Review article
Received: 2006-09-06

UDC 633.852.73:615.01(497.4 Istra)

OLIVE TREE – THE SOURCE OF PHARMACODYNAMICALLY ACTIVE SUBSTANCES

Metoda LIPNIK-ŠTANGELJ
University of Ljubljana, Faculty of Medicine, Department of Pharmacology and Experimental Toxicology, SI-1000 Ljubljana, Korytkova 2
and
University of Primorska, Science and Research Center Koper, SI-6000 Koper, Garibaldijeva 1
E-mail: metoda.lipnik-stangelj@mf.uni-lj.si

Milena BUČAR-MIKLAVČIČ
University of Primorska, Science and Research Center Koper, SI-6000 Koper, Garibaldijeva 1
and
LABS, LLC - Institute for Ecology, Olive Oil and Control, SI-6310 Izola, Zelena ulica 8

Bojan BUTINAR
University of Primorska, Science and Research Center Koper, SI-6000 Koper, Garibaldijeva 1

ABSTRACT

Olive and its products have been recognized as an important component of a healthy diet. Increase olive oil consumption is implicated in reduction of cardiovascular diseases, rheumatoid arthritis and a variety of cancers. In the prevention of these diseases, antioxidant activity of olive oil seems to play the key role. In addition, some compounds from olive oil, particularly polyphenols, show potent effects on cell signalling via modulation of enzymes activity and protein expression, therefore their use became interesting not only in prevention, but also in the treatment of certain diseases.

Key words: olive oil, polyphenols, antioxidants, healthy diet

OLIVO – FONTE DI SOSTANZE FARMACODINAMICAMENTE ATTIVE

SINTESI

L'olivo ed i suoi prodotti vengono riconosciuti come un'importante componente di una dieta salutare. L'aumento del consumo dell'olio d'oliva è implicato nella riduzione delle malattie cardiovascolari, dell'artrite reumatica e di varie forme di cancro. Nella prevenzione di tali malattie, l'attività antiossidante dell'olio d'oliva ha una funzione chiave. Alcuni composti dell'olio d'oliva, in particolare i polifenoli, hanno un potente effetto sulla segnalazione cellulare tramite modulazione dell'attività enzimatica e dell'espressione proteica, pertanto il loro utilizzo è diventato interessante non solo nella prevenzione ma anche nella cura di alcune malattie.

Parole chiave: olio d'oliva, polifenoli, antiossidanti, dieta salutare

INTRODUCTION

Olive and its products have been recognized as an important component of a healthy diet. The number of reports, describing the beneficial properties of olive oil, has dramatically increased in the last couple of years, and recent data have suggested that olive oil has more health benefits that previously thought. The growing popularity of the Mediterranean diet is due to several epidemiological studies that show the lowest incidence of coronary heart disease and certain cancers, *e.g.*, breast and colon cancers in the Mediterranean basin. It has been suggested that this is largely due to the relatively safe and even protective dietary habits of this area, where olive oil represents the principal source of fat in a diet (Hertog *et al.*, 1995; Keys, 1995; Visioli *et al.*, 2005).

Olive oil's vital components, monounsaturated fatty acids and antioxidant and anti-thrombotic substances are attributed with its protective effects against, among others, atherosclerosis and other cardiovascular diseases (Manna *et al.*, 2004; Dell'Agli *et al.*, 2006; Perona *et al.*, 2006), diabetes (Al-Azzawie *et al.*, 2006), certain cancers (Owen *et al.*, 2004; Visioli *et al.*, 2004; Hashim *et al.*, 2005), inflammation diseases (Pattison *et al.*, 2004; Puel *et al.*, 2004; Beauchamp *et al.*, 2005) and age related cognitive decline (Wahle *et al.*, 2004).

This review is focuses on biologically active constituents of olive and particular olive oil and their importance for human health. The molecular mechanisms involved in pharmacologically effects of certain compounds are highlighted.

THE COMPOSITION OF OLIVE OIL

Olive oil is obtained from the drupes of olive tree that is best grown between the 30^0 and the 45^0 parallel. Accordingly, the Mediterranean countries supply more than 95% of the world olive oil production that is around 2.000.000 tons/year (Visioli *et al.*, 2002). Depending on its chemical properties, organoleptic characteristics and its degree of acidity, olive oil is classified into different grades (EEC Council regulations, 1991). From this classification, the most valuable is the extra-virgin oil, obtained from intact olives that are quickly processed and cold-pressed. In this way, activation of cellular lipases and degradation of the triglycerides is minimized (Visioli *et al.*, 2002).

The composition of olive oil is primarily saponifiable glyceridic compounds as triglycerides (Montedoro, 1972), where the oleic acid, a monounsaturated acid (18:1n-9) represents 56 to 84% of total fatty acid, while linoleic acid (18:2n-6) is present in 3 to 21% (Boskou, 2000; Butinar *et al.*, 2004).

The biological effects of monounsaturated fatty acids in olive oil on circulating lipids and lipoproteins in human body are somewhat controversial (Visioli *at al.*, 2002): while the major effects of high monounsaturated fatty acids intakes on serum cholesterol are generally attributed to the associated replacement of saturated fatty acids (Hegsted *et al.*, 1993; Gardner *et al.*, 1995), some studies attributed a direct, although modest cholesterol-lowering effect to monounsaturated fatty acids alone, when they equicalorically replace carbohydrates. Furthermore, monounsaturated fatty acids increase the levels of the protective high-density lipoprotein (HDL) more than polyunsaturated when these two classes of fatty acids replace carbohydrates in the diet (Mensink *et al.*, 1992).

Unsaponifiable compounds represent 0.5 to 1.0% of constituents of minor fraction in olive oil. Among minor constituents of virgin olive oil, there are vitamins such as α- and γ-tocopherols (around 200 ppm) and β-carotene, phytosterols, pigments, terpenoids, flavonoids such as luteolin and quercetin, squalene, and more than 30 different phenolic compounds (Montedoro, 1972; Butinar *et al.*, 1999; Boskou, 2000), some of them with potent antioxidant activity, which is important in the prevention of cardiovascular and cancer diseases and inflammation (Boskou, 1996) (Tab. 1).

The amount of phenolic compounds in olive oil depends on several factors, including cultivar, degree of maturation, possible infestation by the olive fly *Dacus olea*, and climate (Boskou, 2000; Butinar *et al.*, 2000a), and usually decreases with over-maturation of olives (Visioli *et al.*, 2002).

The three phenolic compounds in highest concentration in olive oil are the glycoside oleuropein, hydroxytyrosol (3,4-dihydroxyphenyl ethanol) and tyrosol. These three compounds are related structurally. Hydroxytyrosol and tyrosol are structurally identical except that hydroxytyrosol possesses an extra hydroxy group in the *meta* position (Tuck *et al.*, 2002). Oleuropein is an ester composed of hydroxytyrosol and elenolic acid. Oleuropein is the major phenolic compound in olive drupes, whereas hydroxytyrosol is the major phenolic component in olive oil (Amiot *et al.*, 1996). As the olive drupe matures, the concentration of oleuropein decreases, while hydroxytyrosol, a hydrolysis product of oleuropein, increases (Cimato *et al.*, 1990; Ryan *et al.*, 1999).

Tab. 1: The chemical composition of olive oil.
Tab. 1: Kemična sestava oljčnega olja.

Olive oil	Subfraction	Component
Major fraction (98–99%, saponifiable)	triglycerides	oleic acid, linoleic acid
Minor fraction (1–2%, unsaponifiable)	hydrocarbons	squalene, β-carotene, polycyclic aromatic hydrocarbon
	sterols	β-sitosterol, campesterol, Δ7-stigmasterol, brassicasterol
	terpenic dialcohols	erythroidol, uvaol
	tocopherols	α-tocopherol, β-tocopherol, γ-tocopherol, Δ-tocopherol
	phenolic compounds	tyrosol, hydroxytyrosol, caffeic acid, oleuropein
	others	flavour components

PHARMACODYNAMICALLY EFFECTS OF OLIVE OIL CONSTITUENTS

Recent studies showed that certain olive oil constituents exert strong pharmaco-dynamic effects in human body. They have potent modulatory effect on cell signalling and became interesting not only in prevention, but also in the treatment of certain diseases. So far, several biologic activities of olive oil compounds have been demonstrated, like: scavering of superoxide and other reactive oxygen substances (ROS) (Le Toutour *et al.*, 1992; Aeschbach *et al.*, 1994; Manna *et al.*, 1997) inhibition of low-density lipoprotein (LDL) oxidation (Scaccini *et al.*, 1992; Visioli *et al.*, 1994), inhibition of apoprotein derivatization (Visioli *et al.*, 1995), reduced thromboxan B_2 and leukotriene B_4 production by activated leukocytes, inhibition of platelet aggregation and thromboxane generation (Petroni *et al.*, 1995), inhibition of peroxynitrite-induced DNA damage and inhibition of peroxynitrite-induced tyrosine nitration (Deiana *et al.*, 1999), inhibition of bacterial growth and activity, and decreased isoprostane excretion in humans and in side-stream smoke-exposed rats (Tuck *et al.*, 2002), and others, like scavering of hypochlorus acid, increased nitric oxide production by mouse macrophages, cytostasis, hypotensive action, and increased plasma antioxidant capacity (for review see Visioli *et al.*, 2002).

Antioxidant activity of olive oil constituents

Antioxidant activity of olive oil constituents, particularly polyphenols, seems to play a key role in the beneficial effect in several diseases, like cardiovascular diseases, cancer and inflammation. The main mechanism by which the components of olive oil express their antioxidant activity is inhibition and/or scavenging of ROS, which can activate different signalling pathways that lead to progression of disease. In the excess of ROS, they can also react with different cellular constituents and cause cell damage. ROS are produced during normal metabolism or after oxidative processes and include superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Voetsch *et al.*, 2004). In the increased production of O_2^- and H_2O_2, they react rapidly with nitric oxyde (NO) to form peroxynitrite ($OONO^-$), thus inactivating NO and leading to different physiological dysfunctions (Perona *et al.*, 2006) (Fig. 1).

The formation of ROS is balanced by a range of antioxidant defences, but the excess can overwhelm these systems and leads to oxidative stress, which importantly contributes to the development of certain diseases. Several constituents in olive oil potently modulate the ROS production. Oleic acid and β-sitosterol may reduce intracellular ROS by creating a less-oxidant environment through inhibition of intracellular ROS production. β-Sitosterol may also enhance superoxyde dysmutase activity, hence decreasing O_2^- levels. This reduction has also been observed for the terpenoid oleanolic acid, although the mechanism is not presently known. Tocopherols and phenolic compounds are potent antioxidants that may help reduce lipid peroxidation and scavenge intracellular ROS and free NO, reducing the formation of $OONO^-$. ROS can activate the nuclear factor κB (NFκB), which is then translocated into the nucleus, where it binds to recognition sequences in DNA to induce gene expression. This mobilization of NFκB is blocked by α-tocopheryl succinate but not by α-tocopherol. In contrast, phenolic compounds have been proposed to act blocking the formation of NFκB/DNA binding complexes. NFκB modulates the expression of cytokines, enzymes lipoxygenase (LOX) and cycloxygenase (COX), thereby affecting the levels of adhesion molecules and eicosanoids. However, some of the minor compounds of olive oil may act directly on these enzymes and cytokines. LOX and COX activities are inhibited at different points by phenolics and triterpenoids, whereas interleukin-1β (IL-1β) expression is inhibited by phenolics and tocopherols, contributing to protect the endothelium against vasoconstriction, platelet aggregation and monocyte adhesion. Vasodilatation is also suggested to be enhanced by oleuropein and oleanolic acid through an increase in the production of NO (Tab. 2) (for review see Perona *et al.*, 2006).

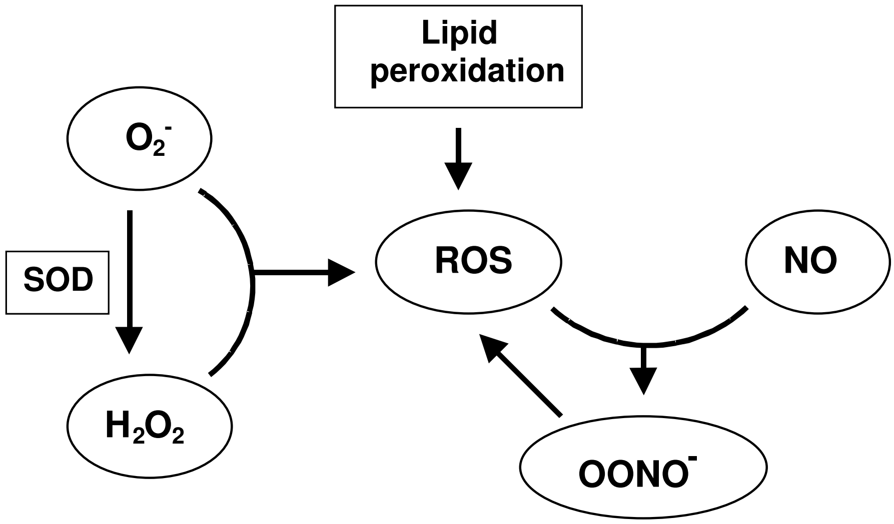

Fig. 1: Different biochemical pathways of reactive oxygen substances (ROS) production in the cell (SOD-superoxyde dysmutase).
Sl. 1: Različne biokemične poti nastanka reaktivnih kisikovih spojin (ROS) v celici (SOD-superoksid-dismutaza).

Tab. 2: Influence of olive oil constituents on biochemical processes in the cell.
Tab. 2: Vpliv sestavin oljčnega olja na biokemične procese v celici.

Olive oil constituent	Mechanism of action
oleic acid	Inhibition of ROS production
polyphenols	Reduction of lipid peroxidation Scavenging intracellular ROS Reduction of OONO- formation Reduction of NFκB/DNA binding complexes formation Inhibition of LOX and COX Inhibition of IL-β expression Increasing NO production
tocopherols	Scavenging intracellular ROS Reduction of OONO- formation Block of NFκB mobilisation to the nucleus Inhibition of IL-β expression
β-sitosterol	Inhibition of ROS production Activation of superoxyde dismutase
terpenoids	Inhibition of LOX and COX

The effects of olive oil constituents in cardiovascular diseases

Olive oil constituents show complex benefit effects in prevention of cardiovascular diseases. Cardiovascular diseases risk factors, like hypercholesterolemia (Ohara *et al.*, 1993; Stokes *et al.*, 2002), diabetes mellitus (Guzik *et al.*, 2002) and hypertension (Just, 1997; Kerr *et al.*, 1999), are all strongly related to increased production of ROS, inactivation of NO and endothelial dysfunction. ROS can also react with polyunsaturated fatty acids contained in lipoproteins in the vessel wall, initiating lipid peroxidation. The hydroperoxides formed in this process can in turn react with NO to form $OONO^-$, inactivating NO, and directly decrease the endothelial synthesis of NO (Chin *et al.*, 1992).

Monounsaturated fatty acids in olive oil increase the levels of the protective HDL and improve therefore lipoprotein profile in the body. Beside this, they reduce the thrombogenic-atherogenic process by various actions on arterial thrombus formation, such as decreased monocyte adhesion, increased fibrinolysis and decreased arterial pressure. Additionally, the antioxidant substances found in olive oil (*i.e.* tocopherols, polyphenols) could influence atherogenesis by inhibition of LDL-cholesterol oxidation, protection against free radicals and their toxic effects, inhibition of platelet aggregation and thromboxane generation, stimulation of anti-inflammatory agents and increased nitric oxide production (Perona *et al.*, 2006).

The effects of olive oil constituents in inflammation

Certain compounds from olive oil have been shown to modulate immune function that might be interesting in the treatment of inflammatory processes, associated with the immune system like rheumatoid arthritis (Pattison *et al.*, 2004; Puel *et al.*, 2004; Beauchamp *et al.*, 2005).

In the inhibition of inflammatory processes by olive oil constituents, the reduction of intracellular ROS production is important, since ROS enhances transcriptional activity via activation of NFκB that modulates the expression of certain pro-inflammatory cytokines like IL-1β, IL-6 and tumour necrosis factor α. Beside this, reduction of ROS production inhibits LOX and COX transcription and activity. Consequently, the cyclooxygenase and lipoxigenase pathway of arachidonic acid metabolism, and the production of prostaglandins and other inflammatory mediators are diminished. Some of the minor compounds of olive oil may act directly on selected enzymes and cytokines, involved in inflammation; LOX and COX activities are inhibited at different points by phenolics and triterpenoids, whereas IL-1β expression is inhibited by phenolics and tocopherols (Beauchamp *et al.*, 2005; Perona *et al.*, 2006).

The effects of olive oil constituents in cancer

In the ethiopathogenesis of cancer disease, there are several factors contributing to the development and progress of the disease. Among them, oncogenic substances, oxidative stress and angiogenesis play an important role.

Several epidemiological studies show beneficial effects of olive oil constituents in prevention of cancer diseases (Owen *et al.*, 2004; Visioli *et al.*, 2004; Hashim *et al.*, 2005). Olive oil constituents inhibit production of ROS and have protective role in conditions, where the excess production of ROS leads to oxidative stress and causes oxidative cell damage. Certain compounds from olive oil also inhibit the expression of pro-oncogenic substances (Nelson, 2005). Recent studies showed inhibitory effect of olive oil polyphenols on proliferation of human promyelocytic leukemia cell by inducing apoptosis and differentiation (Fabiani *et al.*, 2006). Beside this, it was found strong anti-angiogenic effect of oleuropein, which causes irreversible changes in cancer cells, preventing their replication, motility and invasiveness (Hamdi *et al.*, 2005).

These effects may explain the cancer-protective effects of the olive-rich Mediterranean diet and may also have important therapeutic implications in the treatment of cancer disease.

CONCLUSION

Epidemiological studies show that populations consuming a predominantly plant-based Mediterranean-style diet exhibit lower incidences of chronic diseases than those consuming a northern European or North American diet. Although total fat intake in Mediterranean populations can be higher than in other regions, the greater proportion is derived from olive oil and not animals. Increased olive oil consumption is implicated in a reduction in cardiovascular diseases, rheumatoid arthritis and, to a lesser extent, a variety of cancers. Olive oil intake also has been shown to modulate immune function, particularly the inflammatory processes associated with the immune system (Wahle *et al.*, 2004).

Typical of olive oil is combination of high oleic acid content and content of a variety of plant antioxidants, particularly oleuropein, hydroxytyrosol, and tyrosol. In the prevention of diseases, strong antioxidant activity of olive oil seems to play the key role. In addition, some compounds from olive oil, particularly polyphenols, showed potent effects on cell signalling via modulation of enzymes activity and protein expression, therefore their use became interesting not only in prevention, but also in the treatment of certain diseases.

OLJKA – VIR FARMAKODINAMIČNO UČINKOVITIH SNOVI

Metoda LIPNIK-ŠTANGELJ
Univerza v Ljubljani, Medicinska fakulteta, Inštitut za farmakologijo in eksperimentalno toksikologijo, SI-1000 Ljubljana, Korytkova 2
in
Univerza na Primorskem, Znanstveno-raziskovalno središče Koper, SI-6000 Koper, Garibaldijeva 1
E-mail: metoda.lipnik-stangelj@mf.uni-lj.si

Milena BUČAR-MIKLAVČIČ
Univerza na Primorskem, Znanstveno-raziskovalno središče Koper, SI-6000 Koper, Garibaldijeva 1
in
LABS, LLC – Inštitut za ekologijo, oljčno olje in kontrolo, SI-6310 Izola, Zelena ulica 8

Bojan BUTINAR
Univerza na Primorskem, Znanstveno-raziskovalno središče Koper, SI-6000 Koper, Garibaldijeva 1

POVZETEK

Oljka in izdelki iz oljke so že dolgo znani kot pomembna sestavina zdrave prehrane. Dokazani so bili številni koristni učinki sestavin oljčnega olja, plodov in listov oljke. S količinskega vidika so glavna komponenta oljčnega olja umiljivi triacilgliceroli, medtem ko manjši del sestavljajo neumiljive snovi, kot so steroli, terpenski alkoholi, tokoferoli, polifenoli in druge spojine. Večina sestavin (oleinska kislina, polifenoli, tokoferoli, β-sitosterol, terpenoidi) ima močan modulatoren učinek na celično signaliziranje, zato so postale zanimive za preprečevanje in zdravljenje nekaterih bolezni.

Najbolj preučevan učinek je zaščitna vloga oljčnega olja pri boleznih srca in ožilja, kjer imata ključno vlogo moteno delovanje žilnega endotelija in razvoj ateromatoznih plakov. Glavni mehanizem delovanja, po katerem sestavine oljčnega olja vplivajo na delovanje žilnega endotelija, je zaviranje delovanja reaktivnih kisikovih spojin in lovljenje prostih radikalov. To je posledica zaviranja nastanka reaktivnih kisikovih spojin pa tudi aktivacije encima superoksid-dismutaze. Poleg tega oleuropein in oleanolna kislina s povečanjem nastanka NO povzročata tudi razširitev žil.

Polifenoli so učinkoviti tudi pri zaviranju aktivnosti nekaterih pro-vnetnih citokinov, kot je npr. IL-1β, ter encimov ciklo-oksigenaze in lipo-oksigenaze. Ti procesi so posredovani najmanj po dveh poteh. Prvo pot predstavlja zaviranje mobilizacije NFκB, ki je pomembna za izražanje genov številnih pro-vnetnih citokinov in encimov. Poleg tega delujejo polifenoli in tokoferoli tudi kot lovilci prostih radikalov in s tem zavirajo nastanek vnetnih mediatorjev iz arahidonske kisline. Ker polifenoli tako neposredno vplivajo na nastanek vnetnega in/ali imunskega odgovora, so postali zanimivi za zdravljenje bolezni, kot je npr. revmatoidni artritis, ter degenerativnih boleznih živčnega sistema, kakršna je npr. Alzheimerjeva bolezen. Nekatere sestavine oljke, zlasti tokoferoli, skvaleni in polifenoli, pa so zaradi antioksidativnega in protitumorskega učinka zanimive tudi za preprečevanje nastanka raka.

Ključne besede: oljčno olje, polifenoli, antioksidanti, zdrava prehrana

REFERENCES

Aeschbach, R., J. Loliger, B. C. Scott, A. Murcia, J. Butler, B. Halliwell & O. I. Aruoma (1994): Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem. Toxicol., 32, 31–36.

Al-Azzawie, H. F. & M. S. Alhamdani (2006): Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci., 16, 1371–1377.

Amiot, M. J., A. Fleuriet & J. J. Macheix (1996): Importance and evolution of phenolic compounds in olive during growth and maturation. J. Agric. Food Chem., 34, 823–826.

Beauchamp, G. K., R. S. Keast, D. Morel, J. Lin, J. Pika, Q. Han, C. H. Lee, A. B. Smith & P. A. Breslin (2005): Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. Nature, 437(7055), 45–46.

Boskou, D. (1996): In: Olive oil chemistry and technology. AOCS Press, Illinois, p. 115–117.

Boskou, D. (2000): Olive oil. In: Simopoulos, A & F. Visioli (eds.): Mediterranean diets. Wld. Rev. Nutr. Diet, 87, 56–77.

Butinar, B., M. Bučar-Miklavčič & D. Čalija (1999): Tocopherols in olive oils from Slovene Istra in three consecutive years. Annales, Ser. Hist. Nat., 9(2), 37–46.

Butinar, B., M. Bučar-Miklavčič & D. Čalija (1999a): Total polyphenols, hydroxytyrosol and tyrosol in the olive oils of Slovene Istra in two consecutive years (1996, 1997). Annales, Ser. Hist. Nat., 9(2), 27–36.

Butinar, B., M. Bučar-Miklavčič, D. Čalija & E. Bešter (2004): Spremljanje maščobno kislinske sestave oljčnih olj Slovenske Istre v letih 1992–2002. 1. slovenski sadjarski kongres z mednarodno udeležbo. Zbornik referatov. 24.–26. marec, 2004, Krško, str. 711–716.

Chin, J. H., S. Azhar & B. B. Hoffman (1992): Inactivation of endothelial derived relaxing factor by oxidized lipoproteins. J. Clin. Invest., 89, 10–18.

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

Deiana, M., O. I. Aruoma, M. L. P. Bianchi, J. P. E. Spencer, H. Kaur, B. Halliwell, R. Aeschbach, S. Banni, M. A. Dessi & F. P. Corongiu (1999): Inhibition of peroxynitrite dependent DNA base modification and tyrosine nitration by the extra virgin olive oil-derived antioxidant hydroxytyrosol. Free Rad. Biol. Med., 26, 762–769.

Dell'Agli, M., R. Fagnani, N. Mitro, S. Scurati, M. Masciadri, L. Mussoni, G. V. Galli, E. Bosisio, M. Crestani, E. De Fabiani, E. Tremoli & D. Caruso (2006): Minor components of olive oil modulate proatherogenic adhesion molecules involved in endothelial activation. J. Agric. Food Chem., 54(9), 3259–3264.

EEC Council regulations (1991): No 2568/91 on the characteristics of olive oil and olive-pomace oil and the relevant methods of analysis, as last amended by Regulation (EC) No 1989/2003.

Fabiani, R., A. De Bartolomeo, P. Rosignoli, M. Servili, R. Selvaggini, G. F. Montedoro, C. Di Saverio & G. Morozzi (2006): Virgin olive oil phenols inhibit proliferation of human promyelocytic leukemia cells (HL60) by inducing apoptosis and differentiation. J. Nutr., 136(3), 614–619.

Gardner, C. D. & H. C. Kraemer (1995): Monounsaturated versus polyunsaturated dietary fat and serum lipids: a meta-analysis. Arterioscler. Thromb. Vasc. Biol., 15, 1917–1927.

Guzik, T. J., S. Mussa, D. Gastaldi, J. Sadowski, C. Ratnatunga, R. Pillai & K. M. Channon (2002): Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation, 105, 1656–1662.

Hamdi, H. K. & R. Castellon (2005): Oleuropein, a nontoxic olive iridoid, is an anti-tumor agent and cytoskeleton disruptor. Biochem. Biophys. Res. Commun., 334(3), 769–778.

Hashim, Y. Z., M. Eng, C. I. Gill, H. McGlynn & I. R. Rowland (2005): Components of olive oil and chemoprevention of colorectal cancer. Nutr. Rev., 63(11), 374–386.

Hegsted, D. M., L. M. Ausman, J. A. Johnson & G. E. Dallal (1993): Dietary fat and serum lipids: an evaluation of the experimental data. Am. J. Clin. Nutr., 57, 875–883.

Hertog, M. G., D. Kromhout, C. Aravanis, H. Blackburn, R. Buzina, F. Fidanza, S. Giampaoli, A. Jansen, A. Menotti, S. Nedeljkovic *et al.* (1995): Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. Arch. Intern. Med., 155, 381–386.

Just, A. (1997): Nitric oxide and renal autoregulation. Kidney Blood Press. Res., 20, 201–204.

Kerr, S., M. J. Brosnan, M. McIntyre, J. L. Reid, A. F. Dominiczak & C. A. Hamilton (1999): Superoxide anion production is increased in a model of genetic hypertension: role of the endothelium. Hypertension, 33, 1353–1358.

Keys, A. (1995): Mediterranean diet and public health: personal reflections. Am. J. Clin. Nutr., 61, 1321S–1323S.

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

Manna, C., V. Migliardi, P. Golino, A. Scognamiglio, P. Galletti, M. Chiariello & V. Zappia (2004): Oleuropein prevents oxidative myocardial injury induced by ischemia and reperfusion. J. Nutr. Biochem., 15(8), 461–466.

Mensink, R. P. & M. B. Katan (1992): Effect of dietary fatty acids on serum lipids and lipoproteins. Arterioscler. Thromb. Vasc. Biol., 12, 911–919.

Montedoro, G. (1972): Phenolic substances present in virgin olive oil. Note 1. Identification of phenolic acids and their antioxidant power. Sci. Technol. Aliment., 2, 177–186.

Nelson, R. (2005): Oleic acid suppresses overexpression of ERBB2 oncogene. Lancet. Oncol., 6(2), p. 69.

Ohara, Y., T. E. Peterson & D. G. Harrison (1993): Hypercholesterolemia increases endothelial superoxide anion production. J. Clin. Invest., 91, 2546–2551.

Owen, R. W., R. Haubner, G. Wurtele, E. Hull, B. Spiegelhalder & H. Bartsch (2004): Olives and olive oil in cancer prevention. Eur. J. Cancer. Prev., 13(4), 319–326.

Pattison, D. J., D. P. Symmons & A. Young (2004): Does diet have a role in the aetiology of rheumatoid arthritis? Proc. Nutr. Soc., 63(1), 137–143.

Perona, J. S., R. Cabello-Moruno & V. Ruiz-Gutierrez (2006): The role of virgin olive oil components in the modulation of endothelial function. J. Nutr. Biochem., 17(7), 429–445.

Petroni, A., M. Blasevich, M. Salami, N. Papini, G. F. Montedoro & C. Galli (1995): Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. Thromb. Res., 78, 151–160.

Puel, C., A. Quintin, A. Agalias, J. Mathey, C. Obled, A. Mazur, M. J. Davicco, P. Lebecque, A. L. Skaltsounis & V. Coxam (2004): Olive oil and its main phenolic micronutrient (oleuropein) prevent inflammation-induced bone loss in the ovariectomised rat. Br. J. Nutr., 92(1), 119–127.

Ryan, D., K. Robards & S. Lavee (1999): Changes in phenolic content of olive during maturation. Int. J. Food Sci. Tech., 34, 265–274.

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

Stokes, K. Y., D. Cooper, A. Tailor & D. N. Granger (2002): Hypercholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. Free Radic. Biol. Med., 33, 1026–1036.

Le Toutour, B. & D. Guedon (1992): Antioxidative activities of Olea Europaea leaves and related phenolic compounds. Phytochemistry, 31, 1173–1178.

Tuck, K. L. & P. J. Hayball (2002): Major phenolic compounds in olive oil: metabolism and health effects. J. Nutr. Biochem., 13(11), 636–644.

Visioli, F. & C. Galli (1994): Oleuropein protects low density lipoprotein from oxidation. Life Sci., 55, 1965–1971.

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

Visioli, F., A. Poli & C. Galli (2002): Antioxidant and Other Biological Activities of Phenols from Olives and Olive Oil. Med. Res. Rev., 22, 65–75.

Visioli, F., S. Grande, P. Bogani & C. Galli (2004): The role of antioxidants in the mediterranean diets: focus on cancer. Eur. J. Cancer Prev., 13(4), 337–343.

Visioli, F., P. Bogani, S. Grande & C. Galli (2005): Mediterranean food and health: building human evidence. J. Physiol. Pharmacol., 56(Suppl. 1), 37–49.

Voetsch, B., R. C. Jin & J. Loscalzo (2004): Nitric oxide insufficiency and atherothrombosis. Histochem. Cell. Biol., 122(4), 353–367.

Wahle, K. W., D. Caruso, J. J. Ochoa & J. L. Quiles (2004): Olive oil and modulation of cell signaling in disease prevention. Lipids, 39(12), 1223–1231.