2001), with some modifications. About 2.5 mg of DPPH powder was diluted in 100 ml pure methanol with absorption 0.7 (±0.03) at 517 nm. Ethanolic extracts of steamed samples of Section 2.4.1 were diluted 50 times and those of Section 2.4.2 were diluted 80 times, while hot water blanched and UV-irradiated samples were diluted 10 times. An aliquot of 1 ml of 0.004% DPPH solution was added in cuvette with 330 μl of diluted Ol extract. As control, 330 μl of pure ethanol was added instead of olive leaves extract. The reaction mixture was vortex-mixed and was let to stand in the dark at room temperature for 30 min before measuring the decrease in absorbance at 517 nm. Spectrophotometer was calibrated with pure methanol. Antioxidant activity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation:

$$AA(\%) = [(A_0 - A_i)/A_0] \times 100$$

A_0 and A_i are the absorbance of control sample and the sample containing olive leave extract, respectively.