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Effect of the composition of extra virgin olive oils on the differentiation and antioxidant capacities of twelve monovarietals

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ABSTRACT

The effect of the composition of twelve varieties of extra virgin olive oils (EVOOs) on their differentiation based in agronomic criteria and on the antioxidant capacity was studied. Principal component analysis permitted an overview of the samples and their compositions, showing evidence of grouping and correlation between antioxidant capacity, oleuropein and ligstroside derivatives (OLD) and specific extinction at 270. Oleic and linoleic acids, 3,4-DHPEA-EA and p-HPEA-EDA (OLD), unsaturated/saturated ratio and induction time (IT) allowed the correct classification of samples according to year of harvest, ripening stage and variety. The antioxidant capacity of EVOOs was satisfactory predicted through a partial least square model based on ΔK, hydroxytyrosol, pinoresinol, oleuropein derivate and IT. Validation of the model gave a correlation $R > 0.83$ and an error of 7% for independent samples. This model could be a useful tool for the olive industry to highlight the nutritional quality of EVOOs and improve their marketing.

1. Introduction

Adherence to the Mediterranean diet has been associated with longevity and with a reduced risk of morbidity and mortality. This has been attributed to diverse diet components that are thought to be associated with protective health effects. In this context, extra virgin olive oil (EVOO) plays an important role as the main source of fats in the diet (Servili et al., 2014).

There is ample scientific evidence showing that modulation of dietary fat composition affects blood-lipid concentrations. Regarding oleic acid, the main monounsaturated fatty acid in EVOO, the most noticeable effects have been demonstrated in studies where the substitution of saturated fat with oleic acid was tested. The isocaloric replacement of approximately 5% of the energy from saturated fatty acids by oleic acid has been estimated to reduce coronary heart disease risk by 20–40%, mainly via low-density lipoprotein (LDL)-cholesterol reduction. Other beneficial effects on risk factors for cardiovascular disease, such as factors related to thrombogenesis, in vitro LDL oxidative susceptibility and insulin sensitivity, have also been reported (Lopez-Huertas, 2010).

Several studies carried out in the last year have demonstrated that the beneficial effects should also be attributed to the olive phenols. The study of EVOO phenolic compounds has established that these substances show many health benefits, including the reduction of the risk factors of coronary heart disease, the prevention of several chronic diseases (for example, atherosclerosis), cancer, chronic inflammation, strokes and other degenerative diseases (Casaburi et al., 2013; Cicerale, Lucas, & Keast, 2012; López-Miranda et al., 2010; Servili et al., 2014).

EVOO presents a major fraction of triacylglycerides (oleic acid being the main fatty acid), representing more than 98% of the total weight; a minor fraction (approximately 2% of the weight) is composed of a complex set of compounds, including over 230 chemical compounds (aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds, phenols and pigments) (Barjol, 2013).

The main antioxidants in EVOO are represented by lipophilic and hydrophilic phenols, with the presence of a small amount of carotenoids. Alpha-tocopherol, a lipophilic phenol and primary antioxidant, is the main tocopherol in EVOO, with a wide concentration range (23–751 mg/kg) (Servili et al., 2014). Phenols, secondary plant metabolites, are the main antioxidant in EVOO and constitute a complex matrix of compounds where oleuropein and ligstroside derivatives (OLD) are the most abundant in many varietals. The concentration of

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lipophilic and hydrophilic phenols is variable in the oils and depends on agronomic and climatic factors (Romero, Saavedra, Tapia, Sepúlveda, & Aparicio, 2015).

Antioxidants present in EVOO delay its autoxidation by inhibiting the formation of free radicals or by interrupting the propagation of free radicals by several mechanisms. The most effective antioxidants are those that interrupt the free radical chain reaction (Augusto, Dillenburg, De Souza, & Teixeira, 2015; Brewer, 2011). Methods commonly used to determine the total antioxidant capacity fall into two major groups: assays based on a single-electron transfer (SET), monitored through a change in colour as the oxidant is reduced (the degree of colour change is correlated with the sample's antioxidant concentrations), and assays based on a hydrogen atom transfer reaction (HAT), where the antioxidant and the substrate (probe) compete for the free radical. Among SET methods are the total phenols assay by Folin−Ciocalteu reagent (FCR) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging capacity assay. The most employed HAT method is the oxygen radical absorbance capacity (ORAC) assay (Augusto et al., 2015; Huang, Ou, & Prior, 2005).

Several studies have related EVOO phenols composition with the EVOO antioxidant capacity and oxidative stability (Angelino et al., 2011; Artajo, Romero, Morello, & Motilva, 2006; Augusto et al., 2015; Baldioli, Servili, Perretti, & Montedoro, 1996; Montaño, Hernández, Garrido, Llerena, & Espinosa, 2016; Paiva-Martins & Gordon, 2005; Ramos-Escudero, Morales, & Asuero, 2015); however, being a complex matrix, other components of the oil may be influencing the measurement of these properties. The objectives of this study were to investigate the influence of the composition of the EVOO on its differentiation based on agronomic variables such as year of harvest, variety and ripening stage and on its antioxidant capacity measured by DPPH· and $ORAC_{FL}$. Several chemometrics tools were used in a multivariate analysis approach to perform the study.

2. Materials and methods

2.1. Reagents

All reagents were either analytical or HPLC grade (Merck, Darmstadt, Germany). AAPH (2,2′-azobis (2-amidinopropane) dihydrochloride), DPPH· (2,2-diphenyl-1-picrylhydrazyl), FAME M RM-1 (methyl arachidate, methyl linoleate, methyl linolenate, methyl oleate, methyl palmitate, and methyl stearate) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The phenol standards (3-hydroxytyrosol, 2-(4-hydroxyphenyl) ethanol (tyrosol), *p*-coumaric acid, vanillic acid, vanillin, luteolin, apigenin, pinoresinol, *p*-hydroxyphenylacetic acid (internal standard 1), *o*-coumaric acid (internal standard 2) and oleuropein) were also obtained from Sigma-Aldrich. Tocopherol standards were purchased from Calbiochem (Merck). All standards had a purity of 98% or higher.

2.2. Plant material

The germplasm bank of the Huasco Experimental Center of the Instituto de Investigaciones Agropecuarias located in the north of Chile (Atacama, III Region; latitude 28° 34′ 45″ S and longitude 70° 47′ 52″ W, at 453 m above sea level), was created in the year 2000, incorporating 36 different varieties of olive trees used commercially. The trees are distributed at distances of 7 × 7 m, with 5 specimens per variety, randomly distributed. Driven to an axis and irrigated by drip system according to the reference evapotranspirative demand, registered in automatic meteorological station.

Rainfall in the periods 2013–2014 and 2014–2015 was 32.7 and 77.7 mm, respectively, concentrated in winter and being the rest of the seasons dry with no rainfall. The maximum and minimum temperatures in these two periods were 21.6 and 8.9 °C and 22.2 and 9.1 °C, respectively. The olive trees were irrigated according to the reference evapotranspirative demand (ETo) with 856 mm/year in the 2013–2014 season and 1143 mm/year in the 2014–2015 season.

Twelve monovarietals (Arbequina, Arbequina I18, Ascolana de Huasco, Coratina, Kalamata, Koroneiki, Leccino, Manzanilla Chilena, Nocellara del Bélice, Oliva di Cerignola, Picual and Sevillana) from the germplasm bank were sampled during the 2014 and 2015 harvests. From the 2014 harvest, one sample of each variety, all in ripening stage 3-4, were obtained. From the 2015 harvest, three samples by variety in ripening stages 2-3 for 'Coratina', 'Koroneiki' and 'Sevillana'; 4-5 for 'Kalamata'; and 3-4 for the remaining varieties. Only two samples of 'Manzanilla Chilena' were obtained in the 2015 harvest, and no samples from 'Leccino' or 'Ascolana de Huasco' were available. The ripening stage of the fruits harvested was defined based on the coloration of the skin of the fruit, determined visually, according to methodology of the Index of Ferreira, that goes from class 0 (fruit of green skin), to class 7, in that the fruit has black skin and flesh (Uceda & Hermoso, 2001).

At each harvest, 10 kg of olives (experimental unit) was handpicked from the middle portions of three randomly selected trees; the olives were mixed prior to extracting the oil. Only healthy fruits, without any sign of infection or physical damage, were used. In total, 41 samples of EVOO were processed.

2.3. Olive oil extraction

Olive oils were collected at olive mills where olives were processed using Frantoino model Monoblock extraction equipment (Toscana Enologica Mori, Florence, Italy) with a two-phase centrifugation system. The fresh olives (10 kg) were crushed and then slowly mixed for 30 min at 26 ± 2 °C. The resulting paste was centrifuged at 1027*g* for 5 min to separate the oil. All samples were subsequently filtered through hydrophilic cotton, placed in amber glass bottles and stored in the dark at −23 °C until analysis (within 1 month). The samples were analysed in triplicate using the chemical analytical methods described below. All of the olive oils were extra virgin according to official analytical methods and limits (free acidity ≤0.8% in oleic acid, $K_{232} \leq 2.50$, $K_{270} \leq 0.22$, $\Delta K \leq 0.01$; IOOC, 2015).

2.4. Quality parameters

Free fatty acids (Ca 5a-40), peroxide value (Cd 8-53), and specific extinctions of oils (K_{232}, K_{270}, ΔK) (Ch 5-91) were determined according to American Oil Chemists's Society (1993).

2.5. Fatty acid composition

Fatty acids were transformed into methyl esters using potassium hydroxide in methanol, according to International Olive Council (IOOC, 2001), and analysed by gas chromatography (GLC) using an HP 5890 chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with an FID detector. A BPX70 fused silica capillary column (50 m, 0.25 μm film; SGE, Incorporated, Austin, TX, USA) was used. The temperature was programmed between 160 °C and 230 °C at 2 °C/min, and 0.5 μL samples were run with hydrogen as the carrier gas. The injection was carried out in split mode. Standard fatty acid methyl esters (FAME) from Sigma-Aldrich Co. (St. Louis, MO, USA) were used for identification purposes.

2.6. Determination of phenolic compounds

A standard solution (0.5 mL) of *p*-hydroxyphenylacetic (0.12 mg/mL) and *o*-coumaric (0.01 mg/mL) acids in methanol was added to the EVOO (2.5 g). The phenolic compounds were isolated by solid-phase extraction using a Waters diol-bonded phase cartridge (Milford, MA, USA) and analysed by reverse phase HPLC using a Waters HPLC system equipped with a binary pump (model 1525), a diode array UV detector (model 2998), an autosampler (model 2707) and a Waters Spherisorb