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Monitoring the phenolic compounds of Greek extra-virgin olive oils during storage

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This work is in memory of Maria Tasioula-Margari.

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ABSTRACT

Extra virgin olive oil (EVOO) samples, of five Greek olive varieties, were stored in dark glass bottles (headspace 0.5%) in a basement without central heating for 24 months. Quantitative variations of the phenolic compounds and their degradation products were monitored over time. The differences observed in the initial total phenolic compounds concentration (ranging between 250.77 and 925.75 mg/kg) were attributed to extraction system, olive variety, and maturity stage. Even after 24 months, the degree of reduction in total phenolic compounds did not exceed 31%. The reduction was more pronounced in dialdehydic forms of oleuropein and ligstroside aglycones (DAFOA and DAFLA), indicating a more active participation in the hydrolysis and oxidation processes of the more polar secoiridoids. The initial total phenolic content was the main factor correlated to the degradation rate of the phenolic compounds. The decrease in secoiridoid derivatives, gave rise to hydroxytyrosol and tyrosol content and to the formation of four oxidized products.

1. Introduction

High quality extra virgin olive oil (EVOO) is an important ingredient of the Mediterranean diet, thanks to its nutritional and health benefits and its sensory characteristics. Virgin olive oil (VOO) contains minor compounds that are of great sensory and biological importance. Among these, tocopherols (vitamin E), which are related to lipids due to the presence of a hydrophobic side chain in their structure, are important for the nutritional and antiradical properties, whereas the hydrophilic phenols greatly influence not only the biological activity and oxidative stability of VOO but also the taste quality (bitter and pungent attributes) (Bendini et al., 2007; Servili et al., 2004; Tasioula-Margari, 2011).

Several studies have attempted to elucidate the mechanisms through which EVOO-derived phenols might contribute to its healthy properties (de la Torre-Carbot et al., 2010; Menendez et al., 2009; Owen et al., 2000; Tripoli et al., 2005). Even in small quantities, phenolic compounds in VOO are fundamental for protecting glycerides from oxidation; in fact, they exert an intense protective action by exposing themselves to oxidation in the place of the lipid substrate. Phenolic compounds can inhibit oxidation through a variety of mechanisms based on radical scavenging, hydrogen atom transfer and metal-chelating (Tasioula-Margari, 2011).

European Food Safety Authority (EFSA), based on several scientific evidence (Covas et al., 2006; de la Torre-Carbot et al., 2010), recently approved a health claim stating that the dietary intake of VOO polyphenols is able to prevent LDL oxidation (EFSA, 2011). Hydroxytyrosol and its derivatives are the key compounds with such an activity, and to bear the claim olive oil should contain at least 5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil.

The major polar phenolic compounds identified and quantified in olive oil belong to five different classes: phenolic acids (especially derivatives of benzoic and cinnamic acids), flavones (luteolin and apigenin), lignans (pinoresinol, 1-acetoxypinoresinol and syringaresinol), phenyl-ethyl alcohols (hydroxytyrosol and tyrosol) and secoiridoids (aglycon derivatives of oleuropein and ligstroside) (Brenes et al., 2000; Christophoridou, Dais, Tseng, & Spraul, 2005; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Servili & Montedoro, 2002). Hydroxytyrosol (3,4-dihydroxyphenethylalcohol), tyrosol (4-hydroxyphenethylalcohol) and their derivatives with elenolic acid, which derive from the glycosides ligstroside and oleuropein, are the most abundant phenolic compounds in VOO (Servili et al.,

Abbreviations: EVOO, extra virgin olive oil; VOO, virgin olive oil; DAFOA, Dialdehydic Form of Oleuropein Aglycone (decarboxymethylated and carboxymethylated); DAFLA, Dialdehydic Form of Ligstroside Aglycone (decarboxymethylated and carboxymethylated); AFOA, Aldehydic Form of Oleuropein Aglycone; AFLA, Aldehydic Form of Ligstroside Aglycone.

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2004; Tasioula-Margari & Okogeri, 2001). The content of phenolic derivatives in freshly made VOO is influenced by the variety, climatic conditions, fruit ripeness (Amiot, Fleuriet, & Macheix, 1986; Baccouri et al., 2007; Morelló, Romero, & Motilva, 2004) and the oil extraction process (Di Giovacchino, Sestili, & Di Vincenzo, 2002; Ranalli, Contento, Schiavone, & Simone, 2001).

During storage VOO phenolic compounds undergo qualitative and quantitative modifications due to oxidative and hydrolytic reactions (Brenes, Garcia, Garcia, & Garrido, 2001; Daskalaki, Kefi, Kotsiou, & Tasioula-Margari, 2009). Temperature, light, oxygen availability, packaging, and storage duration are the main factors affecting the phenolic composition of aged oils (Cinquanta, Esti, & Notte, 1997; Kanavouras, Hernandez-Munoz, & Coutelieris, 2006; Okogeri & Tasioula-Margari, 2002). The ratio between simple phenols and specific derivatives formed by hydrolysis or oxidation could be useful to determine the freshness or aging ratio of a VOO (Bendini, Cerretani, Salvador, Fregapane, & Lercker, 2009). The formation of oxidized phenolic compounds has been evidenced during auto- and thermo-oxidation (Daskalaki et al., 2009; Ríos, Gil, & Gutiérrez-Rosales, 2005). Hydroxytyrosol and its derivatives are more susceptible to oxidative degradation than tyrosol derivatives at accelerated oxidation conditions (Nissiotis & Tasioula-Margari, 2002). Lavelli, Fregapane, and Salvador (2006) evaluated the degradation of phenolic compounds in EVOO during storage in commercial containers. The results obtained in this study showed that the autoxidation and hydrolytic processes of phenolic compounds, under conditions that mimic its routine commercial storage, differs from those observed under accelerated storage conditions.

Given the importance of the phenolic fraction, with regard to antioxidant activities (Bendini, Cerretani, Vecchi, Carrasco-Pancorbo, & Lercker, 2006), sensory properties (Esti, Contini, Moneta, & Sinesio, 2009) and health benefits (Covas et al., 2006), the changes in the phenolic fraction over time could be an important quality control parameter of VOO. More specifically, the aim of this work was to monitor the changes in the phenolic compounds composition during EVOO storage for 24 months, with regard to five Greek olive varieties.

2. Materials and methods

2.1. Samples and storage

The VOO samples were collected during the harvesting periods 2007–2008 and 2010–2011 from regions of Western Greece: Koroneiki from Zakynthos and Kefalanonia ($n = 14$), Lianolia from Corfu and Preveza ($n = 8$), Asprolia from Lefkada ($n = 8$), Thiaki from Kefalonia ($n = 6$) and Native from Zakynthos ($n = 6$). Olives were processed in selected local olive mills, using two or three phases centrifugation systems. Only samples which were of EVOO quality were chosen for the study. Their initial quality characteristics are given in a previous work, where the same samples were used in order to evaluate the changes in the volatile fraction over time (Kotsiou & Tasioula-Margari, 2015). These samples were transferred in dark glass bottles (headspace 0.5%) and were stored in a basement without central heating for 24 months. During this period the temperatures were lower than 18 °C (12 ± 6 °C) except for the summer period when the temperature reached up to 25 °C (20 ± 5 °C). These conditions simulated real VOO storage conditions.

2.2. Standards, reagents and solvents

Methanol, acetonitrile, water and hexane for phenolic compounds extraction and chromatographic separation were all of HPLC grade and were purchased from Merck (Darmstadt, Germany).

The standards used for identification and quantification of phenolic compounds were: hydroxytyrosol from Extrasynthese (Genay, France), tyrosol (for expression of tyrosol derivatives) from Sigma–Aldrich (Steinheim, Germany), oleuropein (for expression of hydroxytyrosol derivatives) from Extrasynthese (Genay, France), vanillic acid, *p*-coumaric acid, ferulic acid, luteolin and apigenin from Merck-Schuchardt (Hohenbrunn, Germany) and pinoresinol (for expression of 1-acetoxypinoresinol and syringaresinol) was purchased from Separation Research (Turku, Finland). Finally, *p*-hydroxyphenyl-acetic acid from Sigma–Aldrich (Steinheim, Germany) was used as an internal standard.

2.3. Determination of quality indices

The peroxide value, spectroscopic indices (K_{232}, K_{270}) and free acidity analyses were carried out according to the methods described by European Community Regulation, standard methods (EC, 1991).

2.4. Extraction of phenolic compounds

Liquid–liquid extraction of phenolic compounds was performed as previously described (Daskalaki et al., 2009).

2.5. HPLC-UV and LC-DAD-MS analysis

HPLC-UV analysis, used for phenolic compounds quantification, was performed in a JASCO liquid chromatography system (Tokyo, Japan) equipped with a PU-980 pump, a UV 970 (UV/vis) detector and a Rheodyne injection valve (20 µl loop). The columns used were Lichrospher 100RP18 column (4.0 mm id × 250 mm, particle size = 5 µm; Merck) and Luna RP-C18 (4.6 mm id × 250 mm, particle size = 5 µm; Phenomenex, Macclesfield, United Kingdom). The elution solvents used were 2% acetic acid in water (A) and a mixture of methanol and acetonitrile, 1:1, v/v (B). The flow rate was 1 ml/min and run time 80 min. The sample injection volume was 20 µl. The samples were eluted by the following gradient: 95% A and 5% B as initial conditions, 70% A and 30% B at 20 min, 65% A and 35% B at 25 min, 60% A and 40% B at 45 min, 30% A and 70% B at 60 min, 0% A and 100% B at 65 min and up to 75 min and, finally, 95% A and 5% B at 80 min. Detection was performed at 280 nm. Phenolic compounds were quantified using available commercial standards as previously described (Daskalaki et al., 2009). The method was also previously evaluated (Tasioula-Margari & Tsabolatidou, 2015).

LC-DAD-MS, used for phenolic compounds identification, was performed in an Agilent 1100 Series LC/MSD Trap, Model SL (Waldbronn, Germany) equipped with a thermostated column compartment 1100, diode array detector 1100 and a standard autosampler 1100. All of the analyses used the ion-spray source in the negative mode with the following settings: nebulizer gas (N_2) 40.0 psi, drying gas 12 l/min and drying gas temperature 350 °C. Target mass was 350 *m*/*z*. Full scan data were acquired by scanning from *m*/*z* 50 to 800. The mobile phase consisted of solvents A (0.1% acetic acid in water) and B (mixture of methanol and acetonitrile, 1:1, v/v) and the solvent gradient were the same used in HPLC-UV (above). The flow rate was 0.5 ml/min and run time 80 min. The sample injection volume was 10 µl. The UV/vis spectra were recorded in the range of 200–700 nm and chromatograms were acquired at 240, 280 and 340 nm.

2.6. Statistical analysis

SPSS (version 19.0, SPSS Inc. Chicago, IL, USA) was used to perform analysis of variance (ANOVA) and stepwise linear regression analysis (SLRA). Pearson correlation coefficients were calculated