

Olive Oil Phenols and Their Potential Effects on Human Health

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Olive oil is the fat of choice in the Mediterranean area, where the diet has been associated with a lower incidence of coronary heart disease and certain cancers. Phenols in extra virgin olive oil are responsible for its peculiar pungent taste and for its high stability. Recent findings demonstrate that olive oil phenolics inhibit oxidation of low-density lipoproteins (the most atherogenic ones) and possess other potent biological activities that if demonstrated *in vivo*, could partially account for the observed healthful effects of diets that include high-quality olive oil and other foods rich in flavonoids and phenols.

Keywords: *Olive oil; phenols; atherosclerosis; Mediterranean diet; antioxidants; free radicals*

OLIVE OIL

The world production of olive oil is around 2 000 000 tons, representing ~4% of total vegetable oil production (Boskou, 1996). The major olive oil producers are Spain, Italy, Greece, and Maghrebian countries but, due to the increasing popularity of the Mediterranean diet, in which olive oil is the major fat component, its consumption is expanding to nonproducer countries such as United States, Canada, and Japan.

Depending on its chemical and organoleptic properties, olive oil is classified into different grades (EC, 1992) that also serve as guidelines for the consumer in the choice of the preferred kind of oil. In many producing countries, extra virgin olive oil accounts for just 10% of all the oil produced.

Abundance of oleic acid, a monounsaturated fatty acid, is the feature that sets olive oil apart from other vegetable oils. In particular, oleic acid (18:1 n-9) ranges from 56 to 84% of total fatty acids, while linoleic acid (18:2 n-6), the major essential fatty acid and the most abundant polyunsature in our diet, is present in concentrations between 3 and 21% (Tiscornia et al., 1982).

In addition to triglycerols and free fatty acids, olive oil contains a variety of nonsaponifiable compounds that add up to 1–2% of the oil and are important for its stability and unique flavor and taste. In contrast, other edible seed oils lose most of their minor compounds during the refining stages. Analysis of the “minor constituent” fraction is very important for the identification of the area of production of each batch of oil and allows the disclosure of possible adulterations.

THE PHENOLIC FRACTION

Phenols in olive oil amount to up to 1 g/kg although, due to the variety of methods proposed for their determination, the reported values are hardly comparable: the widely employed Folin–Ciocalteau reagent is not specific for phenols and HPLC procedures (an example is given in Figure 1) are limited by the complexity of

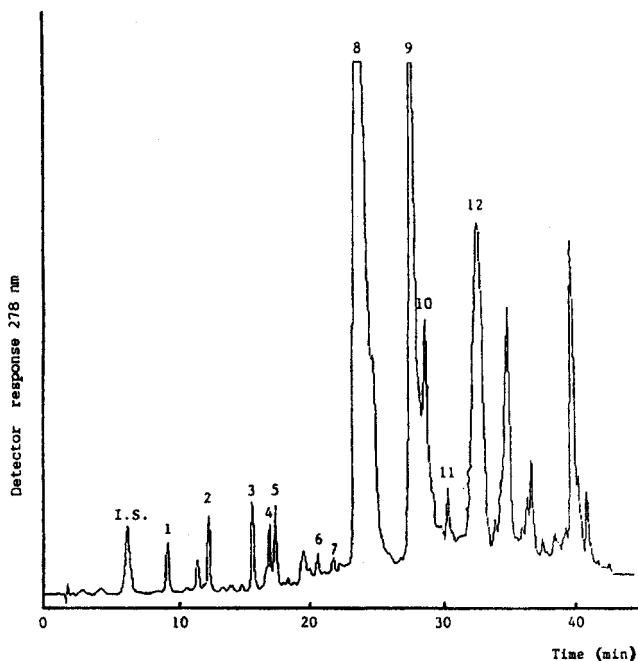


Figure 1. HPLC chromatogram of the phenolic fraction of a virgin olive oil (cultivar: Moraiolo) at 278 nm. (From Montedoro et al., 1993.) Peaks correspond to (I.S.) gallic acid; (1) hydroxytyrosol; (2) tyrosol; (3) vanillic acid; (4) caffeic acid; (5) syringic acid; (6) *p*-coumaric acid; (7) ferulic acid; (8) isomer of oleuropein aglycon; (9) dialdehydic form of elenonic acid linked to hydroxytyrosol; (10) dialdehydic form of elenonic acid linked to tyrosol; (11) cinnamic acid; and (12) unidentified.

the phenolic fraction. Some of the most representative phenolic compounds are hydroxytyrosol (3,4-dihydroxyphenylethanol), tyrosol, oleuropein, and its aglycon, caffeic acid, vanillic acid, syringic acid, protocatechuic acid, and *p*-hydroxyphenylacetic acid (Figure 1).

The absolute concentration of phenols in olive oils is the result of a complex interaction between several factors, including cultivar, degree of maturation, and climate (Cimato et al., 1992). It usually decreases with over-maturation of olives, although there are some exceptions to this rule. For instance, olives grown in warmer climates, despite a more rapid maturation, yield oils that are richer in phenols (Cimato et al., 1992).

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The influence of the elaboration process [see Boskou (1996) for an exhaustive discussion of the various methods] on the phenolic content is yet to be fully elucidated. It appears, for instance, that oils that have been obtained by centrifugation have a lower phenols content (Di Giovacchino et al., 1994), possibly because this process involves the use of large quantities of warm water that wash off a considerable amount of phenols that are thus removed together with the water phase (the so-called wastewater).

It is noteworthy that during the elaboration of olive oil, a considerable amount of phenols, according to their partition coefficient, end up in the wastewater. Thus, olive oil wastewaters, produced in extremely large quantities (~800 000 tons/year in Italy) and currently disposed of, contain powerful, as yet unused, antioxidants that could be recovered and employed in preservative chemistry (Visioli et al., 1995b).

INFLUENCE OF PHENOLS ON THE OIL TASTE

Oleuropein is the bitter principle of olives and is found in olive oil together with its aglycon form. It was first named and studied by Bourquelot and Vintilesco (1908), and effects in humans were subsequently described by Panizzi et al. (1960), who reported on the hypotensive properties of this glucoside. Oleuropein amounts to up to 14% of the dry weight in unripe olives (Amiot et al., 1986) but, during maturation, undergoes hydrolysis and yields several simpler molecules that build up the well-known olive oil complex taste.

Most phenols confer a very bitter and pungent zest to the oil. The effect of bitterness and pungency is the result of complex interactions between the "minor constituents" and the taste buds. In particular, phenolic acids such as phenol and cinnamic acid are responsible for the bitter sensation that can be felt on the lateral and posterior areas of the tongue, while secoiridoids confer the peculiar pungency. As a result, organoleptic feelings that remind of pepper or chili peppers can be found in phenols-rich olive oil, that are most favored by gourmets. This is confirmed by panel tests, in which oils produced from greener olives usually obtain higher scores (Cimato et al., 1992) because of their "fruity" and complex aroma, provided by their high phenols content. Conversely, "sweet" oils are almost devoid of phenols. It should however be noted that a very high load of phenols may result in an excessive and unpleasant bitterness and is not synonymous with quality: the continuous systems employed to extract the oil sampled in the panel test reported by Cimato et al., (1992), despite removing a portion of phenols, prevented the development of off-flavors that can derive from dirty fiber mats or molds and hence lowered the percentage of defective oils. In turn, high phenol levels in virgin olive oils are very likely to exhibit a high stability and a strong, fruity flavor, indicating a high, but not necessarily the most preferred, organoleptic quality of the oil.

PHENOLS AS ANTIOXIDANTS

A linear relationship between phenols content and oxidative stability of virgin oils has been found (Gutfinger, 1981). In particular, hydroxytyrosol concentrations are closely correlated with the stability of the oil, while those of tyrosol are not (Papadopoulos and Boskou, 1991; Tsimidou et al., 1992). *o*-Diphenols such as

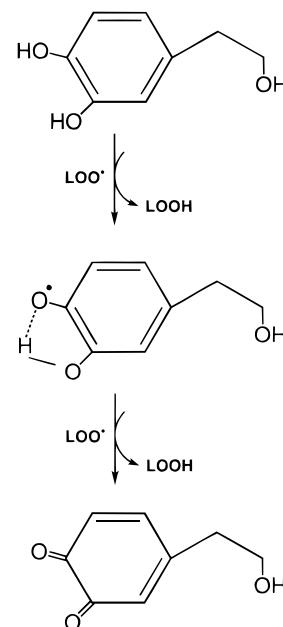


Figure 2. Antioxidant mechanism, by hydrogen donation, of hydroxytyrosol.

hydroxytyrosol, in particular, mostly contribute to the stability of the oil and their relative proportions, together with the hydroxytyrosol/tyrosol ratio, should be taken into consideration during analyses of the polar fraction.

Moreover, a strong metal ion chelating activity (as for most phenolic molecules) has been found for hydroxytyrosol activity (Visioli et al., 1995a): removal of the prooxidant metal ions considerably increases the stability of any lipid substrate, including olive oil, in which traces of some transition metals can cause partial degradation of phenols (Angerosa and Di Giacinto, 1993).

The antioxidant properties of *o*-diphenols can be related to hydrogen donation, i.e., their ability to improve radical stability by forming an intramolecular hydrogen bond between the free hydrogens of their hydroxyl group and their phenoxyl radicals (Figure 2). In fact, although investigations on the structure-activity relationship of olive oil phenols are yet to be carried out, similar studies have been performed on flavonoids and have indicated that in general, the degree of antioxidant activity is correlated with the number of hydroxyl substitutions (Rice-Evans et al., 1996; Cao et al., 1997). In particular, the *o*-diOH substitution confers a high antioxidant capacity, whereas single hydroxyl substitutions, e.g. tyrosol, provide no activity. Tyrosol, for instance, does not protect LDL from chemically induced oxidation (unpublished observations).

The free radical scavenging activities of olive oil phenolics, including their removal of DPPH and superoxide anion, have been recently confirmed by Visioli et al. (1998), who also reported on the scavenging effects of hydroxytyrosol and oleuropein with respect to hypochlorous acid, a potent oxidant produced *in vivo* at the site of inflammation and a major component of chlorine-based bleaches that can often come into contact with food during manufacturing.

POTENTIAL EFFECTS OF OLIVE OIL PHENOLS ON HUMAN HEALTH

Atherosclerosis is a multifactorial disease that represents the primary cause of death worldwide. Elevated levels of circulating low-density lipoprotein (LDL), which are rich in cholesterol and cholesteryl esters, are a well-established risk factor for developing coronary heart disease (CHD) (Ross, 1993). The uptake of cholesterol by cells of the arterial wall—monocytes/macrophages—is generally mediated by a self-regulating receptor, that limits cholesterol deposition when the required intracellular levels are reached. The discovery of a macrophagic “scavenger” receptor, which internalizes oxidatively modified forms of LDL (oxLDL) and is not down-regulated by increasing intracellular cholesterol levels (Brown and Goldstein, 1983), led to the hypothesis that oxLDLs play a crucial role in the onset of the atherosclerotic lesion, due to their uptake by such receptor and the subsequent infiltration and deposition of cholesterol-laden cells (foam cells) into the arterial wall (Steinberg et al., 1989). Evidence of an *in vivo* presence of oxLDL, either circulating or embedded in atherosclerotic plaques, is increasing. Accordingly, there is a growing body of data from epidemiological and controlled studies that correlates a high intake of antioxidants, including vitamins—especially vitamin E, flavonoids, and phenols, with a lower incidence of CHD (Hertog et al., 1993a,b, 1995), although negative results have also been reported (Steinberg, 1995).

The epidemiological evidence of a lower incidence of CHD in the Mediterranean area (Hertog et al., 1995) and the recent availability of pure compounds led us to hypothesize a protective effect of some olive oil phenolics, either provided in pure form by the group of Professor G. F. Montedoro at the University of Perugia and by the group of Professor F. F. Vinceri at the University of Florence or commercially available, with respect to chemically induced oxidation of human LDL.

Oxidation of LDL can be investigated *in vitro* by incubating isolated LDL with a variety of agents that include chemicals such as transition metal ions, cultured cells like macrophages and endothelial cells, or by physical means such as UV light irradiation (Visioli and Galli, 1997a). Several markers of oxidative stress must be taken into account, since they provide information on the oxidation of lipids and apolipoproteins.

Both hydroxytyrosol (HT) and oleuropein (OE) potently inhibit copper sulfate induced oxidation of LDL in a dose-dependent manner, when incubated from 10^{-6} to 10^{-4} M (Visioli et al., 1995a; Visioli and Galli, 1994). The protective effects of HT and OE are demonstrated through the assessment of various markers, such as a reduced formation of short-chain aldehydes (evaluated as thiobarbituric acid-reacting substances, TBARS) and of lipid peroxides, by a higher vitamin E content in the residual LDL (indicating sparing of endogenous antioxidants), and by a reduced formation of malondialdehyde–lysine and 4-hydroxynonenal–lysine adducts, indicating protection of the apoprotein layer (Visioli et al., 1995a; Visioli and Galli, 1994, 1997b).

Figures 3 and 4 depict the effects of luteolin and its aglycon (usually present in low amounts in olive oil), which were extracted and purified from crushed olives, 10^{-5} M on LDL oxidized with CuSO_4 5 μM . It is clear that both compounds protect LDL from oxidation, as shown by a significantly reduced formation of TBARS and lipid peroxides, respectively: a ~70% protection was

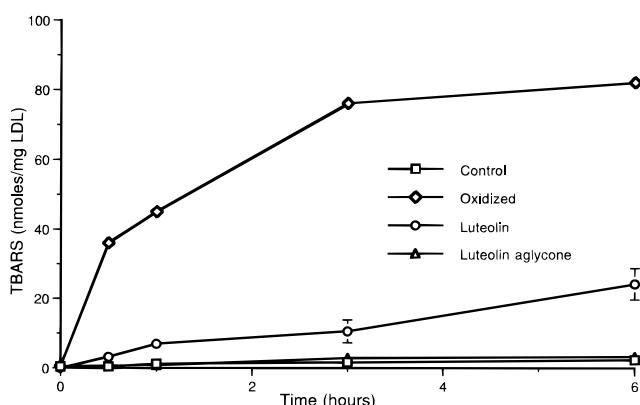


Figure 3. Thiobarbituric acid reacting substances (TBARS) levels in low-density lipoproteins (LDL) oxidized with CuSO_4 5 μM and/or preincubated with luteolin or its aglycon 10^{-5} M. LDL was isolated from human plasma by ultracentrifugation and diluted to reach a concentration of 200 $\mu\text{g}/\text{mL}$. Incubations were carried out at 37 °C and aliquots were withdrawn at the indicated times for analyses. TBARS were quantified according to Balla et al. (Balla et al., 1991).

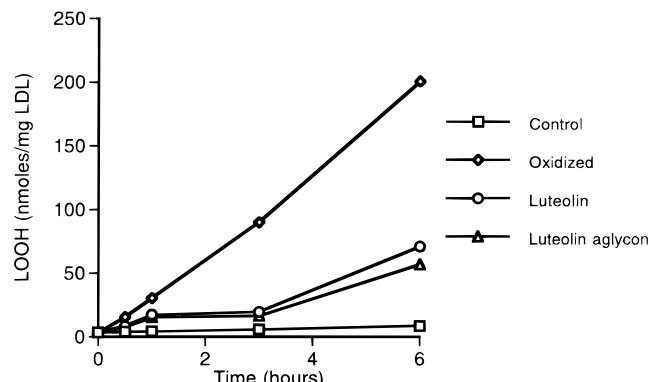


Figure 4. Lipid peroxides (LOOH) levels in LDL oxidized with CuSO_4 5^{-5} M. LOOH were quantified as follows: 100 μM xylenol orange, 250 μM FeSO_4 , 25 mM H_2SO_4 , and 4 mM BHT in 90% methanol were added to the LDL aliquot. After 15 min of incubation at room temperature, absorbance was read at 560 nm and LOOH were quantified by comparison with a standard scale of *tert*-butyl hydroperoxide.

still present after 6 h of incubation. Other experiments attributed the antioxidant properties of hydroxytyrosol to both a strong metal chelation and a free-radical scavenging activity, as demonstrated by the use of metal-independent oxidative systems (Visioli et al., 1995a) and stable free radicals (Visioli et al., 1998).

Additional evidence of the antioxidant properties of hydroxytyrosol was recently provided by Manna et al. (1997) who demonstrated an antioxidant effect of hydroxytyrosol (but not, as expected, tyrosol) in a model of oxidative stress induced in intestinal epithelial cells. In this experimental model tyrosol, which lacks the *o*-diphenolic structure, was found to be ineffective, as it was in the models of LDL oxidation described above (unpublished data).

Hydroxytyrosol was also tested for other biological activities, such as the *in vitro* effect on platelet function, where the compound was proven to inhibit the chemically induced aggregation, the accumulation of the proaggregant agent thromboxane in human serum, the production of the proinflammatory molecules leukotrienes by activated human leukocytes, and the inhibition of arachidonate lipoxygenase (Petroni et al., 1994; Visioli et al., 1994; Kohyama et al., 1997). The potent

Table 1. Effect of Oleuropein on Nitric Oxide Production from Activated Mouse Macrophages

samples ^a	NO production, as percentage of controls
controls	100
LPS + L-NAME	20.3 ± 1.2
LPS + OE 10 ⁻⁶ M	113.2 ± 2.5
LPS + OE 10 ⁻⁵ M	127.8 ± 2.2
LPS + OE 10 ⁻⁴ M	158.7 ± 4.6
LPS + OE 10 ⁻⁴ M + L-NAME	71.2 ± 1.5

^a LPS, bacterial lipopolysaccharide (75 ng/mL); OE, oleuropein; L-NAME, L-nitromethylarginine methyl ester 300 µM.

(EC₅₀s in the 10⁻⁵ M range) inhibitory effect of hydroxytyrosol toward all these parameters discloses unpredicted biological activities of olive oil phenolics in addition to their antioxidant properties.

Finally, when added to murine macrophages together with a bacterial lipopolysaccharide, oleuropein increases the functional activity of these immune-competent cells, as evaluated by a significant increase (+ 58.7 ± 4.6%) in the production of the bactericidal and cytostatic factor nitric oxide (Table 1). A direct tonic effect of oleuropein on the inducible form of the enzyme nitric oxide synthase (iNOS) was demonstrated by Western blot analysis of cell homogenates and by the use of the iNOS inhibitor L-nitromethylarginine methyl ester (Table 1) (Visioli et al., 1998).

Macrophage-derived nitric oxide during acute sepsis and inflammation represents an adaptive response of the organism that reacts to the endotoxin challenge by increasing the production of this mediator. In fact, nitric oxide inhibits platelet aggregation and adherence, and it maintains a proper perfusion rate through increased vasorelaxation. Accordingly, inhibition of nitric oxide synthesis during sepsis increases cellular damage and animal mortality (Lowenstein et al., 1996).

CONCLUSIONS

Olive oil represents a small share of the whole vegetable oil market, but its use is gaining ground together with the increasing popularity of the Mediterranean diet. The olive oil industry is actively trying to improve the overall olive oil quality and stability by selecting the appropriate cultivars and by optimizing each production step, from harvesting to extraction, although this process is somewhat limited by the high number of farmers and mills, each employing its own traditional methods.

The observation that in the Mediterranean area there was a lower incidence of CHD (Keys, 1995; Willet et al., 1995) and certain types of cancers (Trichopoulou, 1995; Lipworth et al., 1997) led to the hypothesis that a diet rich in grain, legumes, fresh fruits, and vegetables, wine in moderate amounts, and olive oil had beneficial effects on human health. To date, this effect has been mainly attributed to the low saturated fat intake of the Mediterranean diet and to its high monounsaturates proportion. Nevertheless, other components of the diet, like fiber, vitamins, flavonoid phenols, and phenols, may play an important role in disease prevention. Thus, a high phenol content in virgin olive oil has been so far sought for because it increases the organoleptic qualities of the oil. The results illustrated in this review, however, suggest that choosing a phenols-rich olive oil would contribute to the dietary intake of biologically active compounds, in estimated quantities that have

been correlated with a reduced risk of developing CHD (Hertog et al., 1995, 1993a).

The question of whether flavonoids and phenols (including olive oil ones) are absorbed and metabolized in vivo is still unanswered. However, in addition to the above-mentioned epidemiological data, experiments with laboratory animals have demonstrated a higher resistance to oxidation of LDL obtained from animals fed virgin olive oil, as compared to animals that were only given a triglyceride preparation with an equivalent amount of oleic acid (Scaccini et al., 1992; Wiseman et al., 1996). Furthermore, absorption and disposition of some flavonoids have been investigated and demonstrated in humans (Hollman et al., 1995; Künzli, 1976). In the future, availability of pure compounds in adequate quantities and development of appropriate methodologies will clarify these important issues.

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