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Original Article

Evaluation of antioxidant activities of phenolic compounds from two extra virgin olive oils

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ABSTRACT

The aim of this work was to investigate the antioxidant properties of two varieties of olive oil (Chétoui and Chemlali), and to study the protective effect of phenolic extract (PE) from these varieties against low density lipoprotein (LDL) oxidation in vitro. Antioxidant activities were examined as well, using different radical scavenging assays: radical scavenging activity by DPPH, and total antioxidant status by ABTS. The antioxidant effect of the oils on human LDL was evaluated by measuring levels of conjugated dienes and polyunsaturated fatty acids. Chemlali oil was less rich in total phenols (158 mg/kg) than Chétoui oil (395 mg/kg) ($p < 0.05$). The highest antioxidant activity was attributed to Chétoui oil (78.56% vs. 37.23% of DPPH and 2.42 vs. 0.61 mmol Trolox/kg; $p < 0.05$). Chétoui PE had a significantly greater inhibitory effect on LDL oxidation than Chemlali PE (lag time = 116 ± 6.05 min vs. 64 ± 11.31 min at 0.3 mg/l of PE respectively; $p < 0.05$). The differences in quantity and quality of the studied oils influenced their biological activities and they could provide beneficial effects in cardiovascular disease by inhibiting LDL oxidation.

1. Introduction

Coronary heart disease is the main cause of mortality in the western world (De Lorgeril and Salem, 2000). It has been observed that oxidized low density lipoproteins (ox LDLs) play an important role in the development of atherosclerosis (Witztum, 1994; Steinberg, 1997; Ehara et al., 2001). In fact, ox LDL is recognized by scavenger receptors which are not regulated by the intracellular cholesterol level, in contrast to the LDL receptor (Goldstein and Brown, 1990). Oxidation of LDL particles lead to a modification of apolipoprotein B (apoB) as products generated from the catabolism of lipid peroxides, such as aldehydes, interact with lysine residues of apoB rendering LDL particles more negatively charged, which result in decreased affinity for LDL receptor and an increased affinity for scavenger receptors (Brown and Goldstein, 1983).

Epidemiological studies show that various dietary sources of antioxidants such as vitamin C, vitamin E and β-carotene minimize the oxidative damage (Rimm et al., 1993; Roberts et al., 2007). An extensive body of scientific evidence indicates that diets rich in fruit, vegetables, legumes, whole grains, fish, low-fat dairy products and monounsaturated fats are associated with a lower incidence of cardiovascular disease, mainly through their influence on blood pressure, lipids and lipoproteins levels, as well as LDL oxidation (Panagiotakos et al., 2003; Trichopoulou and Vasilopoulou, 2000; Covas, 2007). Recently, attention has been focused on a variety of non-vitamin antioxidants, such as phenolic compounds, that might also contribute to prevent oxidative damage (Frankel et al., 1993; Xiuzhen et al., 2007). These compounds, although minor constituents of olive oil, contribute to sensory organoleptic properties and to the prevention of oil auto-oxidation (Gutierrez et al., 2001; Esti et al., 2009). Virgin olive oil is the most commonly used as a cooking fat in the Mediterranean countries; it is producted in large quantities from the fruit "*Olea europea* L." using physical methods. It shows sensory characteristics and nutritional properties that distinguish it from other types of olive oil (Regulation ECC/2568/91, 1991). The oil is characterized by biological criteria such as fatty acid composition and antioxidant composition, particularly polyphenols and secoiridoids, which are powerful natural antioxidants (Pellegrini et al., 2001; Morello et al., 2004; Dabbou et al., 2009). Phenols types and concentrations differ greatly among olive oils. In fact, many factors affect phenolic content in olive oil, such as cultivars, ripening scale, seasonal variation, and light climate (Cartechini et al., 1994; Ranalli et al., 1999; Issaoui et al., 2009, 2010). Previous studies of possible mechanisms of phenol action indicate that these compounds are able to scavenge free radicals and break peroxidation reaction

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chains (Frankel et al., 1993; Angerosa et al., 1999). Phenolic compounds were also found to have antioxidant activity in terms of their capacity to inhibit LDL oxidation in vitro (Fito et al., 2000). Moreover, a mixture of phenols might exert different activity compared to single phenols because of a cooperative effect, thereby modifying biological activity (Reaven and Witztum, 1996). Interactions among phenols also seem to depend on the relative amount of single polyphenols (Romani et al., 2004).

In this context, we hypothesize that consumption of good-quality olive oil (with a high phenolic content) might have an important impact on health by preventing oxidative stress. Literature data on olive oil polyphenols mainly concern purified compounds, while the antioxidant properties of the total olive oil phenolic extract have been poorly investigated. We therefore investigated the free radical scavenging properties of Tunisian Chemlali and Chétoui varieties of extra virgin olive oil (EVOO) and examined the antioxidant effect of their phenolic compounds on human LDL oxidation.

2. Materials and methods

2.1. Olive samples

Samples were obtained from homogeneous olive fruits (*Olea europaea* L.) of two varieties, Chétoui and Chemlali, cultivated in the same pedoclimatic conditions and growing in the "Boughrara" National Collection—in southern Tunisia. Olive fruits were harvested manually at the same ripening stage during the 2004–2005 season in December.

2.2. Oil extraction

Oil was extracted using an Abencor laboratory oil mill equipped with a metal crusher, a mixer and a basket centrifuge. The oil was stored at 4 °C in amber glass bottles in the dark.

2.3. Analytical methods

Free fatty acids, the peroxide index, and specific UV absorbance conventionally indicated by K232 and K270, were determined according to the EC-Regulation for analytical methods (Regulation ECC/2568/91, 1991).

Fatty acids were converted to fatty acid methyl esters (FAMEs) were prepared by dissolving 0.1 g of EVOO in 2 ml of heptane and 0.2 ml of KOH (0.2 N) in methanol and incubating the mixture for 1 h. FAMEs were separated and quantified by gas chromatography on a model 5890 series II instrument (Hewlett-Packard, Palo Alto, CA, USA) equipped with a flame-ionization detector and a HP – INNOWAX fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The column temperature was increased from 170 to 270 °C at a rate of 5 °C/mn. Ultra pure nitrogen was used as the carrier gas. Results are expressed as a relative area percentage of total FAMEs.

Total phenolic content was determined by the Folin–Ciocalteu method (Gutfinger, 1981). A 2.5 g sample of olive oil was extracted with 5 ml of hexane. Then 5 ml of methanol/water (60:40, v/v) was added and the mixture was vortexed vigorously for 2 min. Folin–Ciocalteu reagent (0.5 ml) and 4.8 ml of bi-distilled water were added to the phenolic fraction. Then 1 ml of sodium bicarbonate (35%, w/v) was added and water was used to bring the final volume to 10 ml. The mixture was incubated for 2 h in the dark at room temperature and then the absorbance of the mixture was measured at 725 nm. Spectrophotometric determination of o-diphenols was carried out according to Mateos et al. (2001) and results are expressed as mg/kg of gallic acid. Phenolic compounds were analysed by HPLC as described by Montedero et al. (1992).

The oxidative stability index (OSI) was measured by using a Rancimat model 734 instrument (Metrohmn, Switzerland). A sample of 3 g of oil was heated at 120 °C, and air was bubbled through it at a flow rate of 20 l/h. Results are expressed as the induction time for hydroperoxides decomposition.

For the tocopherol analysis, the sample was diluted with n-hexane (1:10), the mixture was vortexed and 200 μl was transferred to a test tube containing 600 μl of methanol and 200 μl of internal standard (300 μg/ml). HPLC separation was carried out on a Hewlett-Packard system (Waldbronn, Germany) comprising a HP-1100 pump, a Rheodyne model 7725 injector (Cotati, CA, USA, loop volume 20 μl), a HP-1200 M multi-array detector and a Supelcosil ODS-2 column (150 mm × 4.5 mm i.d., film thickness 5 μm) (Gimeno et al., 2000).

Carotenoids and chlorophylls were determined as described by Minguez-Mosquera et al. (1991). In brief, 7.5 g of oil was weighed, dissolved in cyclohexane and made up to a final volume of 25 ml. Carotenoid and chlorophyll pigments were determined by measuring the absorbance at 470 and 670 nm; results are expressed as mg of pheophytin a and lutein per kg of oil, respectively.

2.4. Antioxidant activity assays

2.4.1. DPPH assay

The radical scavenging activity (RSA) was measured following the methodology described by Brand-Williams et al. (1995). The bleaching rate of a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) was monitored in the presence of the sample. A 3.9 ml aliquot of 6×10^{-5} M DPPH$^{\bullet}$ in methanol solution was used and the reaction was started by adding 100 μl of phenolic extract (PE). The UV absorbance of DPPH$^{\bullet}$ was monitored at 515 nm and 25 °C for 1 h.

2.4.2. ABTS assay

The RSA of olive oil PE was also determined using a 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation decolorization assay (Re et al., 1999). ABTS was dissolved in water to a concentration 7 mM and radical cations (ABTS$^{\bullet}$+) were produced by reacting this stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. This solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. Reagent blank reading was taken (A_0). After addition of 3.9 ml of diluted ABTS$^{\bullet}$+ solution (A_{734} nm = 0.700 ± 0.020) to 100 μl of phenolic fraction or trolox, the absorbance was read at 30 °C exactly 6 min after initial mixing (A_t).

2.4.3. Copper mediated LDL oxidation assay

To minimize variability between LDL preparations, blood samples were obtained by venipunture from five to six healthy normolipidemic volunteers after an overnight fast and collected into evacuated tubes. LDL were isolated from pooled fresh plasma by density gradient ultracentrifugation in a vertical rotor (Beckman Vti 50) and dialyzed against degassed phosphate buffered saline (PBS, pH 7.4) for 48 h. The concentration of LDL solutions is reported in terms of total protein concentrations. All LDL pools' preparations ($n = 3$) were oxidized shortly after blood collection, usually within 2–3 weeks and never exceeding 4 weeks after blood collection.

In vitro oxidation of human LDL was monitored as described by Esterbauer et al. (1989). To evaluate the ability of EVOO (PE) to inhibit copper mediated (5 μM Cu^{2+}) LDL (0.05 g/l) oxidation, conjugated dienes were continually monitored (3 min intervals at