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Optimal harvesting period for cvs. Madural and Verdeal Transmontana, based on antioxidant potential and phenolic composition of olives

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ABSTRACT

In the present study we propose to determine an approximate optimum harvesting period for table olives and olive oil of two Portuguese olive cultivars (Madural and Verdeal Transmontana) based on phenolic modifications (HPLC/DAD) and antioxidant activity (scavenging capacity on 2,2-diphenyl-1-picrylhydrazyl and reducing power). Samples were collected from almost edibility to slightly over-mature. The sum of polyphenols, as well as its most abundant components oleuropein and hydroxytyrosol, decreased during this maturation period, more intensively in Madural than Verdeal Transmontana. In their green stages an antioxidant potential loss was gradually observed in both olive cultivars, while in the latter purple-black phases a slight increase in the antioxidant activity was observed. Both phenolic profile and antioxidant activity were highly correlated with the maturation process. A principal component analysis showed the particular effect of maturation in both olive cultivars.

Based on the acquired knowledge we can advance that, for these cultivars and geographical region, olives harvest for table olives, traditionally collected sooner, can be performed in the middle of September. For olive oil harvesting can occur in the first days of November, giving priority to cv. Madural rather than Verdeal Transmontana, in order to enhance the bioactivity, phenolic composition and stability of olive oils.

1. Introduction

Olive products are increasingly popular worldwide, not only for their unique sensorial characteristics but also for the beneficial health effects associated with their consumption, particularly within the Mediterranean diet. An array of olive components have been linked to its beneficial properties: a balanced fatty acid profile, sterols, tocopherols, pigments like chlorophylls and carotenoids, and a very important group of components - the phenolic compounds. Indeed, several biological functions and properties are ascribed to phenolic compounds, particularly within olive products. Apart from their natural roles in plant chemical defense mechanism, as common to other species, they are particularly important for the olive products sensorial attributes, particularly oleuropein for its bitterness (Andrews, Busch, Joode, Groenewegen, & Alexandre, 2003), being also associated with other positive sensorial attributes, such as the spicy, pungency and bitter ones (Dierkes et al., 2012). Obied et al. (2012) reviewed the pharmacology of olives biophenols and discussed their antioxidant, anti-inflammatory, cardiovascular, immunomodulatory, gastrointestinal, endocrine, respiratory, autonomic, central nervous system, antimicrobial, chemotherapeutic, anticancer and chemopreventive effects/properties. Based on these potential benefits, olive products phenolic compounds should be maximized, with careful attention to keep a balanced sensorial profile for consumer's acceptability.

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Several aspects are known to influence olives phenolic composition, with direct repercussions on its derived products, in particular: i) olive cultivar (Malheiro, Sousa, Casal, Bento, & Pereira, 2011); ii) geographical origin (Vinha et al., 2005); iii) agricultural practices (Tovar, Motilva, & Romero et al., 2001); and iv) maturation process (Bouaziz, Chamkha, & Sayadi, 2004; Morelló, Romero, & Motilva, 2004; Ryan, Robards, & Lavee, 1999). The maturation process assumes a special importance when high quality olives are intended for future processing. During olives maturation a series of metabolic and enzymatic reactions prompts a decrease in many phenolic compounds. Indeed, advanced maturation results in a clear reduction of positive sensorial attributes and oxidative stability due to the decline on photosynthetic pigments (chlorophylls and carotenoids) and phenolic compounds (Morelló et al., 2004), directly influencing olive products quality. Several studies devoted to the study of phenolic composition of olives during maturation indicate that phenols content increases progressively during the so-called green-phase, corresponding to the fruit growth period. When olives are purple and black the phenols content decrease sharply (Morelló et al., 2004).

Madural, Verdeal Transmontana, and Cobrançosa, are the main cultivars used for the production of the Protected Designation of Origin (PDO) "Azeite de Trás-os-Montes" olive oil, in Northeast of Portugal. These cultivars account for more than 90% of olives cultivation area in this region and are also cultivated in others olive producing regions of Portugal. There is a lack of information on the chemical characteristics of Madural and Verdeal Transmontana olives as regards to antioxidant capacity and phenolic composition throughout maturation. The aim of this investigation is to study the effect of the maturation process in the phenolic profile and biological properties of the olive fruit, particularly its antioxidant potential, in order to maximize olive products quality and biological properties, being, for the author's knowledge, the first report of this kind in these two olive cultivars.

2. Material and methods

2.1. Reagents and standards

Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and iron (III) chloride were obtained from Sigma–Aldrich (St. Louis, USA). Methanol (HPLC grade), sodium dihydrogen phosphate dihydrate, potassium hexacyanoferrate (III), formic acid (98–100%) were purchased from Merck (Darmstadt, Germany). Hydrochloric acid and di-sodium hydrogen phosphate dihydrate were obtained from Panreac (Barcelona, Spain). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA). Hydroxytyrosol, chlorogenic acid, verbascoside, oleuropein, rutin, apigenin 7-*O*-glucoside and luteolin standards, used for phenolic profile identification were obtained from Extrasynthèse (Genay, France).

2.2. Sampling

Five representative olive trees from cvs. Madural and Verdeal Transmontana were selected in an olive grove at Paradela, Mirandela (Northeast of Portugal), in 2009. Olive grove characteristics: 3 ha; planting density of 7 × 7 m; trees more than 40 years old; pruned every three years; rain-fed; soil tilled 2–3 times/year. Five sampling dates (29th September, 13th and 27th October, and 10th and 18th November) were chosen to monitor the maturation process, corresponding to potentially edible olives from slightly green to over-mature ones. From each tree and sampling date olives were handpicked (1 kg). Samples were divided in two parts, one part used for maturation index estimation and moisture content (oven drying at 105 °C), and the remaining olives depulped, frozen at −20 °C and freeze-dried (Ly-8-FM-ULE, Snijders) for subsequent chemical analysis. Maturation index (MI) was determined on each olive cultivar and sampling date as described by Hermoso, Uceda, Frias, and Beltrán (2001).

2.3. Identification and quantification of phenolic compounds

2.3.1. Extraction procedure

For each olives sample, three powdered pulp fruit sub samples (~1.5 g; sieve size 0.841 mm) were extracted by stirring with 50 mL of methanol, for 1 h at 150 rpm, and filtered through Whatman N°. 4 paper. The residue was re-extracted similarly with three additional 50 mL portions of methanol. The combined methanolic extracts were vacuum-evaporated (Stuart RE3000, United Kingdom) at 35 °C, redissolved in methanol, filtered through a 0.2 μm Nylon membrane (Whatman) and analyzed by HPLC. Previous tests with hydro-methanolic and water extracts were also assayed according to the above extraction conditions. Once methanolic extract profile comprises more phenolic compounds of several different polarities than the others, it was chosen for the quantification purposes.

2.3.2. Chromatographic conditions

Phenolic profile was performed by HPLC analysis on a Knauer Smartline separation module equipped with a Knauer smartline autosampler 3800 (with a cooling system set to 4 °C) and a Knauer DAD detector 2800. A reversed-phase Spherisorb ODS2 column was used (250 mm × 4 mm I.D., 5 μm particle diameter, end-capped Nucleosil C18 (Macherey–Nagel)) and its temperature was maintained at 30 °C. The solvent system used was a 66 min gradient program of formic acid/water (50 mL/L) (A) and methanol (B) at 0.9 mL/min (Vinha et al. 2005). Spectral data from all peaks were accumulated in the 200–600 nm range. Phenolic compounds quantification was performed at 280 nm and achieved by external standard calibration curves using authentic standards.

2.4. Antioxidant activity

2.4.1. Extraction procedure

For each sample, three freeze dried powdered sub-samples (~5 g; sieve size 0.841 mm) were extracted with 250 mL of water, under boiling for 45 min, and filtered through Whatman N°. 4 paper (Malheiro et al. 2011). The aqueous extracts were frozen, lyophilized, and weighed. From the dry extract, aqueous solutions ranging from 0.01 to 3 g/L were prepared for antioxidant activity assays.

2.4.2. Scavenging activity assay

The capacity to scavenge DPPH free radicals was monitored according to the method of Hatano, Kagawa, Yasuhara, and Okuda (1988) with modifications. The extract solution (0.3 mL) was mixed with 2.7 mL of methanolic DPPH radicals (6×10^{-5} mol/L) solution. The mixture was shaken vigorously, monitoring continuously the absorbance decrease at 517 nm, read against a blank, until stable absorbance values were obtained. DPPH scavenging effect was calculated as a percentage of DPPH discoloration using the following equation: % scavenging effect $= [(A_{DPPH}\text{-}A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (EC_{50}) was calculated and converted to pulp mass based on the extract weight at 2.4.1.

2.4.3. Reducing power assay

The reducing power was determined according to the method of Berker, Güçlü, Tor, and Apak (2007). The extract solution (1 mL) was