Nutrition Research Reviews (2005), **18**, 98–112 DOI: 10.1079/NRR200495
© The Authors 2005

The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health

Elisa Tripoli, Marco Giammanco*, Garden Tabacchi, Danila Di Majo, Santo Giammanco and Maurizio La Guardia

Institute of Physiology and Human Nutrition, Faculty of Pharmacy, University of Palermo, Via Augusto Elia 3, 90 127, Palermo, Italy

The Mediterranean diet is rich in vegetables, cereals, fruit, fish, milk, wine and olive oil and has salutary biological functions. Epidemiological studies have shown a lower incidence of atherosclerosis, cardiovascular diseases and certain kinds of cancer in the Mediterranean area. Olive oil is the main source of fat, and the Mediterranean diet's healthy effects can in particular be attributed not only to the high relationship between unsaturated and saturated fatty acids in olive oil but also to the antioxidant property of its phenolic compounds. The main phenolic compounds, hydroxytyrosol and oleuropein, which give extra-virgin olive oil its bitter, pungent taste, have powerful antioxidant activity both *in vivo* and *in vitro*. The present review focuses on recent works analysing the relationship between the structure of olive oil polyphenolic compounds and their antioxidant activity. These compounds' possible beneficial effects are due to their antioxidant activity, which is related to the development of atherosclerosis and cancer, and to anti-inflammatory and antimicrobial activity.

Olive oil: Antioxidants: Cardiovascular diseases: Phenolic compounds: Oleuropein

Introduction

Olive oil, a product of the mechanical extraction from the fruit of *Olea europeae* L. (Oleaceae family), is composed of a glycerol fraction, constituting approximately 90–99 %, and of a non-glycerol or unsaponifiable fraction (0·4–5 %). Oleic acid, a MUFA (18 : 1*n*-9), represents 70–80 % of the fatty acids present in olive oil. Epidemiological studies have shown a lower incidence of atherosclerosis, cardiovascular diseases and certain kinds of cancer in the Mediterranean area than in other areas. The results of these studies have been in part attributed to the characteristic kind of diet of the local population. The traditional Mediterranean diet contains, unlike the Northern European and American diet, a considerable proportion of vegetables, cereals, fruit, fish, milk, wine and olive oil. The substantial difference between the two kinds of diet – despite the similarity between the classic risk factors for cardiovascular pathologies, such as high plasma cholesterol levels – has been associated with a lower risk of their development (Keys, 1995; Trichopoulou, 1995; Willet *et al.* 1995; Lipworth *et al.* 1997; Visioli & Galli, 1998*a*; Trichopoulou *et al.* 1999; Visioli *et al.* 2000*b*).

It is known that an increased consumption of MUFA instead of PUFA reduces the risk of atherosclerosis because it makes the circulating lipoprotein less sensitive to peroxidation (Reaven *et al.* 1991; Bonanome *et al.* 1992; Moreno & Mitjavilab, 2003).

Also, the inclusion in the diet (approximately 15 % of total energy) of oleic acid reduces plasma levels of the complex LDL-cholesterol and increases HDL-cholesterol. However, the protective role of the Mediterranean diet is much higher than that of the single foods that characterise it, and the protective role played by many of these foods has still to be defined. Recent studies have demonstrated that other constituents of certain characteristic Mediterranean diet foods have beneficial biological effects on health. It has been established that olive oil has beneficial effects as regards breast and colon cancer (Owen *et al.* 2000*b*), diabetes accompanied by hypertriacylglycerolaemia, inflammatory, and autoimmune diseases such as rheumatoid arthritis (Alarcon de la Lastra *et al.* 2001).

We will therefore consider the unsaponifiable fraction of extra-virgin olive oil, which is rich in tocopherols, aromatic hydrocarbon compounds and sterols. In particular, we will study the biological functions of its polyphenolic compounds.

The phenolic compounds

The beneficial effects of the Mediterranean diet can be attributed not only to the high relationship between

Abbreviations: HMG, 3-hydroxy3-methylglutaryl; ROS, reactive oxygen species.

*** Corresponding author:** Professor M. Giammanco, fax +39 091 6236407, email giammanco@unipa.it

unsaturated and saturated fatty acids of olive oil, but also to the antioxidant property of its phenolic compounds. The pulp of olives contains these compounds, which are hydrophilic, but they are also found in the oil. The class of phenols includes numerous substances, such as simple phenolic compounds like vanillic, gallic, coumaric and caffeic acids, tyrosol and hydroxytyrosol and more complex compounds like the secoiridoids (oleuropein and ligstroside), and the lignans (1-acetoxypinoresinol and pinoresinol).

Chemical structure

The main antioxidants of virgin olive oil are carotenoids and phenolic compounds, which are both lipophilic and hydrophilic. The lipophilics include tocopherols, while the hydrophilics include flavonoids, phenolic alcohols and acids, secoiridoids and their metabolites. The polyphenols include phenolic alcohols and acids, secoiridoids and their metabolites and the lignans; however, since some of these (tyrosol) do not possess two hydroxyl groups, it would be incorrect to put them in this class (Visioli *et al.* 2002).

The flavonoids include the glycosides of flavonol (luteolin-7-glucoside and rutin), anthocians, cyanidin and the glucosides of delphinidin.

The polyphenols can be distinguished as simple or complex. In the first class, 3,4-dihydroxyphenyl-ethanol, or hydroxytyrosol, and *p*-hydroxyphenyl-ethanol, or tyrosol, are the most abundant phenolic alcohols in olives (Fig. 1 (B)).

Other phenolic acids, with the chemical structure C6–C1 (benzoic acids) and C6–C3 (cinnamic acid), are also present in olives (Garrido Fernández *et al.* 1997).

Historically, these compounds (caffeic, vanillic, syringic, protocatechuic, *p*-coumaric and *o*-coumaric, 4-hydroxybenzoic acids) represent the first group of simple phenols observed in virgin olive oil (Montedoro, 1972; Vasquez Roncero, 1978).

The presence of simple phenolic acids as secondary components in olive oil has been widely reported (Solinas & Cichelli, 1981; Tsimidou *et al.* 1996). The presence of gallic acid has also been documented (a substance also present in tea) (Mannino *et al.* 1993).

The secoiridoids oleuropein, demethyloleuropein, ligstroside and nüzhenide, the main complex phenols in virgin olive oil, are secondary glycosidic compounds similar to coumarins; secoiridoids are characterised by the presence of elenolic acid in its glucosidic or aglyconic form, in their molecular structure (Bianco & Uccella, 2000) (Fig. 1).

The secoiridoids, which are glycosidated compounds, are produced from the secondary metabolism of terpenes as precursors of several indole alkaloids (Soler-Rivas *et al.* 2000).

Oleuropein is the ester between 2-(3′,4′-dihydroxyphenyl)ethanol (hydroxytyrosol) and the oleosidic skeleton common to the glycosidic secoiridoids of the Oleaceae (Fig. 1 (A)).

Hydroxytyrosol can be present as a simple or esterified phenol with elenoic acid, forming oleuropein and its aglycone, or as part of the molecule of verbascoside (Amiot *et al.* 1986; Servili *et al.* 1999*b*); it can also be present in several glycosidic forms, depending on the hydroxyl group

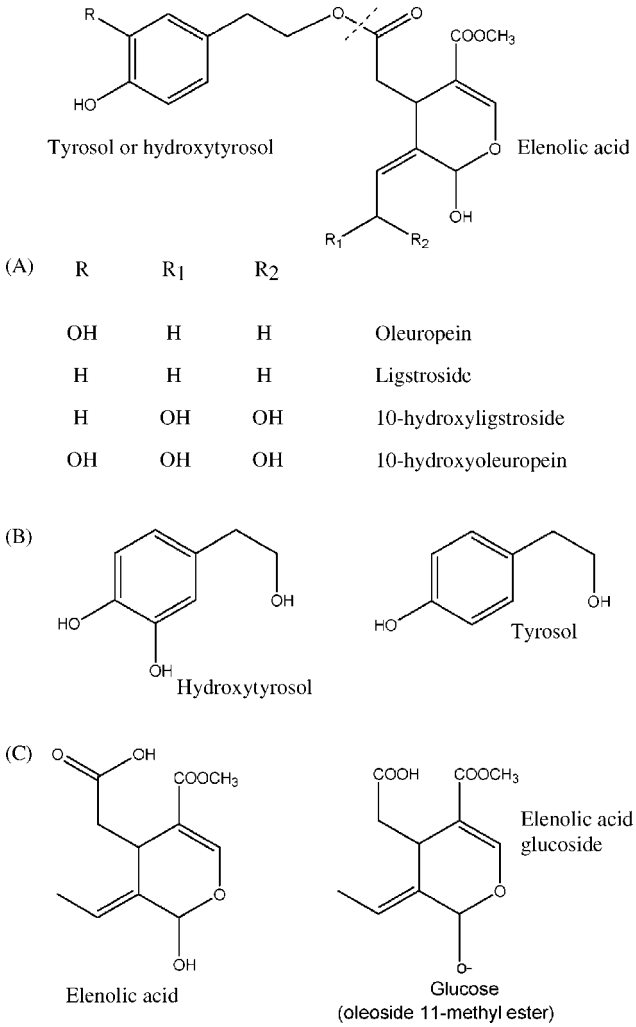

Fig. 1. (A) Chemical structures of oleuropein, ligstroside, 10-hydroxyligstroside and 10-hydroxyoleuropein. Hydroxytyrosol and tyrosol derive from the hydrolysis of oleuropein. (B) Chemical structures of hydroxytyrosol and tyrosol. (C) Chemical structures of elenolic acid and elenolic acid glucocoside.

to which the glucoside is bound (Bianco *et al.* 1998*a*,*b*; Ryan *et al.* 2001).

While tocopherols, phenolic acids, phenolic alcohols and flavonoids are present in many fruits and vegetables belonging to several botanical families, secoiridoids are present exclusively in plants of the family of Olearaceae.

Oleuropein, demethyoleuropein and verbascoside are present in all the constituent parts of the fruit, but more abundantly in the pulp (Soler-Rivas *et al.* 2000) (Fig. 2 (A) and (B)). Nüzhenide has been only found in the seed (Servili *et al.* 1999*a*) (Fig. 2 (C)).

Hydroxytyrosol is one of the main phenolic compounds in olives, virgin oil and waste water obtained during the production of olive oil. In fresh virgin oil, hydroxytyrosol mostly occurs esterified, while in time the non-esterified form prevails owing to hydrolytic reactions (Angerosa *et al.* 1995; Cinquanta *et al.* 1997) (Fig. 1 (A)).

Another group of substances present in the phenolic fraction has been isolated by MS and NMR from

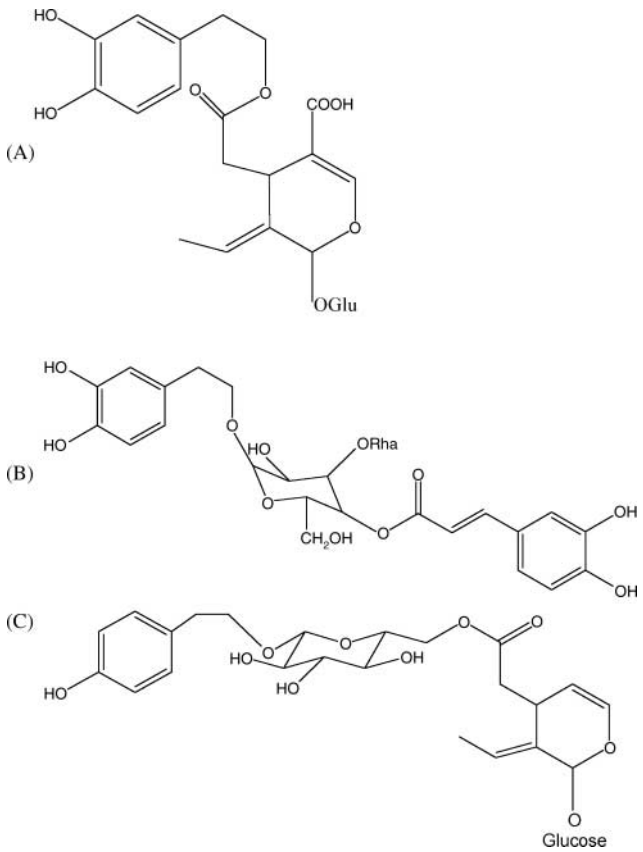

Fig. 2. Chemical structures of demethyloleuropein (A), (B) verbascoside and (C) nüzhenide. OGlu, O-glucose.

extra-virgin olive oil, i.e. lignans, (+)-1-acetoxypinoresinol and (+)-pinoresinol (Owen *et al.* 2000*c*).

The substance (+)-pinoresinol is a common compound of the lignan fraction of several plants, such as the seeds of the species *Forsythia* (Oleaceae family) (Davin *et al.* 1992) and *Sesamum indicum* (sesame) (Kato *et al.* 1998), while (+)-1-acetoxypinoresinol, (+)-1-hydroxypinoresinol and their glycosides have been found in the bark of the *Olea europeae* L. (olive) (Tsukamoto *et al.* 1984, 1985). How lignans are transformed into the main component of the phenolic fraction of olive oil is not known.

They are not present in the pericarp of the olive drupe or in the leaves and sprigs that can be present in the residual vegetable after pressing the olives. It has been recently shown that (+)-pinoresinol is an important component of the phenolic fraction of the olive kernel (Owen *et al.* 2000*c*) (Fig. 3).

Content of phenolic compounds in olive oil

It is necessary to point out that refined oils do not have a significant content of polyphenols. The data on the concentrations of the phenolic compounds, which are responsible for the sensory and antioxidant properties of high-quality olive oils, are not always in agreement. The lack of a suitable analytic methodology is the main cause of inaccuracies in the quantitative evaluation of the phenolic compounds of olive oil. Currently, the commonest methods for evaluating olive oil polyphenol content are the Folin–Ciocalteau colorimetric test and liquid chromatography (Montedoro *et al.* 1992). The former method gives imprecise results because of the reagent's low specificity towards phenolic compounds; also, such methods do not yield quantitative information about single phenolic compounds. On the contrary, HPLC is very sensitive and specific but requires time to perform the analysis (approximately 1 h). It does not supply information regarding phenolic molecules. Standards are therefore not available (Visioli *et al.* 2002).

Mosca *et al.* (2000) described an enzymic test for the quantitative determination of the phenolic compounds of olive oil. This method is rapid and easy to perform; it is more sensitive and specific for phenolic compounds than the Folin–Ciocalteau method, but it supplies only quantitative information and does not detect the important 'minor constituents', i.e. cinnamic and vanillic acids.

Finally, a fast and sensitive method for estimating olive oil phenolic compounds is the combination of MS with atmospheric pressure chemical ionisation. This methodology (Caruso *et al.* 2000) analyses a crude methanolic extract of olive oil, avoiding a complex analytical workup, and also allows quantification of the oleuropein aglycone (Table 1).

In spite of these limits, it is possible to establish some fundamental principles. The quality of the olives and the oil is affected by the amount of oleuropein and its hydrolytic products (Limiroli *et al.* 1995). In turn, the phenolic compound content of the oil depends on the place of cultivation, the climate, the variety, and the olives' level of maturation at the time of harvesting (Cinquanta *et al.* 1997; Visioli & Galli, 1998*b*; Brenes *et al.* 1999). Their level usually diminishes with over-ripening of olives (Monteleone *et al.* 1998; Gutierrez *et al.* 1999), even if there are some exceptions to this rule. For example, olives cultivated in warmer climates, in spite of their faster maturation, produce oils richer in phenols (Visioli *et al.* 1998); also, as we will show later, the phenolic content of olive oil is influenced by the production process.

Oleuropein is the main polyphenol found in olive oil, both in this form and as the aglycone. In nature, it accumulates in the fruit of the olive tree during the growth phase up to 14 % of net weight (Amiot *et al.* 1986); on the contrary, as the olive turns greener, the amount reduces. Finally, when the olive turns dark brown owing to the presence of anthocyanins, the reduction in oleuropein concentration becomes more evident. It has been shown that the oleuropein content is higher in the first stages of fruit maturation and in green cultivars than in black olives.

During the reduction in the levels of oleuropein and other oleosides, such as the quantitatively less important ligstroside, it is possible to observe an increase of other compounds – some more complex, like flavonoids and verbascosides, and others simpler, like single phenols. The reduction in the oleuropein level is also accompanied by an increase in the levels of its glycosylated secondary products, which reach maximum levels in black olives (Amiot *et al.* 1986, 1989; Bianco *et al.* 1993; Soler-Rivas *et al.* 2000). In nature, the concentration of hydroxytyrosol and tyrosol increases as the fruits ripen, in parallel with the hydrolysis of compounds of higher molecular weight, while the total amount of phenolic compounds and α-tocopherol decreases

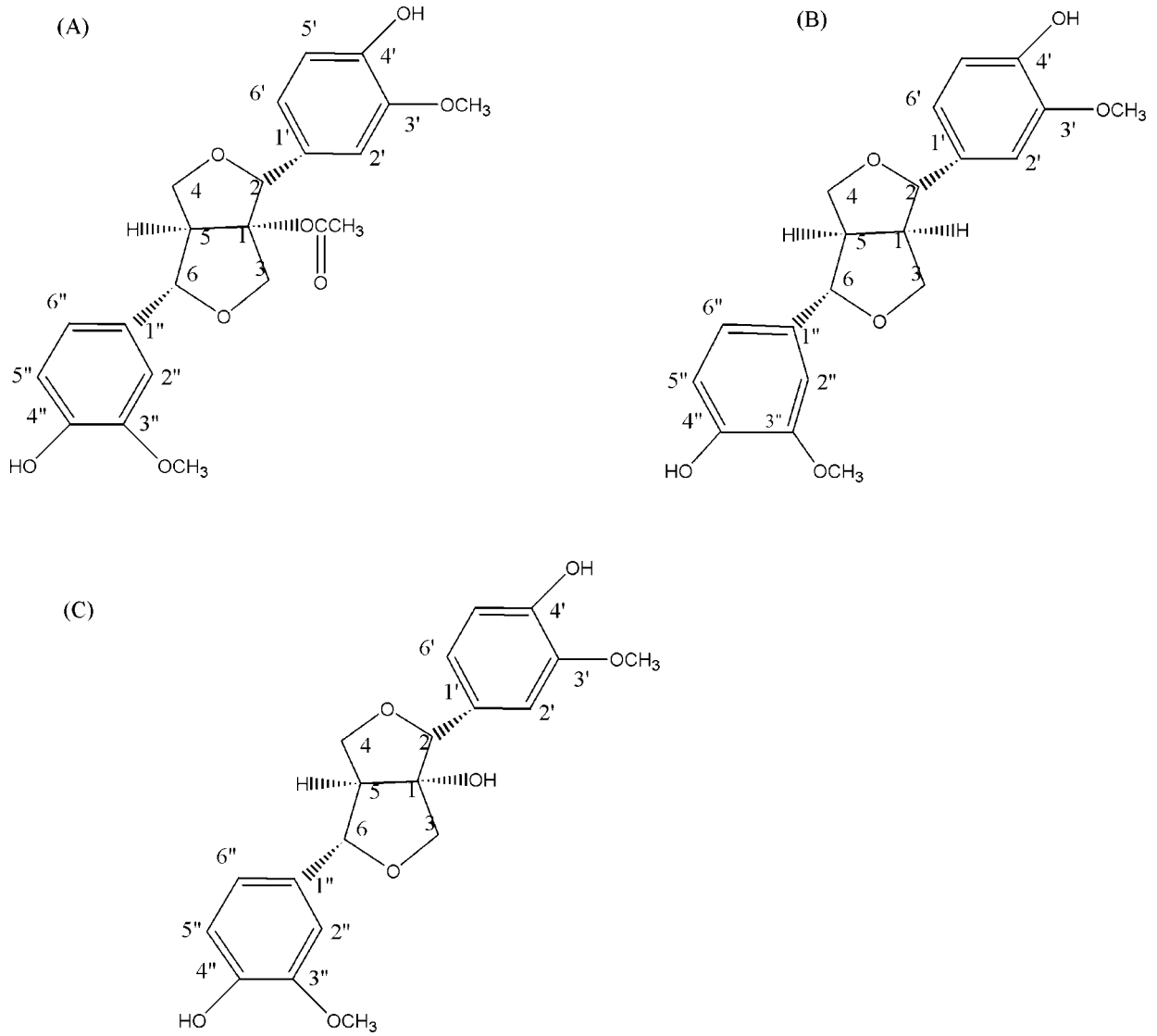

Fig. 3. Chemical structures of the lignans. (A) (+)-1-Acetoxypinoresinol; (B) (+)-1-pinoresinol; (C) (+)-1-hydroxypinoresinol.

as the fruits ripen (Climato *et al.* 1990; Angerosa *et al.* 1995; Limiroli *et al.* 1996; Esti *et al.* 1998; Brenes *et al.* 1999; Gutierrez *et al.* 1999).

Lignans, (+)-1-acetoxypinoresinol and (+)-pinoresinol are not present in seed oils and are virtually absent from refined virgin oils but are present in extra-virgin olive oil up to a concentration of 100 mg/kg. As occurs in simple phenols and secoiridoids, a considerable variation in lignans concentrations between olive oils of various origins also occurs in this case, the reasons probably being related to

Table 1. Methods for the evaluation of the olive oil polyphenols content

Polyphenolic compound	Method employed	Phenol content	Reference
Total phenols	Enzymic assay	566·0–0·8 ppm (mg caffeic acid/kg oil)	Mosca *et al.* (2000)
Oleuropein and its isomers, ligstroside and oleuropein aglycones, deacetoxyligstroside and deacetoxyoleuropein aglycones, 10-hydroxy-oleuropein	APCI–MS		Caruso *et al.* (2000)
Hydroxytyrosol, tyrosol, vanillic, caffeic, syringic, *p*-coumaric, ferulic, cinnamic and elenolic acids	HPLC	Low concentration (total phenols 50–200 mg/kg); medium concentration (total phenols 200–500 mg/kg); high concentration (total phenols 500–1000 mg/kg)	Montedoro *et al.* (1992)

ppm, Parts per million; APCI, atmospheric pressure chemical ionisation.

differences between the production zones, in the climate, in the varieties of olives and in the oil production techniques.

Any alteration in the concentration of the various chemicals changes olive oil's particular taste. Phenolic compounds, and in particular oleuropein, give the oil a bitter taste (Visioli & Galli, 2001).

Effect of oil extraction processes on the content of phenolic compounds

As has been shown, the concentration of phenolic compounds in olive oil is the result of a complex interaction of various factors; for example, the cultivar, the level of maturation and the climate (Cinquanta *et al.* 1997; Esti *et al.* 1998; Monteleone *et al.* 1998; Visioli & Galli, 1998*b*; Visioli *et al.* 1998; Brenes *et al.* 1999; Gutierrez *et al.* 1999). It is also affected by the extraction process. Nowadays, various methods are used to extract olive oil: the traditional discontinuous cycle of pressure; continuous centrifugation; systems of percolation–centrifugation. The crushing of the olives, the pressure applied to the paste, the extraction, the separation of vegetation water and the purification process are all steps common to the three systems of manufacture. Through these three processes, oil, sansa (the solid refuse) and vegetable water are obtained. In the traditional cycle, a grindstone (or stone hammer) is used to mill and press the olives. In continuous cycles, metallic crushers that use hammer, disc and roller are used to mill the olives, and a decanter with a centrifuge, horizontally placed, is used for centrifugation of the paste. A vertical centrifuge is used to separate the oily paste into oil and water (Ranalli *et al.* 1999). Extra-virgin olive oil is obtained from the first physical cold pressure of the olive paste and is rich in phenolic compounds (Visioli *et al.* 1998). Virgin olive oil, obtained through percolation (first extraction), has a higher content in phenols, *o*-diphenols, hydroxytyrosol and tyrosol aglycones, and tocopherols than oils obtained through centrifugation (second extraction) (Ranalli *et al.* 1997, 1998, 1999). The type of rolling-mill used for the pressure and the centrifugation has an important effect. The hammer is more effective in the extraction of phenolic compounds of the olives and should be used for the extraction of oil from olives that have a low content of phenolic compounds, in order to avoid the production of oils with a bitter foretaste. The stone rolling-mill produces oils with a stability towards oxidation similar to that obtained with the hammering-mill, and can be used in order to prepare oil from olives that generally yield oil characterised by a bitter taste (Alloggio & Caponio, 1997).

Oils obtained through centrifugation have a lower phenolic content, probably because this process involves the use of large amounts of hot water that remove a considerable proportion of the phenols that is then eliminated in the watery phase (Lo Scalzo *et al.* 1993; Visioli & Galli, 1998*a*). This vegetable water is regarded as a toxic residue and a pollutant for plants, because the phenolic compounds, hydroxytyrosol, tyrosol and other polyphenols (Capasso *et al.* 1992), have phytotoxic activity (Capasso *et al.* 1995). However, this vegetable water could be used as a good source of phenolic antioxidants (Limiroli *et al.* 1996) or as a bactericidal solution to protect other crops from parasites and from diseases caused by parasites (Capasso *et al.* 1995).

Absorption and pharmacokinetics of polyphenols

It is essential to establish whether olive oil phenolic compounds are absorbed in the intestine and how they are distributed in the organism, to verify if they have the same effects both *in vivo* and *in vitro*. To this purpose, many studies *in vitro* have been carried out, but the results are not satisfactory. An intestinal perfusion technique *in situ* has been developed to estimate oleuropein absorption, both in iso-osmotic and in hypotonic luminal conditions (Edgecombe *et al.* 2000). This technique makes it possible to exclude the influence of the hepatic and renal metabolism and other factors that usually complicate the quantitative evaluation of absorption (Stretch *et al.* 1999). In iso-osmotic conditions, oleuropein is absorbed, with an apparent permeability coefficient (Papp) of 1·47 (SE 0·13) $\times 10^{-6}$ cm/s. The mechanism of absorption is not clear; transcellular transport (carrier Na-dependent glucose transporter 1) or paracellular movement may be involved. In hypotonic conditions, the permeability of oleuropein is significantly higher (5·92 (SE 0·49) $\times 10^{-6}$ cm/s; $P < 0{\cdot}001$). This is probably due to an increase in paracellular movement facilitated by the opening of the paracellular junctions in response to hypotonicity. In an iso-osmotic solution, oleuropein is absorbed at a constant rate of $-0{\cdot}023$ per min (r^2 0·962). Its stability is dependent on pH, since absorption occurs at pH 7. Absorption of oleuropein in such circumstances occurs mainly by way of a transcellular pathway. Since oleuropein is to some extent polar, it is unlikely that it diffuses rapidly through the lipid bilayer of the epithelial cell membrane; a carrier therefore has to be used (Edgecombe *et al.* 2000). As it is a glycoside, oleuropein can probably use a glucose carrier. Three carriers in the epithelial cells of the small intestine have been identified. Two of these (Glut2 and Glut5) carry glucose by facilitated diffusion, while the third is Na-dependent glucose transporter 1, which actively carries the glucose across a concentration gradient (Takata, 1995). Both Glut5 and Na-dependent glucose transporter 1 are on the apical side of intestinal epithelial cells; however, Glut5 is specific for the transport of the fructose, and it is therefore unlikely that it is involved in the absorption of oleuropein (Burant *et al.* 1992; Kane *et al.* 1997).

Glut2 has been localised on the basolateral side of epithelial cells and it probably mediates the passage of glucose and similar substrates from epithelial cells into the circulation (Kayano *et al.* 1990; Nomoto *et al.* 1998). In a study on the absorption and pharmacokinetics of hydroxytyrosol performed in the rat, it was found that the absorption of a single dose of hydroxytyrosol was very rapid: the maximum plasma concentration was obtained in 5–10 min, while after 60 min the concentration was much reduced. However, the concentration of hydroxytyrosol in rat plasma was smaller than the amount administered. This discordance is presumably due to the fact that the experiment did not take into account the presence of hydroxytyrosol metabolites (Bai *et al.* 1998). Studies on the transport kinetics of radiolabelled hydroxytyrosol (^{14}C) performed using

differentiated cells Caco-2 have demonstrated that the transport occurs by passive diffusion (Manna *et al.* 2000).

The metabolic fate of hydroxytyrosol and tyrosol *in vivo* has also been evaluated by administration to rats, both by mouth and intravenously, of the radiolabelled polyphenols. Also in this case, hydroxytyrosol appeared in the plasma, at maximum levels, as soon as 10 min after oral administration. Hydroxytyrosol is quickly eliminated from the plasma and excreted in the urine, as a free compound, and bound to glucuronic acid; to a smaller extent (5 %) it is also eliminated in the faeces (D'Angelo *et al.* 2001; Tuck *et al.* 2001). Conjugation with glucuronic acid is generally regarded as the common final metabolic step of the intact phenolic compounds (Bourne & Rice-Evans, 1998). Other studies carried out *in vivo* in human subjects evaluated the intestinal absorption and urinary excretion of tyrosol and hydroxytyrosol. It was observed that the amount of absorption of these phenols was dose-dependent and that their urinary excretion mostly occurred by conjugation with glucuronic acid (Visioli *et al.* 2000*c*). Urinary excretion of both free phenolic compounds was much higher in the first 4 h and was correlated with the intake: high doses of phenolic compounds increased their rate of conjugation with glucuronide (Visioli *et al.* 2000*c*; Miró Casas *et al.* 2001). In the particular case of hydroxytyrosol, excretion from the human organism occurred in a short time. The estimated hydroxytyrosol elimination half-life was 2·43 h. Free forms of these phenolic compounds were not detected in plasma samples (Miró Casas *et al.* 2003).

The entire quantity of tyrosol or hydroxytyrosol administered was obviously not found in the urine. It remains to be established the quantity not absorbed and that accumulated in organs or erythrocytes, as well as the quantity eliminated after 24 h. Other antioxidants in olive oil could also compete with its intestinal absorption (Tuck *et al.* 2001). The future development of suitable techniques will have to clarify this point.

In the rat, hydroxytyrosol is converted enzymically into four oxidised and/or methylated derivatives. These metabolites have been identified as homovanillic alcohol and acid, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxyphenylacetaldehyde and its sulfate conjugate. Also, a significant fraction of total radioactivity is associated with the sulfate-conjugated derivatives that represent the main urinary products of excretion (D'Angelo *et al.* 2001).

On the basis of the results reported, the pathway of hydroxytyrosol metabolism has been proposed with the participation of catechol-O-methyltransferase (an enzyme involved in the catabolism of the catecholamines), alcohol dehydrogenase, aldehyde dehydrogenase and phenolsulfotransferase (Tuck & Hayball, 2002) (Fig. 4).

After administration of virgin olive oil to healthy volunteers, a significant increase was observed in homovanillic alcohol and acid urinary excretion over 24 h. This suggests that also in man these compounds undergo the action of catechol-O-methyltransferase. Also, the increase in homovanillic acid excretion indicates that in man the ethanolic derived compound of hydroxytyrosol and/or homovanillic alcohol is oxidised (Caruso *et al.* 2001) (Fig. 4).

One should be cautious before extrapolating these results and associating them with the typical Mediterranean diet. The daily intake of olive oil is on average less than 50 ml, an amount that Visioli *et al.* (2000*c*) gave to the subjects in their study in a single dose, and the phenolic content of the oils they used was higher than that of typical virgin olive oil. However, it cannot be excluded that continuous exposure to the phenols in olive oil can in the long run cause phenomena of accumulation, since the absorption of simple phenols (at least at the doses used) appears to be dose-dependent and not saturable (Tuck *et al.* 2001).

The antioxidant activity of the polyphenolic compounds

The 'reactive oxygen species' (ROS), which are continuously formed as a result of normal metabolic processes, can oxidise and damage cellular macromolecules, possibly leading to the development of degenerative diseases (for example, atherosclerosis, cancer, diabetes, rheumatoid arthritis and inflammatory diseases). Exogenous antioxidants are important because they have a twofold function, preventing food oxidation – and in particular lipid oxidation – and at the same time increasing the amount of antioxidant agents present in the organism, protecting against degenerative diseases. The most important dietary antioxidants are certain vitamins (ascorbic acid, tocopherols, carotenes) and phenolic compounds, which are present in various foods of vegetable origin characteristic of the Mediterranean diet, such as olive oil (Berra *et al.* 1995).

Phenolic compounds can act as antioxidants in various ways. In oxidative systems using transition metals such as Cu and Fe, they can chelate metallic ions, which can prevent their involvement in Fenton reactions that can generate high concentrations of hydroxyl radicals (Halliwell & Gutterige, 1990; Halliwell *et al.* 1995). However, the most important antioxidant activity is related to the free radical-scavenging ability, by breaking the chain of reactions triggered by free radicals. The antioxidant properties of the *o*-diphenols are associated with their ability to form intramolecular hydrogen bonds between the hydroxyl group and the phenoxylic radicals (Visioli & Galli, 1998*b*) (Fig. 5). As similar studies on the flavonoids have already shown, the degree of antioxidant activity is correlated with the number of hydroxyl groups (Rice-Evans *et al.* 1996; Cao *et al.* 1997). The number of –OH groups and their positions on the ring are important for both flavonoids and phenols. From the study of the resonance structures formed during the oxidation processes, it can be observed that the *ortho*- and *para*-substitutes of the radicals are more stable than the *meta*-substitute (Finotti & Di Majo, 2003). In particular, *ortho*-diphenolic substitution gives high antioxidant ability, while a single hydroxyl substitution, as in tyrosol, does not confer any activity, since tyrosol does not protect LDL from chemically induced oxidation.

Although olive oil contains a relatively low concentration of α-tocopherol, it is known to be highly resistant to oxidative degradation. This is due, in part, to the relatively low content of PUFA and also to the high concentration of polyphenolic antioxidants, particularly in extra-virgin olive oil. The antioxidant activity of olive oil phenolic compounds, and in particular of oleuropein and its by-product hydroxytyrosol, has been studied in many experimental models: with the use of transition metals; the

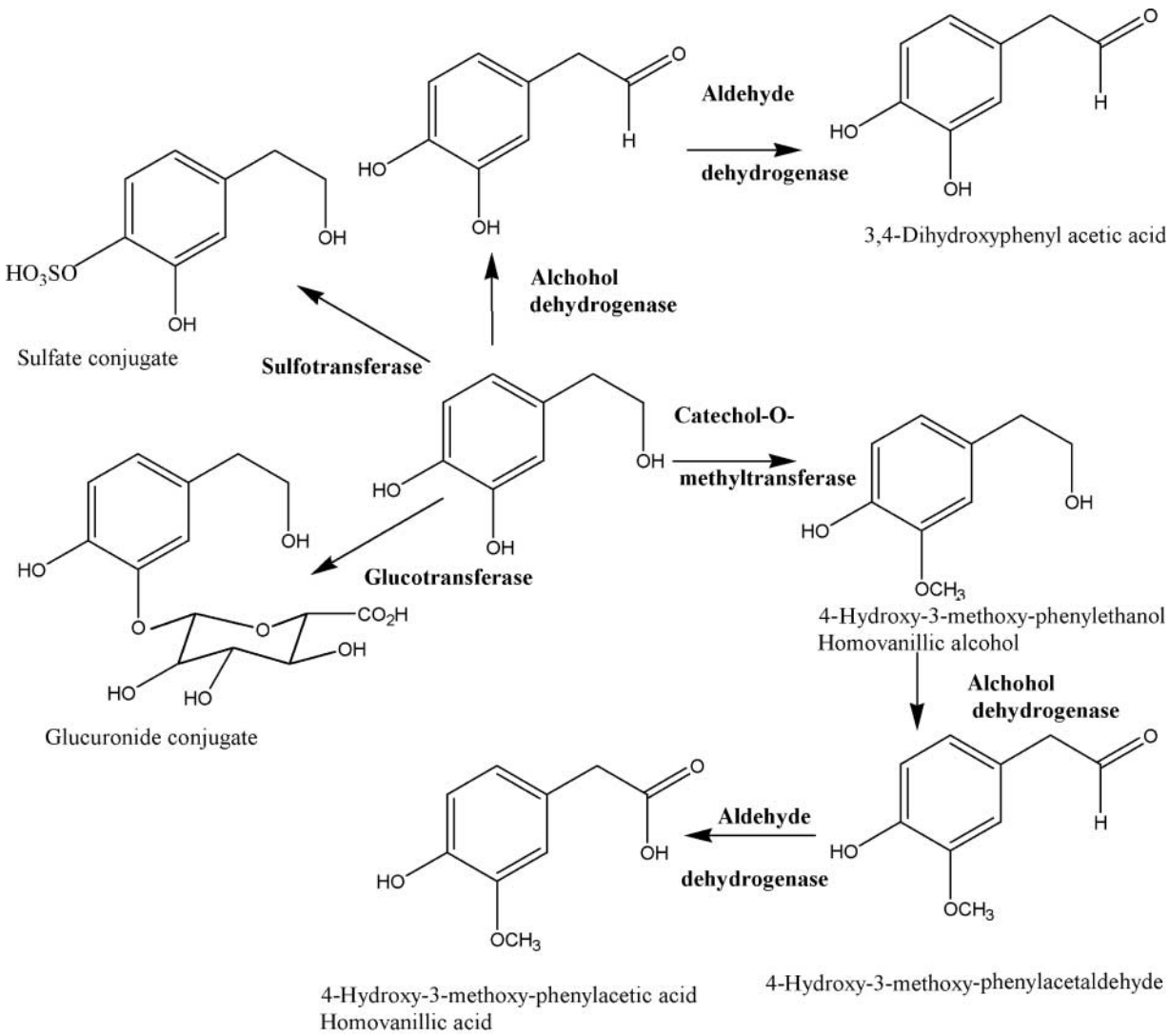

Fig. 4. Postulated enzymic pathways for the metabolites of hydroxytyrosol *in vivo*.

chemically induced oxidation of LDL; ROS formation, for example the radicals superoxide and trichlormethylperoxylic, and hypochlorous acid (Aeschbach *et al.* 1994; Salami *et al.* 1995; Visioli *et al.* 1995*a*, 1998; Aruoma *et al.* 1998). By estimating the antioxidant activity of these polyphenolic compounds on the basis of their ability to inhibit the formation of peroxides, it has been shown that hydroxytyrosol and caffeic and protocatechuic acids have a higher protective activity (Papadopoulos & Boskou, 1991). The antioxidant activity of oleuropein and hydroxytyrosol has also been demonstrated in cellular models and animals (Manna *et al.* 1997; Speroni *et al.* 1998).

Some polyphenols can contribute to the regeneration of vitamin E, as has been demonstrated by treating human lipoproteins *in vitro* with peroxides.

In a recent study, the antioxidant activity of α-tocopherol and phenolic extracts from olives and olive oil was compared over time. It was demonstrated that in the first 15 min the scavenger activity of α-tocopherol was higher but soon terminated. The extract from stoned olives and oil contained compounds that continued to reduce the concentration of these radicals more slowly; when on the other hand the reaction time was delayed to 60 min, all the extracts of the olives were much more active than α-tocopherol. On day 6 the extracts of the olives and the oil continued to be more effective than α-tocopherol (Keceli & Gordon, 2001).

The biological activity of phenolic compounds of olive oil is not limited to their antioxidant ability but extends to their interaction with important enzymic systems. In particular, it has been found out that olive oil phenols:

inhibit platelet aggregation;
reduce pro-inflammatory molecule formation such as thromboxane B_2 and leucotriene B_4;
inhibit the use of oxygen in human neutrophils;
increase NO production by the macrophages of rats

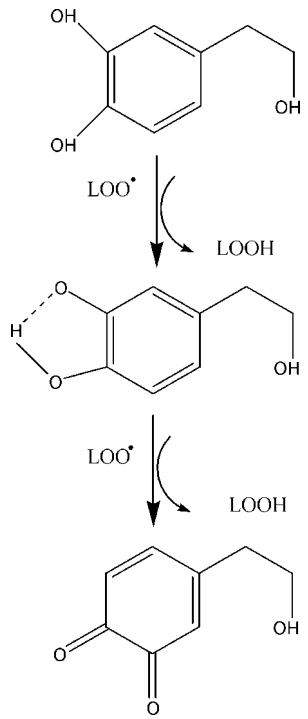

Fig. 5. Lipoperoxidation. LOO•, lipoperoxyl radical.

exposed to endotoxin – they therefore act by up regulating the immune system.

Other biological actions of phenolic compounds have been discovered that can be important for their effects on human health. For example, caffeic acid could have cytoprotective effects on endothelial cells, correlated not only with its action as an antioxidant agent but also with its ability to block the increase of the concentration of intracellular Ca^{2+} in response to lipoprotein oxidation (Vieira *et al.* 1998). The ability of polyphenolic compounds to react with metal ions could make them pro-oxidant. It has in fact been widely observed that caffeic acid, a simple polyphenol with an *ortho*-diphenolic structure, can have pro-oxidant activity on LDL oxidation induced by Cu^{2+} (Yamanaka *et al.* 1997). However, this pro-oxidant activity has been found only in the propagation phase of oxidation, and not in the initiation phase, in which caffeic acid inhibits lipoprotein oxidation, as has been found in previous studies (Laranjinha *et al.* 1994; Nardini *et al.* 1995).

The effects of the antioxidant activity of olive oil polyphenols on the integrity and function of the cells have been studied in erythrocytes and intestinal cells (Caco-2). The capacity of polyphenols to prevent damage in these cells was verified when they were exposed to oxidative stress, as in treatment with H_2O_2. Human erythrocytes were chosen because they are the cells most exposed to oxidative risk, since their specific role is to carry oxygen. The main target of H_2O_2 is Hb, which is oxidised to methaemoglobin. Exposure of erythrocytes to H_2O_2 also causes lipid peroxidation, and alterations in proteins, for example the formation of carbonyl dimers. As a consequence of this oxidative damage, the shape of the erythrocytes changes, causing haemolysis. The spontaneous oxidation of Hb produces superoxide anion radicals that cause the dismutation of H_2O_2. In the presence of reduced metal ions, especially Fe, these compounds form the highly reactive hydroxyl radical that can damage the cellular membrane, with consequent haemolysis (Sadrzadeh *et al.* 1984; van Dyke & Saltman, 1996). Some studies on isolated erythrocyte membranes have demonstrated that the ATP-dependent ion transport (such as amino acid transport) is considerably compromised by oxidative damage (Rohn *et al.* 1993). Under physiological conditions, ROS are quickly removed by both enzymic and non-enzymic systems; however, if ROS production is excessive, or if antioxidant defence is impaired, serious oxidative damage can occur, to both the plasma membrane and the cytosol, which finally leads to haemolysis. Erythrocytes pre-treated with phenols extracted from extra-virgin olive oil show significantly less lipid oxidation and haemolysis after treatment with H_2O_2.

In erythrocytes pre-treated with H_2O_2 and incubated in the presence of [^{3}H]methionine or [^{3}H]leucine, there is a marked reduction in the absorption of both the amino acids compared with control erythrocytes.

3,4-Dihydroxyphenyl-ethanol, or hydroxytyrosol, prevents the alteration of amino acid transport by H_2O_2 in intact erythrocytes (Manna *et al.* 1999). Similarly in intestinal tumour cells (Caco-2) treated with H_2O_2, pre-treatment with olive oil polyphenols exerts a strong antioxidant effect. H_2O_2 induces a clear increase in the intracellular concentration of malonyldialdehyde and the paracellular transport of inulin, respectively indicating the occurrence of lipid peroxidation and changes in cellular permeability. Pre-incubation of the Caco-2 cells with hydroxytyrosol totally prevents the alterations induced by H_2O_2 (Manna *et al.* 1999).

Polyphenolic compounds in the prevention of atherosclerosis

Plasma LDL is atherogenic only after oxidative modification (Brown & Goldstein, 1983; Parthasarathy, 1991); some studies have shown that oxidative stress provokes the onset of atherosclerosis by inducing lipid peroxidation (Halliwell, 1997). From this point of view, antioxidants that can prevent lipid peroxidation can have an important role in preventing oxidative modification of LDL. Human LDL contain a variety of antioxidants capable of inhibiting peroxidation, such as α-tocopherol, ubiquinol-10, β-carotene, lycopene and other hydroxy-carotenoids. α-Tocopherol is the most abundant antioxidant in LDL (Princen *et al.* 1992; Abbey *et al.* 1993; Reaven *et al.* 1993; Jialal *et al.* 1995); however, it has been demonstrated that other antioxidants are also able to protect LDL from oxidation (Cominacini *et al.* 1991; Esterbauer *et al.* 1992). On the basis of previous epidemiological studies pointing out the direct correlation between the Mediterranean diet and a lower incidence of cardiovascular diseases (Hertog *et al.* 1993), various studies performed *in vitro* and *in vivo* (Table 2) have shown that the polyphenolic compounds of extra-virgin olive oil play an important role in the prevention of atherosclerotic damage through their inhibition of LDL oxidation (Visioli *et al.* 1995*a*; Rice-Evans *et al.* 1996; Cao *et al.* 1997; Masella *et al.*

Table 2. Biological properties of olive oil phenolics

Polyphenolic compound	Mechanism of action	Salutary effect on human health
Oleuropein, hydroxytyrosol, caffeic acid, protocatechuic acid and 3,4-dihydroxy-phenylethanol-elenolic acid	Inhibition of LDL oxidation, both *in vitro* and *in vivo*; inhibition of HMG-CoA reductase; inhibition of thromboxane B_2 and consequently platelet aggregation	Prevention of cardiovascular diseases
Secoiridoids (hydroxytyrosol and tyrosol) and lignans	Inhibitory action on activity of xanthine oxidase and reduction of superoxide formation; lignans act as anti-oestrogens and increase sex hormone-binding globulin	Prevention of tumoral diseases
Hydroxytyrosol and other polyphenolics	Inhibitory action on cyclo-oxygenase and lipo-oxygenase; reduce pro-inflammatory molecule formation such as thromboxane B_2 and leucotriene B_4	Anti-inflammatory activity
Oleuropein; verbascoside (hydroxytyrosol and tyrosol)	Inhibition of viral and bacterial growth and activity	Antimicrobial and antiviral activity

HMG, 3-hydroxy3-methylglutaryl.

1999). In a sample of LDL, the vitamin E oxidation induced by $CuSO_4$ was prevented by the addition of hydroxytyrosol or the secondary compounds of oleuropein; this effect was linearly correlated with the hydroxytyrosol concentration. In LDL, the addition of polyphenolic compounds caused significant reduction in lipid peroxide formation. In LDL not treated with polyphenolic compounds, these lipid peroxides are formed at the same time as the reduction of vitamin E levels. This vitamin E depletion by LDL occurs before massive lipid peroxidation. Phenolic compounds thus delay the beginning of the oxidative process, preserving the endogenous antioxidant pool (Visioli *et al.* 1995*a*, 2000*a*).

The antioxidant effect of the various polyphenolic compounds of olive oil has recently been compared. The results show that protocatechuic and 3,4-dihydroxy-phenylethanol-elenolic acids have an antioxidant activity comparable with that of caffeic acid, oleuropein and 3,4-dihydroxyphenyl-ethanol in hydroxytyrosol (Masella *et al.* 1999). Some studies of the antioxidant effect of polyphenolic compounds on plasma LDL have been performed, in an attempt to simulate as well as possible the situation *in vivo*. Plasma was incubated with various olive oil phenols; LDL was subsequently isolated and subjected to the action of free radicals, in order to test the relative resistance to oxidation. The results indicate that hydroxytyrosol and oleuropein are more effective than monohydroxyphenols (tyrosol and ligstroside aglycone), confirming previous results (Rice-Evans *et al.* 1996; Cao *et al.* 1997). However, the concentration of antioxidants added to whole plasma to inhibit LDL oxidation was substantially higher than in previous studies, where the antioxidants were directly added to isolated LDL (Leenen *et al.* 2002). These data confirm other studies performed *in vivo* on animals fed with phenol-rich olive oils; in these animals, the lipoproteins were much more resistant to oxidation than in other control animals fed with equal amounts of oleic acid (Scaccini *et al.* 1992), care being taken to maintain constant levels of vitamin E (Wiseman *et al.* 1996).

Another important risk factor for the onset of atherosclerosis is a high blood concentration of cholesterol. The regulation of plasma cholesterol is related to the activity of 3-hydroxy3-methylglutaryl (HMG)-CoA reductase, the first enzyme involved in the synthesis of cholesterol. The use of substances inhibiting HMG-CoA reductase (statins) is very effective in blood cholesterol reduction. Some studies have focused attention on the effect of the polyphenolic compounds contained in virgin olive oil on cholesterol metabolism, and recently it has been demonstrated that the activity of HMG-CoA reductase (Table 2) is significantly diminished in the liver microsomes of rats fed with the polyphenolic compounds. The inhibition of HMG-CoA reductase by polyphenolic compounds may thus represent a beneficial effect through olive oil ingestion and play an important role in the prevention of cardiovascular diseases. However, further studies are necessary in order to test the concentration of polyphenolic compounds capable of eliciting a therapeutic response (Benkhalti *et al.* 2002).

Polyphenolic compounds in the prevention of cancer

Many vegetable foods contain substances possessing anticancer properties (Huang *et al.* 1994; Johnson *et al.* 1994; Pezzuto, 1997), most of them active as antioxidants (Aruoma, 1994). Since ROS have been implicated in the genesis of tumours, the study of the antitumoral activity of olive oil phenolic compounds is very interesting.

Peroxynitrites ($ONOO^-$) are highly reactive compounds capable of inducing peroxidation in lipids, oxidising methionine and damaging the DNA by deamination and nitration (Yermilov *et al.* 1995). Peroxynitrites are formed by reaction between NO and $O_2^{\cdot -}$ (superoxide radical). The deamination of guanine and adenine causes breaks in the DNA chain, with consequent mutations (de Rojas-Walker *et al.* 1995); DNA oxidation is also potentially mutagenic (Newcomb & Loeb, 1998). *In vitro*, the presence of hydroxytyrosol reduces the biochemical effects of peroxy-nitrites, such as the deamination of adenine and guanine in some cell lines (Deiana *et al.* 1999).

The antioxidant activity of virgin olive oil extracts, shown *in vitro* by their ability to inhibit the effect of oxygen radicals on salicylic acid, is apparent at concentrations much lower than those of the single antioxidant compounds tested individually; this is probably due to the presence of other polyphenolic compounds, some of which are still unknown (Owen *et al.* 2000*a*). In addition to this action, extracts of

virgin olive oil show an inhibitory action on the activity of xanthine oxidase (Table 2), with a consequent reduction in superoxide formation. This action cannot be demonstrated for simple polyphenolic compounds (tyrosol and hydroxytyrosol) but it is due to secoiridoids and lignans (Owen *et al.* 2000*a*). An adequate intake of olive oil therefore has a double action: it gives protection from the effects of oxygen radicals and reduces the activity of xanthine oxidase, an enzyme potentially involved in carcinogenesis (Tanaka *et al.* 1997).

Among the substances possessing anticancer activity, the lignans are of special interest. It has been demonstrated that they inhibit the development of various kind of tumours: cutaneous, mammary, colonic, and pulmonary (Hirano *et al.* 1990; Kardono *et al.* 1990). In animals, the administration of flax seeds (a notable source of lignans) prevents the onset of mammary carcinoma (Serraino & Thompson, 1991, 1992; Thompson *et al.* 1996). The antitumoral effect of the lignans is based both on their antioxidant activity (Prasad, 1997; Owen *et al.* 2000*b*) and on their antiviral activity (Schroder *et al.* 1990). Also, the structural similarity to oestradiol and the synthetic anti-oestrogen tamoxifen suggests that the lignans can act, in part, as anti-oestrogens (Table 2). This is because they are able to inhibit the synthesis of oestradiol in the placenta (Adlercreutz *et al.* 1993) and adipose tissue (Wang *et al.* 1994), as well as the proliferation of breast cancer cells induced by oestrogens (Mousavi & Adlercreutz, 1992), and to increase sex hormone-binding globulin (a plasma protein carrier of sexual steroids) levels, with a consequent reduction in the biologically active levels of free oestrogens (Adlercreutz *et al.* 1992).

Some of these effects are particularly important in the pathogenesis of mammary carcinoma in obese women. In obesity, the plasma levels of sex-hormone-binding globulin are reduced, with consequent higher plasma levels of free oestrogens. The mammary cells, which are typically hormone-sensitive, are constantly exposed to the action of high amounts of oestrogens (Schapira *et al.* 1991; Colditz, 1993; Maggino *et al.* 1993; Kissebah & Krakower, 1994; Hankinson *et al.* 1995). Also, inhibition by lignans of oestrogen synthesis in adipose tissue is fundamental in the prevention of breast cancer in obese woman, since adipose tissue is not only an energy-store tissue but also carries out an important endocrine function. It picks up and metabolises steroid hormones, converting androstenedione into oestrone (E1) and testosterone into 17-β-oestradiol (E2) (De Pergola *et al.* 1996).

The anticancer effect of the lignans is therefore probably due to their action on the metabolism of oestrogens.

Phenolic components as compounds with anti-inflammatory activity

Lipid radicals are also produced during reactions involved in the metabolism of arachidonic acid, during the synthesis of the eicosanoids by the action of the lipo-oxygenase and cyclo-oxygenase (Table 2). During these reactions, the radicals that are generated are partially inactivated by glutathione peroxidase (Eling *et al.* 1986; Mirochnitchenko *et al.* 2000). Some studies hypothesise an inhibitory activity on cyclo-oxygenase (Petroni *et al.* 1995; de La Puerta *et al.* 2000) and lipo-oxygenase by olive oil phenolic compounds (Kohyama *et al.* 1997; De La Puerta *et al.* 1999; Martinez-Dominguez *et al.* 2001). Considering the functions of the prostaglandins and leucotrienes, the results of these studies have important implications for the genesis of the inflammatory response and for atherosclerosis. In one of these studies, the effects of hydroxytyrosol and of the polyphenols extracted from waste waters were examined *in vitro* in parameters of platelet activity. It was found that the hydroxytyrosol and polyphenols extracted from waste waters inhibited *in vitro* platelet aggregation induced by collagen and thromboxane B_2 production. The effectiveness of hydroxytyrosol in inhibition of the aggregation induced by collagen is similar to that of aspirin, a drug that is well known for its powerful activity in platelet anti-aggregation and cyclo-oxygenase inhibition (Petroni *et al.* 1995).

Polyphenols as compounds with antimicrobial activity

The bacteriostatic and bactericidal activities (Table 2) of oleuropein and the hydrolysis products, hydroxytyrosol and tyrosol, have been studied *in vitro* in comparison with many pathogenic micro-organisms: bacteria, fungi, viruses and protozoa (Hirschman, 1972; Federici & Bongi, 1983; Bisignano *et al.* 1999). Oleuropein and the hydrolysis products are able to inhibit the development and production of enterotoxin B by *Staphylococcus aureus*, the development of *Salmonella enteritidis* and the germination and consequent development of spores of *Bacillus cereus* (Walter *et al.* 1973; Tassou *et al.* 1991; Tranter *et al.* 1993; Tassou & Nychas, 1994, 1995). Oleuropein and other phenolic compounds (*p*-hydroxybenzoic, vanillic and *p*-coumaric acids) completely inhibit the development of *Klebsiella pneumoniae*, *Escherichia coli* and *B. cereus* (Aziz *et al.* 1998). Verbascoside shows antibacterial activity against *Staphylococcus aureus*, *E. coli* and other bacteria of clinical interest; it also shows antiviral activity against the syncytial virus, which affects the human respiratory system (Calis *et al.* 1988; Pardo *et al.* 1993; Chen *et al.* 1998; Kernan *et al.* 1998). Hydroxytyrosol is highly toxic to *Pseudomonas syringae pv savastanoi* and *Corynebacterium michiganense*, which are both phytopathogenic, and tyrosol may act as a mycotoxin (Venkatasubbaiah & Chilton, 1990; Capasso *et al.* 1995). Both phenols therefore protect the drupe from attack by pathogenic agents.

It is not clear why the polyphenolic compounds of olive oil have such a wide antimicrobial activity. They may cause surface activity that damages the membranes of bacterial cells (Juven *et al.* 1972). However, oleuropein, in spite of tyrosol, is ineffective against some bacterial chains (*Moraxella catarrhalis* and *Haemophilus influenzae*): in fact the presence in its chemical structure of the glycosidic group is responsible for the steric hindrance, which blocks the passage through the cell membrane. This simply does not make sense. Whatever the case, the antibacterial activity of olive oil's phenolic compounds is due to the presence of the *ortho*-diphenolic system (catechol) (Bisignano *et al.* 1999).

These data indicate that the active compounds of olive oil, in addition to their use as food additives, could also be used

as a potential antimicrobial agent in the treatment of some infections. Oleuropein can also interfere with the synthesis of amino acids necessary for viral activity, and in this way it prevents the diffusion, development and attack on the cell membrane, it inhibits reproduction and, in the case of retroviruses, it inhibits the production of reverse transcriptase and protease. Finally, oleuropein stimulates phagocytosis as a response of the immune system against pathogenic micro-organisms (Hirschman, 1972).

While a bactericidal effect has been observed on a wide range of bacteria, no effect has been observed on yeasts (Beuchat & Golden, 1989). However, oleuropein has some influence, though only slight, on the delay of the development and sporulation of *Aspergillus parasiticus*; also, the production of aflatoxin is notably reduced (Gourama & Bullerman, 1987).

Conclusion

The positive correlation between the Mediterranean diet and the low incidence of cardiovascular diseases and certain kinds of cancer (breast, prostate, intestine and skin cancer) leads us to conclude that a diet rich in grain, legumes, fresh fruit, vegetables, wine in moderate amounts and olive oil has beneficial effects on human health.

On the one hand, these effects are due to the high MUFA:saturated fatty acid ratio; on the other hand, some components of the Mediterranean diet, such as fibre, vitamins, flavonoids and polyphenolic compounds, play an important role in the prevention of these diseases (Visioli, 2000).

The normal consumption of extra-virgin olive oil, which is rich in polyphenolic compounds, antioxidant substances that combat the free radicals, could contribute, in appropriate amounts (three to five spoonfuls per d, in a balanced diet), together with other biologically active compounds, to reduce the risk of development of these pathologies.

Finally, nowadays the interest of the pharmaceutical industries in natural antioxidants is constantly growing; the waste waters produced by the processing of olive oil could represent a cheap source of polyphenolic compounds, as yet unused (Visioli *et al.* 1995*b*; Capasso *et al.* 1999; Mulinacci *et al.* 2001).

References

Abbey M, Nestel PJ & Baghurst PA (1993) Antioxidant vitamins and low density lipoprotein oxidation. *American Journal of Clinical Nutrition* **58**, 52.

Adlercreutz H, Bannwart C, Wähälä K, Makela T, Brunow G, Hase T, Arosemena PJ, Kellis JT Jr & Vickery LE (1993) Inhibition of human aromatase by mammalian lignans and isoflavoid phytooestrogens. *Journal of Steroid Biochemistry and Molecular Biology* **44**, 147–153.

Adlercreutz H, Mousavi Y, Clark J, Hockerstedt K, Hamalainen E, Wähälä K, Makela T & Hase T (1992) Dietary phytoestrogens and cancer: in vitro and in vivo studies. *Journal of Steroid Biochemistry and Molecular Biology* **41**, 331–337.

Aeschbach R, Loliger J, Scott BC, Murcia A, Butler J, Halliwell B & Aurome OI (1994) Antioxidant actions of tymol, carvacrol, 6-gingerolo, zingerone and hydroxytyrosol. *Food and Chemical Toxicology* **32**, 31–36.

Alarcon de la Lastra C, Barranco MD, Motilova V & Herrerias JM (2001) Mediterranean diet and health: biological importance of olive oil. *Current Pharmaceutical Design* **7**, 933–950.

Alloggio V & Caponio F (1997) The influence of olive paste preparation techniques on the quality of olive oil. II. Evolution of phenolic substances and of some quality parameters referred to the ripening of drupes in virgin olive oil from the Coratina cv. *Rivista Italiana Sostanze Grasse* **74**, 443–447.

Amiot MJ, Fleuriet A & Macheix JJ (1986) Importance and evolution of phenolic compounds in olive during growth and maturation. *Journal of Agricultural and Food Chemistry* **34**, 823–826.

Amiot MJ, Fleuriet A & Macheix JJ (1989) Accumulation of oleuropein derivatives during olive maturation. *Phytochemistry* **28**, 67–69.

Angerosa F, D'Alessandro N, Konstantinou P & Di Giacinto L (1995) GC–MS evaluation of phenolic compounds in virgin olive oil. *Journal of Agricultural and Food Chemistry* **43**, 1802–1807.

Aruoma OI (1994) Nutrition and health aspects of free radicals and antioxidants. *Food and Chemical Toxicology* **32**, 671–683.

Aruoma OI, Deiana M, Jenner A, Halliwell B, Harparkash K, Banni S, Corongiu FF, Dessì MA & Aeschbach R (1998) Effect of hydroxytyrosol found in extra virgin olive oil on DNA damage and on low-density lipoprotein oxidation. *Journal of Agricultural and Food Chemistry* **46**, 5181–5187.

Aziz NH, Farag SE, Mousa LAA & Abo Zaid MA (1998) Comparative antibacterial and antifungal effects of some phenolic compounds. *Microbios* **93**, 43–54.

Bai C, Yan X, Takenake M, Sekiya K & Nagata T (1998) Determination of synthetic hydroxytyrosol in rat plasma by GC-MS. *Journal of Agricultural and Food Chemistry* **46**, 3998–4001.

Benkhalti F, Prost J, Paz E, Perez-Jimenez F, El Modafar C & El Boustani E (2002) Effects of feeding virgin olive oil or their polyphenols on lipid of rat liver. *Nutrition Research* **22**, 1067–1075.

Berra B, Caruso D, Cortesi N, Fedeli E, Rasetti MF & Galli G (1995) Antioxidant properties of minor polar components of olive oil on the oxidative processes of cholesterol in human LDL. *La Rivista Italiana delle Sostanze Grasse* **72**, 285–288.

Beuchat LR & Golden DA (1989) Antimicrobials occurring naturally in foods. *Food Technology* **43**, 134–142.

Bianco A, Lo Scalzo R & Scarpati ML (1993) Isolation of cornoside from Olea europaea and its transformation into halleridone. *Phytochemistry* **32**, 455–457.

Bianco A & Uccella N (2000) Biophenolic components of olives. *Food Research International* **33**, 475–485.

Bianco AD, Mazzei RA, Melchioni C, Romeo G, Scarpati ML & Uccella N (1998*a*) Microcomponents of olive oil. Part II: digalactosyldiacylglycerols from *Olea europaea*. *Food Chemistry* **62**, 343–346.

Bianco AD, Mazzei RA, Melchioni C, Romeo G, Scarpati ML & Uccella N (1998*b*) Microcomponents of olive oil-III. Glucosides of 2 (3, 4-dihydroxy-phenyl)ethanol. *Food Chemistry* **63**, 461–464.

Bisignano G, Tomaino A, Lo Cascio R, Crisafi G, Uccella N & Saija A (1999) On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol. *Journal of Pharmacy and Pharmacology* **51**, 971–974.

Bonanome A, Pagnan A, Biffanti S, Opportuno A, Sorgato F, Dorella M, Maiorino M & Ursini F (1992) Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. *Arteriosclerosis and Thrombosis* **12**, 529–533.

Bourne LC & Rice-Evans CA (1998) Urinary detection of hydroxycinnamates and flavonoids in humans after high dietary intake of fruit. *Free Radical Research* **28**, 429–438.

Brenes M, Garcia A, Garcia P, Rios JJ & Garrido A (1999) Phenolic compounds in Spanish olive oils. *Journal of Agricultural and Food Chemistry* **47**, 3535–3540.

Brown MS & Goldstein JL (1983) Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annual Review of Biochemistry* **52**, 223.

Burant C, Takeda J, Brot-Laroche E, Bell G & Davidson N (1992) Fructose transporter in human spermatozoa and small intestine is Glut5. *Journal of Biological Chemistry* **267**, 14523–14526.

Calis I, Saracoglu I, Zor M & Alacam R (1988) Antimicrobial activities of some phenylpropanoid glycosides isolated from Scrophularia scopoli. *Turk Tip Eczacilik Dergisi* **12**, 230–233.

Cao G, Sofic E & Prior RL (1997) Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radical Biology and Medicine* **22**, 749–760.

Capasso R, Cristinzio G, Evidente A & Scognamiglio F (1992) Isolation, spectroscopy and selective phytotoxic effects of polyphenols from vegetable waste waters. *Phytochemistry* **31**, 4125–4128.

Capasso R, Evidente A, Avolio S & Solla F (1999) A highly convenient synthesis of hydroxytyrosol and its recovery from agricultural waste waters. *Journal of Agricultural and Food Chemistry* **47**, 1745–1748.

Capasso R, Evidente A, Schivo L, Orru G, Marcialis MA & Cristinzio G (1995) Antibacterial polyphenols from olive oil mill waste waters. *Journal of Applied Bacteriology* **79**, 393–398.

Caruso D, Colombo R, Patelli R, Giavarini F & Galli G (2000) Rapid evaluation of phenolic component profile and analysis of oleuropein aglycon in olive oil by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS). *Journal of Agricultural and Food Chemistry* **48**, 1182–1185.

Caruso D, Visioli F, Patelli R, Galli C & Galli G (2001) Urinary excretion of olive oil phenols and their metabolites in humans. *Metabolism Clinical and Experimental* **50**, 1426–1428.

Chen J, Blanc P, Stoddart CA, Bogan M, Rozhon EJ, Parkinson N, Ye Z, Cooper R, Balick M, Nanakorn W, Kernan MR, Chen JL & Ye ZJ (1998) New iridoids from the medicinal plant Barleria prionitis with potent activity against respiratory syncytial virus. *Journal of Natural Products* **61**, 1295–1297.

Cinquanta L, Esti M & La Notte E (1997) Evolution of phenolic compounds in virgin olive oil during storage. *Journal of the American Oil Chemists Society* **74**, 1259–1264.

Climato A, Mattei A & Osti M (1990) Variation of polyphenol composition with harvesting period. *Acta Horticulturae* **286**, 453–456.

Colditz GA (1993) Epidemiology of breast cancer. Findings from the Nurses' Health Study. *Cancer* **71**, 1480–1489.

Cominacini L, Garbin U, Cenci B & Davoli A (1991) Predisposition to LDL oxidation during copper-catalyzed oxidative modification and its relation to α-tocopherol content in humans. *Clinica Chimica Acta* **204**, 57–68.

D'Angelo S, Manna C, Migliardi V, Mazzoni O, Morrica P, Capasso G, Pontoni G, Galletti P & Zappia V (2001) Pharmacokinetics and metabolism of hydroxytyrosol, a natural antioxidant from olive oil. *Drug Metabolism and Disposition* **29**, 1492–1498.

Davin BD, Bedgar DL, Katayama T & Lewis NG (1992) On the stereoselective synthesis of (+)-pinoresinol in *Forsythia suspensa* from its achiral precursor, coniferyl alcohol. *Phytochemistry* **31**, 3869–3874.

Deiana M, Aruoma OI, Bianchi MDLP, Spencer JPE, Kaur H, Halliwell B, Aeschbach R, Banni S, Dessi MA & Corongiu FP (1999) Inhibition of peroxynitrite dependent DNA base modification and tyrosine nitration by the extra virgin olive oil-derived antioxidant hydroxytyrosol. *Free Radical Biology and Medicine* **26**, 762–769.

De la Puerta R, Martínez-Domínguez E & Ruíz-Gutiérrez V (2000) Effect of minor components of virgin olive oil on topical antiinflammatory assays. *Zeitschrift für Naturforschung* **55**C, 814–819.

De la Puerta R, Ruiz Gutierrez V, Robin J & Hoult S (1999) Inhibition of leukocyte 5-lipoxygenase by phenolics from virgin olive oil. *Biochemical Pharmacology* **57**, 445–449.

De Pergola G, Giorgino F, Garruti G, Cignarelli M & Giorgino R (1996) Rapporto tra variabili antropometriche, ormoni sessuali e complicanze dell'obesità (Relationship between anthropometric variables, sex hormones and complications in obesity). *Metabolismo Oggi* **13**, 138–145.

de Rojas-Walker T, Tamir S, Ji H, Wishnock J & Tennanbaum SR (1995) Nitric oxide induces oxidative damage in addition to deamination in macrophages DNA. *Chemical Research in Toxicology* **8**, 473–477.

Edgecombe SC, Stretch GL & Hayball PJ (2000) Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. *Journal of Nutrition* **130**, 2996–3002.

Eling TE, Curtis JF, Harman LS & Mason RP (1986) Oxidation of glutathione to its thiyl free radical metabolite by prostaglandin H synthase. *Journal of Biological Chemistry* **261**, 5023–5028.

Esterbauer H, Gebicki J, Puhl H & Jurgens G (1992) The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine* **13**, 341.

Esti M, Cinquanta L, Notte EI & La Notte E (1998) Phenolic compounds in different olive varieties. *Journal of Agricultural and Food Chemistry* **46**, 32–35.

Federici F & Bongi G (1983) Improved method for isolation of bacterial inhibitors from oleuropein hydrolysis. *Applied and Environmental Microbiology* **46**, 509–510.

Finotti E & Di Majo D (2003) Influence of solvents on the antioxidant property of flavonoids. *Nahrung/Food* **47**, 186–187.

Garrido Fernández A, Fernández Díez MJ & Adams MR (1997) *Table Olives: Production and Processing*, pp. 67–109. London: Chapman & Hall.

Gourama H & Bullerman LB (1987) Effects of oleuropein on growth and aflatoxin production by Aspergillus parasiticus. *Lebensmittel Wissenschaft Technologie* **20**, 226–228.

Gutierrez F, Jimenez B, Ruiz A & Albi MA (1999) Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties picual and hojiblanca and on the different components involved. *Journal of Agricultural and Food Chemistry* **47**, 121–127.

Halliwell B (1997) Antioxidants and human disease: a general introduction. *Nutrition Reviews* **55**, 44–52.

Halliwell B, Aeschbach R, Loliger J & Aruoma OI (1995) The characterization of antioxidants. *Food and Chemical Toxicology* **33**, 601–617.

Halliwell B & Gutterige MC (1990) Role of free radicals and catalytic metal ions in human disease: an overview. *Methods in Enzymology* **186**, 1–85.

Hankinson SE, Willett WC, Manson JE, Hunter DJ, Colditz GA, Stampfer MJ, Longcope C & Speizer FE (1995) Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. *Journal of the National Cancer Institute* **87**, 1297–1302.

Hertog MLG, Feskens EJM, Katan MB & Kromhout D (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **342**, 1007.

Hirano T, Fukuoka F, Oka K, Naito T, Hosaka K, Mitsuhashi H & Matsumoto Y (1990) Antiproliferative activity of mammalian lignan derivatives against the human breast carcinoma cell line, ZR-75-1. *Cancer Investigation* **8**, 592–602.

Hirschman SZ (1972) Inactivation of DNA polymerases of murine leukaemia viruses by calcium elenolate. *Nature: New Biology* **238**, 277–279.

Huang MT, Osawa T, Ho CT & Rosen RT (1994) Food phytochemicals for cancer prevention. In *Fruits and Vegetables. ACS Symposium Series* no. 46. Washington, DC: American Chemical Society.

Jialal I, Fuller CJ & Huet BA (1995) The effect of α-tocopherol supplementation on LDL oxidation. A dose-response study. *Arteriosclerosis, Thrombosis, and Vascular Biology* **15**, 190–198.

Johnson IT, Williamson G & Musk SRR (1994) Anticarcinogenic factors in plant foods. A new class of nutrients. *Nutrition Research Reviews* **7**, 1–30.

Juven B, Henis Y & Jacoby B (1972) Studies on the mechanism of the antimicrobial action of oleuropein. *Journal of Applied Bacteriology* **35**, 559–567.

Kane S, Seatter M & Gould G (1997) Functional studies of human Glut5: effect of pH on substrate selection and an analysis of substrate interactions. *Biochemical and Biophysical Research Communications* **238**, 503–505.

Kardono LB, Tsauri S, Pezzuto JM & Kinghorn AD (1990) Cytotoxic constituents of the bark of Plumeria rubra collected in Indonesia. *Journal of Natural Products* **53**, 1447–1455.

Kato MJ, Chu A, Davin LB & Lewis NG (1998) Biosynthesis of antioxidant lignans in *Sesamum indicum* seeds. *Phytochemistry* **47**, 583–591.

Kayano T, Burant C, Fukumoto H, Gould G, Fan Y, Eddy R, Byers M, Shows T, Seino S & Bell G (1990) Human facilitative glucose transporters. *Journal of Biological Chemistry* **265**, 13276–13282.

Keceli T & Gordon MH (2001) The antioxidant activity and stability of the phenolic fraction of green olives and extra virgin olive oil. *Journal of the Science of Food and Agriculture* **81**, 1391–1396.

Kernan MR, Amarquaye A, Chen J, Chan J, Sesin DF, Parkinson N, Ye Z, Barrett M, Bales C, Stoddart CA, Sloan B, Blanc P, Limbach C, Mrisho S, Rozhon EJ, Chen JL & Ye ZJ (1998) Antiviral phenylpropanoid glycosides from the medicinal plant Markhamia lutea. *Journal of Natural Products* **61**, 564–570.

Keys A (1995) Mediterranean diet and public health: personal reflections. *American Journal of Clinical Nutrition* **61**, 1321–1323.

Kissebah AH & Krakower GR (1994) Regional adiposity and morbidity. *Physiological Reviews* **74**, 761–811.

Kohyama N, Nagata T, Fujimoto S & Sekiya K (1997) Inhibition of arachidonate lipoxygenase activities by 2- (3, 4-dihydroxyphenyl)ethanol, a phenolic compound from olives. *Bioscience, Biotechnology, and Biochemistry* **61**, 347–350.

Laranjinha JA, Almeida LM & Madeira VM (1994) Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low density lipoprotein peroxidation. *Biochemical Pharmacology* **48**, 487–494.

Leenen R, Roodenburg AJ, Vissers MN, Schuurbiers JA, van Putte KP, Wiseman SA & van de Put FH (2002) Supplementation of plasma with olive oil phenols and extracts: influence on LDL oxidation. *Journal of Agricultural and Food Chemistry* **50**, 1290–1297.

Limiroli R, Consonni R, Ottolina G, Marsilio V, Bianchi G & Zetta L (1995) ^{1}H and ^{13}C NMR characterization of oleuropein aglycones. *Journal of the Chemical Society, Perkin Transactions 1* **5**, 1519–1523.

Limiroli R, Consonni R, Ranalli A, Bianchi G & Zetta L (1996) ^{1}H NMR study of phenolics in the vegetation water of three cultivars of Olea europaea: similarities and differences. *Journal of Agricultural and Food Chemistry* **44**, 2040–2048.

Lipworth L, Martinez ME, Angell J, Hsien CC & Trichopoulos D (1997) Olive oil and human cancer an assessment of evidence. *Preventive Medicine* **26**, 181–190.

Lo Scalzo R, Scarpati ML & Scalzo RI (1993) A new secoiridoid from olive wastewaters. *Journal of Natural Products* **56**, 621–623.

Maggino T, Pirrone F, Velluti F & Bucciante G (1993) The role of the endocrine factors and obesity in hormone-dependent gynecological neoplasias. *European Journal of Gynaecological Oncology* **14**, 119–126.

Manna C, Galletti P, Cucciolla V, Moltedo O, Leone A & Zappia V (1997) The protective effect of the olive oil polyphenol (3, 4-dihydroxyphenyl)ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. *Journal of Nutrition* **127**, 286–292.

Manna C, Galletti P, Cucciolla V, Montedoro GF & Zappia V (1999) Olive oil hydroxytirosol protects human erythrocytes against oxidative damages. *Journal of Nutritional Biochemistry* **10**, 159–165.

Manna C, Galletti P, Misto G, Cucciolla V, D'Angelo S & Zappia V (2000) Transport mechanism and metabolism of olive oil hydroxytyrosol in Caco-2 cells. *FEBS Letters* **470**, 341–344.

Mannino S, Cosio MS & Bertuccioli M (1993) High performance liquid chromatography of phenolic compounds in virgin olive oil using amperometric detector. *Italian Journal of Food Science* **4**, 363–370.

Martinez-Dominguez E, de la Puerta R & Ruiz-Gutierrez V (2001) Protective effects upon experimental inflammation models of a polyphenol-supplemented virgin olive oil diet. *Inflammation Research* **50**, 102–106.

Masella R, Cantafora A, Modesti D, Cardilli A, Gennnaro L, Bocca A & Coni E (1999) Antioxidant activity of 3, 4-DHPEA-EA and protocatechuic acid: a comparative assessment with other olive oil biophenols. *Redox Report* **4**, 113–121.

Miró Casas E, Albadalejo MF, Covas Planells MI, Colomer MF, Lamuela Raventós RM & de la Torre Fornell R (2001) Tyrosol bioavailability in humans after ingestion of virgin olive oil. *Clinical Chemistry* **47**, 341–343.

Miró Casas E, Covas MI, Farre M, Fito M, Ortuno J, Weinbrenner T, Roset P & de la Torre R (2003) Hydroxytyrosol disposition in humans. *Clinical Chemistry* **49**, 945–952.

Mirochnitchenko O, Prokopenko O, Palnitkar U, Kister I, Powell WS & Inouye M (2000) Endotoxemia in transgenic mice overexpressing human glutathione peroxidases. *Circulation Research* **87**, 289–295.

Montedoro GF (1972) I costituenti fenolici presenti negli oli vergini di oliva. Nota 1: identificazione di alcuni acidi fenolici e loro potere antiossidante (Phenolic constituents present in virgin olive oils. Part 1: identification of some phenolic acids and their antioxidant ability). *STA* **3**, 177–186.

Montedoro G, Servili M, Baldioli M & Miniati E (1992) Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry* **40**, 1571–1576.

Monteleone E, Caporale G, Carlucci A & Pagliarini E (1998) Optimisation of extra virgin olive oil quality. *Journal of the Science of Food and Agriculture* **77**, 31–37.

Moreno JJ & Mitjavilab MT (2003) The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (Review). *Journal of Nutritional Biochemistry* **14**, 182–195.

Mosca L, De Marco C, Visioli F & Cannella C (2000) Enzymatic assay for the determination of olive oil polyphenol content: assay conditions and validation of the method. *Journal of Agricultural and Food Chemistry* **48**, 297–301.

Mousavi Y & Adlercreutz H (1992) Enterolactone and estradiol inhibit each others proliferative effect on MCF-7 breast cancer

cells in culture. *Journal of Steroid Biochemistry and Molecular Biology* **41**, 615–619.

Mulinacci N, Romani A, Galardi C, Pinelli P, Giaccherini C & Vincieri FF (2001) Polyphenolic content in olive oil waste waters and related olive samples. *Journal of Agricultural and Food Chemistry* **49**, 3509–3514.

Nardini M, D'aquino M, Tomassi G, Gentili V, Di Felice M & Scaccini C (1995) Inhibition of human low-density lipoprotein oxidation by caffeic acid and other idroxycinnamic acid derivatives. *Free Radical Biology and Medicine* **19**, 541–552.

Newcomb TG & Loeb LA (1998) Mechanism of mutagenicity of oxidatively-modified bases. In *Molecular Biology of Free Radicals in Human Diseases*, pp. 137–166 [OI Aruoma and B Halliwell, editors]. Saint Lucia: OICA International.

Nomoto M, Yamada K, Haga M & Hayashi M (1998) Improvement of intestinal absorption of peptide drugs by glycosylation: transport of tetrapeptide by the sodium ion-dependent D-glucose transporter. *Journal of Pharmaceutical Sciences* **87**, 326–332.

Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B & Bartsch H (2000*a*) The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *European Journal of Cancer* **36**, 1235–1247.

Owen RW, Giacosa A, Hull WE, Haubner R, Wurtele G, Spiegelhalder B & Bartsch H (2000*b*) Olive-oil consumption and health: the possible role of antioxidants. *Lancet Oncology* **1**, 107–112.

Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B & Bartsch H (2000*c*) Identification of lignans as major components in the phenolic fraction. *Clinical Chemistry* **46**, 976–988.

Papadopoulos G & Boskou D (1991) Antioxidant effect of natural phenols on olive oil. *Journal of the American Oil Chemists Society* **68**, 669–671.

Pardo F, Perich F, Villarroel L & Torres R (1993) Isolation of verbascoside, an antimicrobial constituent of Buddleja globosa leaves. *Journal of Ethnopharmacology* **39**, 221–222.

Parthasarathy S (1991) Novel atherogenic oxidative modification of low density lipoprotein. *Diabetes/Metabolism Reviews* **7**, 163.

Petroni A, Blasevich M, Salami M, Papini N, Montedoro GF & Galli C (1995) Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thrombosis Research* **78**, 151–160.

Pezzuto JM (1997) Plant-derived anticancer agents. *Biochemical Pharmacology* **53**, 121–133.

Prasad K (1997) Hydroxyl radical-scavenging property of secoisolar-iciresinol diglucoside (SDG) isolated from flaxseed. *Molecular and Cellular Biochemistry* **168**, 117–123.

Princen HMG, van Poppel G, Vogelazang C, Buytenhek R & Kok FJ (1992) Supplementation with vitamin E but not β-carotene in vivo protects low density lipoprotein from lipid peroxidation in vitro. *Arteriosclerosis and Thrombosis* **12**, 554.

Ranalli A, De Mattia G & Ferrante ML (1997) Comparative evaluation of the olive oil given by a new processing system. *International Journal of Food Science and Technology* **32**, 289–297.

Ranalli A, De Mattia G & Ferrante ML (1998) The characteristics of percolation olive oils produced with a new processing enzyme aid. *International Journal of Food Science and Technology* **33**, 247–258.

Ranalli A, Ferrante ML, De Mattia G & Costantini N (1999) Analytical evaluation of virgin olive oil of first and second extraction. *Journal of Agricultural and Food Chemistry* **47**, 417–424.

Reaven PD, Khouw A, Beltz WF, Parthasarathy S & Witztum J (1993) Effect of dietary antioxidant combinations in humans. Protection of LDL by vitamin E but not by β-carotene. *Arteriosclerosis and Thrombosis* **13**, 590.

Reaven PD, Parthasarathy S, Grasse BJ, Miller E, Almazan F, Mattson FH, Khoo JC, Steinberg D & Witztum JL (1991) Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. *American Journal of Clinical Nutrition* **54**, 701–706.

Rice-Evans CA, Miller NJ & Paganga G (1996) Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine* **20**, 933–956.

Rohn TT, Hinds TR & Vincenzi FF (1993) Ion transport ATPases as targets for free radical damage. Protection by an aminosteroid of the Ca21 pump ATPase and Na1/K1 pump ATPase of human red blood cell membranes. *Biochemical Pharmacology* **46**, 525–534.

Ryan D, Lawrence H, Prenzler PD & Antolovic M (2001) Recovery of phenolic compounds from *Olea europeae*. *Analytica Chimica Acta* **445**, 67–77.

Sadrzadeh SMH, Graf E, Panter SS, Hallaway PE & Eaton JW (1984) Hemoglobin. A biologic Fenton reagent. *Journal of Biological Chemistry* **259**, 14354–14356.

Salami M, Galli C, De Angelis L & Visioli F (1995) Formation of F2-isoprostanes in oxidized low density lipoprotein. Inhibitory effects of hydroxytyrosol. *Pharmacological Research* **31**, 275–279.

Scaccini C, Nardini M, D'Acquino M, Gentili V, Di Felice M & Tomassi G (1992) Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. *Journal of Lipid Research* **33**, 627–633.

Schapira DV, Kumar NB & Lyman GH (1991) Obesity, body fat distribution, and sex hormones in breast cancer patients. *Cancer* **67**, 2215–2218.

Schroder HC, Merz H, Steffen R, Muller WE, Sarin PS, Trumm S, Schulz J & Eich E (1990) Differential in vitro antiHIV activity of natural lignans. *Zeitschrift Naturforschung* **45**, 1215–1221.

Serraino M & Thompson LU (1991) The effect of flaxseed supplementation on early risk markers for mammary carcinogenesis. *Cancer Letters* **60**, 135–142.

Serraino M & Thompson LU (1992) The effect of flaxseed consumption on the initiation and promotional stages of mammary carcinogenesis. *Nutrition and Cancer* **17**, 153–159.

Servili M, Baldioli M, Selvaggini R, Macchioni A & Montedoro GF (1999*a*) Phenolic compounds of olive fruit: one- and two-dimensional nuclear magnetic resonance characterization of nüzhenide and its distribution in the constitutive parts of fruit. *Journal of Agricultural and Food Chemistry* **47**, 12–18.

Servili M, Baldioli M, Selvaggini R, Miniati E, Macchioni A & Montedoro GF (1999*b*) HPLC evaluation of phenols in olive fruit, virgin olive oil, vegetation waters and pomace and 1D and 2D-NMR characterization. *Journal of the American Oil Chemists Society* **76**, 873–882.

Soler-Rivas C, Espin JC & Wichers HJ (2000) Oleuropein and related compounds. *Journal of the Science of Food and Agriculture* **80**, 1013–1023.

Solinas M & Cichelli A (1981) Sulla determinazione delle sostanze fenoliche dell'olio di oliva (On the determination of phenolic substances of olive oil). *Rivista Italiana Sostanze Grasse* **58**, 159–164.

Speroni E, Guerra MC, Minghetti A, Crespi-Perellino N, Pasini P, Piazza F & Roda A (1998) Oleuropein evaluated in vitro and in vivo as an antioxidant. *Phytotherapy Research* **12**, 98–100.

Stretch G, Nation R, Evans A & Milne R (1999) Organ perfusion techniques in drug development. *Drug Development Research* **46**, 292–301.

Takata K (1995) Glucose transporters in the transepithelial transport of glucose. *Journal of Electron Microscopy* **45**, 275–284.

Tanaka T, Makita H, Kawamori T, Kawabata K, Mori H, Murakami A, Satoh K, Hara A, Ohigashi H & Koshimizu K (1997) A xanthine oxidase inhibitor 10 acetoxychavicol acetate inhibits azoxymethane-induced colonic aberrant crypt foci in rats. *Carcinogenesis* **18**, 1113–1118.

Tassou CC & Nychas GJE (1994) Inhibition of Staphylococcus aureus by olive phenolics in broth and in a model food system. *Journal of Food Protection* **57**, 120–124.

Tassou CC & Nychas GJE (1995) Inhibition of Salmonella enteritidis by oleuropein in broth and in a model food system. *Letters in Applied Microbiology* **20**, 120–124.

Tassou CC, Nychas GJE & Board RG (1991) Effect of phenolic compounds and oleuropein on the germination of Bacillus cereus T spores. *Biotechnology and Applied Biochemistry* **13**, 231–237.

Thompson LU, Seidl MM, Rickard SE, Orcheson LJ & Fong HH (1996) Antitumorigenic effect of a mammalian lignan precursor from flaxseed. *Nutrition and Cancer* **26**, 159–165.

Tranter HS, Tassou SC & Nychas GJ (1993) The effect of the olive phenolic compound, oleuropein, on growth and enterotoxin B production by Staphylococcus aureus. *Journal of Applied Bacteriology* **74**, 253–259.

Trichopoulou A (1995) Olive oil and breast cancer. *Cancer Causes Control* **6**, 475–476.

Trichopoulou A, Vasilopoulou E & Lagiou A (1999) Mediterranean diet and coronary heart disease: are antioxidants critical? *Nutrition Reviews* **57**, 253–255.

Tsimidou M, Lytridou M, Boskou D, Paooa-Lousi A, Kotsifaki F & Petrakis C (1996) On the determination of minor phenolic acids of virgin olive oil by RP-HPLC. *Grasas y Aceites en la Nutrición Humana* **47**, 151–157.

Tsukamoto H, Hisada S & Nishibe S (1984) Lignans from bark of the *Olea* plants, 1. *Chemical and Pharmaceutical Bulletin* **32**, 2730–2735.

Tsukamoto H, Hisada S & Nishibe S (1985) Lignans from bark of the Olea plants, 2. *Chemical and Pharmaceutical Bulletin* **33**, 1232–1241.

Tuck KL, Freeman MP, Hayball PJ, Stretch GL & Stupans I (2001) The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. *Journal of Nutrition* **131**, 1993–1996.

Tuck KL & Hayball PJ (2002) Major phenolic compounds in olive oil: metabolism and health effects. *Journal of Nutritional Biochemistry* **13**, 636–644.

van Dyke BR & Saltman P (1996) Hemoglobin: a mechanism for the generation of hydroxyl radicals. *Free Radical Biology and Medicine* **20**, 985–989.

Vasquez Roncero A (1978) Les polyphenols de l'huile d'olive et leur influence sur les characteristiques de l'huile (Polyphenols of olive oil and their influence on oil characteristics). *Revue Française des Corps Gras* **25**, 21–26.

Venkatasubbaiah P & Chilton WS (1990) Phytotoxins of Botryosphaeria obtusa. *Journal of Natural Products* **53**, 1628–1630.

Vieira O, Laranjinha J, Madeira V & Almeida L (1998) Cholesteryl ester hydroperoxide formation in myoglobin-catalyzed low density lipoprotein oxidation: concerted antioxidant activity of caffeic and p-coumaric acids with ascorbate. *Biochemical Pharmacology* **55**, 333–340.

Visioli F (2000) Antioxidants in Mediterranean diets. *World Review of Nutrition and Dietetics* **87**, 43–55.

Visioli F, Bellomo G, Montedoro GF & Galli C (1995*a*) Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis* **117**, 25–32.

Visioli F, Bellomo GF & Galli C (1998) Free radical scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications* **247**, 60–64.

Visioli F, Bordone R, Perugini C, Bagnati M, Cau C & Bellomo G (2000*a*) The kinetics of copper-induced LDL oxidation depend upon its lipid composition and antioxidant content. *Biochemical and Biophysical Research Communications* **268**, 818–822.

Visioli F, Borsani L & Galli C (2000*b*) Diet and prevention of coronary heart disease: the potential role of phytochemicals. *Cardiovascular Research* **47**, 419–425.

Visioli F & Galli C (1998*a*) Olive oil phenols and their potential effects on human health. *Journal of Agricultural and Food Chemistry* **46**, 4292–4296.

Visioli F & Galli C (1998*b*) The effect of minor constituents of olive oil on cardiovascular disease: new findings. *Nutrition Reviews* **56**, 142–147.

Visioli F & Galli C (2001) Antiatherogenic components of olive/olive oil. *Current Atherosclerosis Reports* **3**, 64–67.

Visioli F, Galli C, Bornet F, Mattei A, Patelli R, Galli G & Caruso D (2000*c*) Olive oil phenolics are dose-dependently absorbed in humans. *FEBS Letters* **468**, 159–160.

Visioli F, Poli A & Galli C (2002) Antioxidant and other biological activities of phenols from olives and olive oil. *Medicinal Research Reviews* **22**, 65–75.

Visioli F, Vinceri FF & Galli C (1995*b*) "Waste waters" from olive oil production are rich in natural antioxidants. *Experientia* **51**, 32–34.

Walter WM Jr, Flemming HP & Etchells JL (1973) Preparation of antimicrobial compounds by hydrolysis of oleuropein from green olives. *Applied Microbiology* **26**, 773–776.

Wang C, Mäkelä T, Hase T, Adlercreutz H & Kurzer MS (1994) Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. *Journal of Steroid Biochemistry and Molecular Biology* **50**, 205–212.

Willet WC, Sacks S, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsinf E & Trichopoulos D (1995) Mediterranean diet pyramid: a cultural model for healthy eating. *American Journal of Clinical Nutrition* **61**, 1402–1406.

Wiseman SA, Mathot JNNJ, De Fouw NJ & Tijburg LBM (1996) Dietary non-tocopherol antioxidants present in extra virgin olive oil increase the resistance of low density lipoproteins to oxidation in rabbits. *Atherosclerosis* **120**, 15–23.

Yamanaka N, Oda O & Nagao S (1997) Green tea catechins such as (−)-epicatechin and (−)-epigallocatechin accelerate Cu^{2+}-induced low-density lipoprotein oxidation in propagation phase. *FEBS Letters* **401**, 230–234.

Yermilov V, Rubio J & Ohshima H (1995) Formation of 8-nitroguanine in DNA treated with peroxynitrite in vitro and its rapid removal from DNA by depurination. *FEBS Letters* **376**, 207–210.