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Phenolic compounds of 'Galega Vulgar' and 'Cobrançosa' olive oils along early ripening stages

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ABSTRACT

In this study, the lipophilic and hydrophilic phenol composition of virgin olive oils (VOO) obtained from olives from two of the most important Portuguese cultivars ('Galega Vulgar' and 'Cobrançosa'), harvested at different ripening stages and under two irrigation schemes (rain fed and irrigated), was evaluated. Phenolic alcohols (hydroxytyrosol and tyrosol), phenolic acids and derivatives and flavonoids (luteolin and apigenin), as well as tocopherols were quantified. Lipophilic (>300 mg kg^{-1}) and hydrophilic phenols (>600 mg kg^{-1}) were present in high contents in both VOO, for early ripening stages. Gamma-tocopherol content is higher in 'Galega Vulgar' VOO. Total phenols showed a decrease between ripening index 2.5 and 3.5. The dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), also known as oleacein, was the major phenolic compound identified in both oils. The concentration of free hydroxytyrosol and tyrosol in both VOO is very low while their esterified derivatives, like 3,4-DHPEA-EDA and *p*-HPEA-EDA, are much more abundant.

1. Introduction

The benefits of consuming olive oil were traditionally attributed to its high content in oleic acid (Gurr, 2000). However, now it is well known that these benefits may also be ascribed to the phenol compounds of extra virgin olive oil (EVOO) due to their antioxidant, anti-inflammatory and anti-microbial activities. For some activities of EVOO phenolic compounds, the scientific evidence is already strong enough to enable the legal use of health claims on labelling (Martín-Peláez, Covas, Fitó, Kušar, & Pravst, 2013).

Lipophilic and hydrophilic phenols are the most important antioxidants in EVOO. Lipophilic phenols in EVOO are tocopherols, which are molecules with a chroman head (with one phenolic and one heterocyclic ring) and a phytyl tail. The different tocopherols vary in the number of methyl substituents and the patterns of substitution in the phenolic ring. Among them, α-tocopherol is the most abundant (90%) but β- and γ-tocopherols are also present (Beltrán et al., 2010). Claims have been made for the preventive activity of tocopherols against reactive oxygen species (ROS) in biological systems, namely their positive effect on cell aging, some cancer types, immune system maintenance and cardiovascular diseases (Bramley et al., 2000). Moreover, apart from their action as lipid radical scavengers, they also inhibit the photooxidation by reacting with singlet oxygen. Variability in tocopherol contents by crop year is explained by the rainfall levels, showing that oils from drier crop years have higher tocopherol content, in spite of a cultivar-dependent effect (Beltrán et al., 2010). However, the content of tocopherols in virgin olive oils (VOO) is relatively low when compared with several seed oils. In fact, hydrophilic phenols

Abbreviations: ACVA, vanillic acid; APG, apigenin; 3,4-DHPEA, hydroxytyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol or oleacein; 3,4-DHPEA-EA, oleuropein aglycone; EA, elenolic acid; EAME, elenolic acid methyl ester; EVOO, Extra Virgin Olive Oil; HYT, hydroxytyrosol; IR, irrigated; LUT, luteolin; MUFA, monounsaturated fatty acids; *p*-HPEA, tyrosol; *p*-HPEA-EA, aldehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol or oleocanthal; OCUM, *o*-Coumaric acid; PCUM, *p*-Coumaric acid; PDO, Protected Designations of Origin; POD, peroxidases; PPO, polyphenol oxidase; PUFA, polyunsaturated fatty acids; PVP, polyvinylpyrrolidone; RF, rain fed; RI, ripening index; SFA, saturated fatty acids; TYR, tyrosol; VAN, Vanillin; VOO, virgin olive oil.

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are the compounds that most differentiate EVOO from other vegetable oils. The most important phenolic compounds that have been identified in olive oil are phenolic alcohols (hydroxytyrosol (HYT) and tyrosol (TYR)), secoiridoid derivatives, such as the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) (oleacein), the dialdehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EDA) (oleocanthal), the aldehydic form of elenolic acid linked to tyrosol (p-HPEA-EA), 4-(acetoxyethyl)-1, 2-dihydroxybenzene (3,4-DHPEA-AC), oleuropein aglycone (3,4-DHPEA-EA) and its methylated form (methyl 3,4-DHPEA-EA), phenolic acids and derivatives (such as vanillic acid and vanillin, respectively), lignans (pinoresinol and acetoxypinoresinol) and flavonoids such as luteolin and apigenin (Bendini et al., 2007; Kanakis et al., 2013; Servili & Montedoro, 2002).

Olive oil phenol composition is quite different from that of the olive drupe and of the olive paste (Kanakis et al., 2013). In contrast to olive fruits, olive oil contains neither anthocyanins nor flavonols. During the extraction process, the glycosidic oleuropein, dimethyloleuropein and ligstroside are hydrolyzed by endogenous β-glucosidases to form aldehydic aglycones. The aglycones become soluble in the oil phase, whereas the glycosides remain in the water phase (Servili & Montedoro, 2002). The main source of lignans was demonstrated to be the stone and not the pulp (Oliveras López et al., 2008).

EVOO phenolic compounds play also an important role in organoleptic properties namely in attributes related to bitterness and pungency (Peyrot des Gachons et al., 2011). The phenolic compounds 3,4-DHPEA-EA, *p*-HPEA-EA, 3,4-DHPEA-EDA, *p*-HPEA-EDA, elenolic acid (EA), and elenolic acid methyl ester (EAME) showed high correlations with bitterness and pungency (Dierkes et al., 2012). Moreover, oleocanthal causes a pungency perceived as an unusual irritation in the pharynx, consequence of both the specificity of this molecule for a single sensory receptor and the anatomical restriction of this sensory receptor to the pharynx (Peyrot des Gachons et al., 2011).

Olive endogenous enzymes such as oxidoreductases, polyphenol oxidase (PPO) and peroxidase (POD), which oxidize phenolic compounds may be a biochemical factor affecting the phenol content of VOO (García-Rodríguez, Romero-Segura, Sanz, Sánchez-Ortiz, & Pérez, 2011; Hbaieb et al., 2015).

The ripening stage of olives has a high impact on the oil's yield, quality, stability and sensory characteristics. Irrigation also plays an important role in the productivity of olives and consequently in fruit ripening, and therefore in phenol and volatile composition (Gómez-Rico, Salvador, & Fregapane, 2009). Moreover, when early frosts occur, oils extracted from frosted fruits develop sensory defects (Guillaume, Ravetti, & Gwyn, 2010). So, in the last years a lot of attention has been drawn to the main changes on the characteristics of olives and olive oils along fruit ripening, in order to decide the best harvest time (Dag, Harlev, Lavee, Zipori, & Kerem, 2014; Jiménez, Sánchez-Ortiz, Lorenzo, & Rivas, 2013).

Early ripening has been a recommendation in the center of Portugal (Beira Baixa) for organic olive growing. The predominance of 'Galega Vulgar' cv., which is highly susceptible to pests and diseases, is the main reason for this procedure (Peres et al., 2010). However early ripening corresponds to lower yields, so it is crucial to determine how early the harvest can be, in order to have good quality, high nutritional value and sensory scores and a reasonable yield.

The aim of the present study was to investigate the effect of early harvest corresponding to olive ripening index lower than 4.5, on phenol compound levels in virgin olive oils from 'Galega Vulgar' and 'Cobrançosa' fruits, two of the most important Portuguese cultivars for olive oil extraction, grown in rainfed or irrigated orchards.

2. Materials and methods

2.1. Olives Characterization

Portuguese olive fruits (*Olea europaea* L.) of 'Cobrançosa' and 'Galega Vulgar' cultivars used in this study were produced according to the Integrated Production rules, in Beira Baixa Region (Centre-Interior of Portugal), in two types of farming: rainfed orchard (RF) (39° 49′N, 7° 27′W) and irrigated orchards (IR) (39° 50′N, 7° 42′W). 'Galega Vulgar' orchards have 100–123 trees/ha while 'Cobrançosa' orchards have 200–300 trees/ha. For the irrigated orchards, the irrigation drip system was performed as a function of soil moisture and meteorological conditions and controlled by weekly soil water balance. From measurements of soil moisture, a maximum irrigation quantity was determined as the difference between field capacity and the actual soil water content. This maximum value was then taken as an indication for deciding about the amount of water to be supplied by irrigation. Olive fruits were picked from the beginning of October till the second fortnight of November. The annual accumulated precipitation of the year under study was 737.5 mm, which was very similar to the values reported for the period 1981–2010 in this region (783.2 mm). Their ripening indices (RI) were determined following the guidelines of Estación de Olivicultura y Elaiotecnia, Jaén, Spain (Hermoso, Uceda, Frias, & Beltran, 1997); moisture and fat content (by Soxtec) of the fruits were also evaluated. Only healthy fruits were selected for fruit characterization and for olive oil extraction.

2.2. Enzymatic activity assays

Fruits were destoned with a manual destoner and the kernel was cut with a pipe cutter and the seed removed. Extracts were prepared by homogenizing olive pulp and seeds with cold acetone (−20 °C) in an ultraturrax homogeneizer (2 min), followed by filtration in fiber glass filters, washing the pellet with cold acetone (−20 °C) until total removal of pigments, and by drying samples at room temperature with N_2 (Saraiva, Nunes, & Coimbra, 2007). For enzymatic assay, 0.4 g of acetone powder were suspended in 5 mL of extraction buffer (0.05 M potassium phosphate, pH 6.2 containing 1 M KCl) (Servili et al., 2007) and 2% (w/w) of PVP and stirred for 30 min, 4 °C, 400 rpm; the suspension was centrifuged at 12,000 rpm for 30 min and filtered (0.45 μm). PPO activity was evaluated using catechol (30 mM) as substrate, following the increase in absorbance at 420 nm, during 1 min (Oktay, Kufrevioglu, Kocaçaliskan, & Sakiroglu, 1995). One unit of PPO was defined as the quantity of enzyme that causes the absorbance variation of 0.001 min^{-1} mL^{-1} of enzyme extract, at room temperature. Results were expressed as Ug^{-1} FW (fresh weight).

POD activity was performed following the increase in absorbance at 470 nm (2 min) using 30 mM guaiacol and 4 mM H_2O_2 as substrates (Gajewska, Skłodowska, Słaba, & Mazur, 2006). One unit of POD was defined as the consumption of 1 μmol of guaiacol min^{-1} mL^{-1} of enzyme extract, at room temperature using a molar absorptivity (ε) for tetraguaiacol of 26.6 mM^{-1} cm^{-1}. Results were expressed as Ug^{-1} FW.

2.3. Olive oil extraction

Olive oils were extracted in a laboratory oil extraction system (Abencor analyser; MC2 Ingenieria y Sistemas S.L., Seville, Spain) under optimized conditions (Peres, Martins, & Ferreira-Dias, 2014). The olives were crushed with a hammer mill equipped with a 4 mm sieve at 3000 rpm. Malaxation of the pastes was performed at 27–30 °C, during 30 min, and centrifugation at 3500 rpm for 3 min. After centrifugation, the olive oil was separated by settling