



Phenolic content and antioxidant activity of olive by-products and antioxidant film containing olive leaf extract



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ABSTRACT

The antioxidant activity of olive leaf (OL) and cake (OC) extracts with different solvents was evaluated. 70% of aqueous ethanol extract of OL was chosen as the most antioxidant extract based on antiradical activity (DPPH) ($95.4 \pm 0.3\%$) and oxygen radical absorbance capacity (ORAC) (0.82 ± 0.07 g equivalent Trolox per g of solution) assays. This OL extract was incorporated in two multilayer materials consisting of (i) polyethylene/polyethylene (PE/PE) film and (ii) polyethylene/paper (PE/P). These multilayers were exposed to a gas stream enriched in free radicals to evaluate the scavenging capacity of both materials. PE/PE film exhibited the highest scavenging activity of free radicals (78.8%). Migration of the phenolic compounds from olive by-products into two simulants was performed and demonstrated a non-migrating behavior. The limits of detection and quantification for oleuropein were $0.5 \mu\text{g kg}^{-1}$ and $1.7 \mu\text{g kg}^{-1}$ and for Luteolin-7-O-glucoside $1.3 \mu\text{g kg}^{-1}$ and $4.3 \mu\text{g kg}^{-1}$ respectively.

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1. Introduction

Dietary lipids naturally occurring in raw food materials or added during food processing play an important role in food nutrition. Lipid oxidation is the most important cause of the food quality deterioration, the destruction of valuable nutrients and the generation of toxic compounds. During the last decades, the lipid oxidation has been considered as an important challenge for manufacturers and researchers to avoid the food degradation (Kanner & Rosenthal, 1992; Shahidi & Zhong, 2005). A wide variety of organic molecules are susceptible to chemical attack by oxygen and among them more attention has been paid to lipids due to the importance of their oxidative damage (McClements & Decker, 2000; Shahidi & Zhong, 2005; Waraho, McClement, & Decker, 2011). Synthetic antioxidants are frequently used to stabilize fats, oils, and lipids containing foods, and these antioxidants are also included in polymer processing to improve the properties of these materials (Byun, Kim, & Whiteside, 2010; Wanasundara & Shahidi, 2005). However, the use of some synthetic antioxidants in food has been questioned by the scientific community (Gomez-Estaca, Lopez-de-Dicastillo, Hernandez-Munoz, Catalá, & Gavara, 2014; Wanasundara & Shahidi, 2005) because of the potential toxicity over the foodstuff.

Nowadays, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are limited by Codex Alimentarius (FAO/WHO Food Standards, 2005) as well as by European Regulation (Directive 2006/52/EC, 2006) and FDA Food Additive Status List (US Food and Drug Administration, 2006). The alternative approach is the use of natural antioxidants, such as tocopherol, plant extracts, and essential oils from herbs and spices. These natural compounds and mixtures have been used for producing antioxidant packaging materials (Akrami et al., 2015; Laguerre, López-Giraldó, Lecomte, Pina, & Villeneuve, 2007; Li, Miao, Wu, Chen, & Zhang, 2014; Licciardello, Wittenauer, Saengerlaub, Reinelt, & Stramm, 2015; Marcos et al., 2014; Pereira de Abreu, Losada, Maroto, & Cruz, 2010; Pezo, Salafraña, & Nerín, 2008). As previously reported, the active films containing natural antioxidants efficiently enhanced the stability of both myoglobin and fresh meat against oxidation processes (Nerín, Tovar, & Salafraña, 2008; Nerín et al., 2006). These evidences accelerated the search of natural antioxidants, which led to the identification of natural resources and isolation of active antioxidant molecules (Katalinic, Milos, Kulisic, & Jukic, 2006).

Olive oil industry generates a large amount of by-products such as crude olive cake, vegetation water, twigs and leaves (10% of the total weight of the olives). Olive leaf is also one of the by-products of olive grove farming, which accumulate during pruning of olive

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trees (Guinda, Albi, Perez Camino, & Lanzon, 2004). Olive leaf and cakes are always used as animal feed but they can be used in other applications with higher added value such as cosmetic, therapeutic and food industries.

Many studies have been published about the antioxidant effects of olive leaf and cake extracts due to their phenolic composition, oleuropein, luteolin and hydroxytyrosol among others (Bouaziz, Fki, Jemai, Ayadi, & Sayadi, 2008). These bioactive compounds can be incorporated into edible films or food packaging materials to maximize their additional properties such as their antioxidant capacities (Licciardello et al., 2015; Marcos et al., 2014).

The purpose of this study was: (1) to evaluate the antioxidant capacity of several extracts of olive leaf and cakes with different solvents and to identify the main phenolic compounds responsible for the antioxidant activity of these extracts. (2) To select the best antioxidant extract and to incorporate it into a packaging material. (3) To evaluate the efficiency of the new packaging as radical scavenger. (4) To study the migration behavior from the active packaging material containing olive leaf extract.

2. Materials and methods

2.1. Chemicals

DPPH (2,2-Diphenyl-1-picrylhydrazyl, CAS 1898-66-4) was provided by Sigma-Aldrich (Germany). Methanol (high-performance liquid chromatography (HPLC) grade) CAS 67-56-1, acetone (high-performance liquid chromatography (HPLC) grade) CAS 67-64-1 and ethanol (high-performance liquid chromatography (HPLC) grade) CAS 64-17-5 were provided by Scharlab (Mollet del Vallés, Spain). Ultrapure water was obtained from a Millipore Milli-QPLUS 185 system (Madrid, Spain). DPPH solution was freshly prepared in methanol and was stored in hermetically sealed amber glass bottle.

AAPH (2,2'-azobis (2methylpropionamide) dihydrochloride; 97%, CAS 2997-92-4); fluorescein (3,6'-dihydroxypirolo [isobenzofuran-1[3H],9'[9H]-xanthen]-3-one; Standard Fluka, CAS 518-47-8); and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; 98%, CAS 258-422-8) were purchased from Sigma Aldrich Química S.A. (Madrid, Spain). Disodium hydrogen phosphate dehydrate (99.5%, CAS 100028-24-7) and sodium dihydrogen phosphate hydrate (99%, CAS 7558-80-7) were supplied by Merck (Madrid, Spain).

Hydrogen peroxide (>50%, CAS 7722-84-1) were purchased from Scharlab, Sodium salicylate (>99.5%, CAS 54-21-7) and 2, 5-dihydroxybenzoic acid (>99%, CAS 490-79-9) were supplied by Sigma-Aldrich Química S.A. Orthophosphoric acid (85%, reagent grade CAS 7664-38-2) and sodium hydroxide (0.01 mol L⁻¹ CAS 1310-73-2) were purchased from Scharlab.

Luteolin-7-O-Glucoside (LUT-7-O-G) (>98% CAS 5373-11-5), and Oleuropein (OLE) (>98% CAS 32619-42-4), were all supplied by sigma Aldrich Química S.A.

All the working solutions for the oxygen radical absorbance capacity ORAC test were prepared in sodium phosphate buffer (75 mM, pH 7.0). A stock solution of fluorescein (100 µg/g) was prepared in phosphate buffer (PBS) (75 mM, pH 7.0), and then stored in complete darkness under refrigeration conditions. The working solution (2.3 µg/g) was prepared daily by dilution of the stock solution in phosphate buffer. The AAPH radical (34.4 mg/g) was daily prepared. A stock solution of Trolox (1000 µg/g) was prepared when necessary, to carryout the external calibration.

2.2. Plant material

Olive leave samples (*Olea europaea*, L var. Chemlal) were collected after fruit harvesting during December 2012 from an olive

tree located in EL Kseur, Bejaia (Algeria). Olive cake was supplied by a local olive oil mill. Collected leaves and cakes were air dried, grounded and sieved (Retsch Analytical sieve shaker AS 200) to pass through a 500 µm sieve, and then were defatted by soxhlet with hexane (boiling point 68.5 °C).

2.3. Sample treatment

Several extraction solvents such as ethanol and acetone (both at 50% and 70%) and distilled water (DW) were used. The extraction was performed according to previously described procedure (Oomah, Blanchard, & Balasubramanian, 2008). After 2 h extraction time by magnetic stirring, the extracts were filtered and centrifuged for 30 min at 11,000g. In order to obtain a dried extract, the extraction solvent (acetone, ethanol) was removed by using a rotary evaporator at (40 °C) with a 60 rpm rotation under vacuum. Then, solvent free extracts were dried by using a freeze drier system (ALPHA 1-2 LD plus) at -65 °C and 0.044 mbar. The DW extracts were directly dried by using the freeze drier system. The powder extracts were stored in light protected glass flasks until further use.

For the chromatographic study of phenolic compounds of olive leaves and cakes, the extract of *Olea Europaea* was dissolved in the initial conditions of the extraction. These solutions were filtered through a 0.22 µm nylon syringe filter.

For the ORAC tests the extracts were dissolved in methanol (500 µg/g), diluted with sodium phosphate buffer, and filtered using 0.22 µm nylon syringe filter.

For the free radicals test, the samples were prepared according to the procedure described by Pezo, Salafranca, and Nerín (2006), Pezo et al. (2008). The active films were used to prepare plastic bags with internal dimensions of 15 cm × 15 cm by thermo sealing with an impulse sealer (PFS-200, Zhejiang Dongfeng Packing Machine Co., Wenzhou, Zhejiang, China).

2.4. Antioxidant packaging material

Two different materials were studied: A multilayer film composed by 2 film layers of PE (polyethylene) glue with the proper adhesive, and a second multilayer composed by PE and paper (P) also glue with adhesive.

Two different concentrations 1% and 2% (m/m) of the extract were incorporated in the adhesive formula used for PE/P while five different concentrations 1%, 2%, 3%, 5% and 10% (m/m) were used for the PE/PE. The production of multilayer films was as follows: The OL extract was incorporated into an aqueous dispersion adhesive formula, where the components of OL were anchored. This adhesive was then used to glue two different layers of PE-PE in such a way that the OL components were sandwiched in the middle of the two plastic layers. The production was made at laboratory scale using a coating machine K control coater, RK print. Similar procedure was used for building the laminate PE/P.

Using this active adhesive the multilayers above described were built. As usual, the adhesive was sprayed on the whole surface before applying the second layer. Details about the adhesive formula cannot be disclosed here for confidentiality reason.

2.5. UPLC-MS-QTOF analysis for identification and quantification of polyphenols in extracts

UPLC used was an Acquity system coupled to an ESI probe to a Xevo G2 QTOF (Time-of-flight mass spectrometer) supplied by Waters (Milford, MA, USA). A UPLC BEH C18 column of 1.7 µm particle size (2.1 × 100 mm) also from Waters (Milford, MA, USA) was used for identification of the compounds. Injection volume was 10 µL. Chromatography was carried out at 0.4 mL min⁻¹ column

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