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Nutraceutical, cosmetic, health products derived from olive

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Abstract. It is widely recognized that the Mediterranean diet is associated with a reduced rates of chronic disease (i.e. cardiovascular disease, atherosclerosis, some types of cancers, and Alzheimer's disease), and a higher life expectancy. These health benefits have been partially attributed to the dietary consumption of olive oil by populations residing in the Mediterranean region, and more specifically the phenolic compounds naturally present in olive fruit and oil. *In vitro* and *in vivo* studies have demonstrated that these phenolic compounds have potentially beneficial biological effects resulting from their antimicrobial, antioxidant and anti-inflammatory activities. This paper summarizes the modes of action by which olive phenolic compounds beneficially influence health parameters.

Keywords. Health product – Olive – Nutraceuticals – Cosmetic.

Produits nutraceutiques, cosmétiques et de santé dérivés de l'olive

Résumé. *Il est largement reconnu que le régime méditerranéen est associé à un taux réduit de maladies chroniques (i.e. les maladies cardiovasculaires, l'athérosclérose, certains types de cancers et la maladie d'Alzheimer), et à une espérance de vie plus élevée. Ces avantages pour la santé sont partiellement attribuables à la consommation alimentaire de l'huile d'olive par les populations résidant dans la région méditerranéenne, et plus particulièrement aux composés phénoliques naturellement présents dans les fruits et l'huile d'olive. Des études,* in vitro *et* in vivo*, ont démontré que ces composés phénoliques ont des effets biologiques potentiellement bénéfiques résultant de leurs activités antimicrobiennes, anti-oxydantes et anti-inflammatoires. Cet article résume le mode d'action par lequel les composés phénoliques présents dans les olives, ont un effet bénéfique sur les paramètres de la santé.*

Mots-clés. *Produits de santé – Olive – Nutraceutiques – Cosmétiques.*

I – Introduction

It is widely recognised that those residing in the Mediterranean region experience reduced rates of chronic disease [i.e. cardiovascular disease (CVD), atherosclerosis, some types of cancers, and Alzheimer's disease)], and a higher life expectancy in comparison to other worldwide populations (Trichopoulou *et al.,* 2003). The Mediterranean style of eating encompasses a number of dietary components that are thought to contribute protective health effects, including the consumption of 25-50 ml/day of olive oil. Recently, Trichopoulou *et al.* (2009) using a prospective cohort study, investigated the relative importance of the individual components of the Mediterranean diet in relation to overall mortality. The dominant components of the Mediterranean diet that were predictors of lower mortality were moderate consumption of ethanol, low consumption of meat and meat products, and high consumption of vegetables, fruits and nuts, olive oil and legumes.

In addition, scientists, investigating these positive "disease preventing" properties of Mediterranean diet, have begun to identify specific food components, called phytochemicals, that may better explain the role of some foods in the prevention and treatment of diseases.

Although phytochemicals are not yet classified as nutrients, they are substances that can positively affect human function and reduce the risk of disease.

About 30 classes of chemicals with disease-preventive effects that may have practical implications also in reducing cancer incidence in human population have been described, such as carotenoids, chlorophyll, flavonoids, indoles, polyphenolic compounds, protease inhibitors, sulphides, and terpens.

In particular, olive fruit's average composition includes water (50%), protein (1.6%), oil (22%), carbohydrate (19.1%), cellulose (5.8%), inorganic substances (1.5%) and phenolic compounds (1-3%). Other important compounds present in olive fruit are pectin, organic acids, and pigments (Cicerale *et al.*, 2009). Organic acids show metabolic activity and are intermediate products resulting from formation and degradation of other compounds (Cunha *et al.*, 2001). Variation in the amount of these compounds may be caused by numerous factors including: variety, region in which the olive is grown, agricultural techniques used to cultivate the olive, maturity of the olive fruit at harvest, and processing (Covas, 2008).

II – Phenolic components

Phenolic components of olive fruits, present in large amounts also in olive oil, are currently receiving much attention because of their beneficial health effects related to their antioxidant, anti-inflammatory, antiestrogenic, cardioprotective, cancer chemopreventive, and neuroprotective properties. Historically, the healthful properties of virgin olive oil were attributed to a high proportion of monounsaturated fatty acids (MUFAs), namely oleic acid, which represents 55-83% of the fatty acids present in virgin olive oil (Tripoli *et al.*, 2005). However, several seed oils (including sunflower, soybean, and rapeseed) rich in MUFA have been demonstrated to be ineffective in beneficially altering chronic disease risk factors (Aguilera *et al.*, 2004; Harper *et al.*, 2006). In addition to MUFA, virgin olive oil contains a minor, yet significant phenolic component that other seed oils lack. Thus, the phenolic fraction of virgin olive oil has generated much interest regarding its health promoting properties. Subsequent studies (human, animal, in vivo and in vitro) have demonstrated that olive oil phenolics have positive effects on certain physiological parameters, possibly reducing the risk of chronic disease development (Boskou *et al.*, 2006).

Plant phenolic compounds are well known secondary metabolites and they are synthesized naturally by plants in response to stress conditions such as infection, wounding, and UV radiation (Naczk *et al.*, 2004).

Both lipophilic and hydrophilic phenolics are distributed in olive fruit. The main lipophilic phenols are cresols while the major hydrophilic phenols include phenolic acids, phenolic alchohols, flavonoids and secoiridoids; they are present in almost all parts of the plant and in olive oil. However, their nature and concentration varies greatly depending on several factors including maturation stage, part of the fruit, variety, season, packaging, storage, climatologic conditions and the degree of technology used in olive oil production (Covas *et al.*,2006).

Many factors can have a marked effect on phenolic composition and concentration in olive fruit and oil (Table 1). The 3,4-dihydroxyphenyl-ethanol (3,4-DHPEA) and *p*-hydroxyphenyl-ethanol (*p*-HPEA) are the main phenolic alcohols of virgin olive oil (VOO). Their concentration is usually low in fresh oils but increases during oil storage due to the hydrolysis of VOO secoiridoids such as 3,4-DHPEA-EDA 3,4-dihydroxyphenyl-ethanol linked to dialdehydic form of elenolic acid, *p*-HPEA-EDA (*p*-hydroxyphenylethanol linked to dialdehydic form of elenolic acid) and 3,4-DHPEA-EA (3,4-dihydroxyphenyl-ethanol linked to elenolic acid) into hydroxytyrosol (3,4-dihydroxyphenylethanol) (3,4-DHPEA) and tyrosol (*p*-Hydroxyphenylethanol) (*p*-HPEA). During ripening of olives, oleuropein is completely degraded, and is almost undetectable when the fruit darkens, but hydroxytyrosol, tyrosol, and verbascoside increase.

Table 1. Main classes of phenolic compounds in olive fruit and olive oil

Phenolic acids (i.e. Caffeic acid, *p*-Hydroxybenzoic acid, Vanilic acid, Syringic acid, Ferulic acid, Cinnamic acid, Gallic acid) divided into three subgroups:		
1. benzoic acid derivatives (basic skeleton C_6-C_1)	2. cinnamic acid derivatives (basic skeleton of C_6–C_3)	3. Others
Phenolic alcohols • characterized by a hydroxyl group attached to an aromatic hydrocarbon group		
Secoiridoids (i.e. Oleuropein, Demethyloteuropein, Ligstroside, Nuzhenide) • phenolic group characterized by the presence of either elenolic acid or elenolic acid derivatives in their molecular structure		
Flavonoids (i.e. Quercetin-3-rutinoside, Luteolin-7-glucoside, Luteolin-5-glucoside, Apigenin-7-glucoside) • polyphenolic compounds containing two benzene rings joined by a linear three carbon chain. (chemical structure of C_6–C_3–C_6), divided into two groups:		
1. flavones	2. flavanols	
Hydroxy-isocromans (i.e. Verbascoside) • 3,4-dihydro-1H-benzo[*c*]pyran derivatives mainly naturally occurring in nature as part of a complex fused ring system		
Lignans (i.e. (+)-1-acetoxypinoresinol, (+)-pinoresinol) • the exact structure not well understood but based on the condensation of aromatic aldehydes		

III – Bioavailabity of olive phenolics

The degree to which phenolic components are bioavailable (absorbed, metabolised, distributed and eliminated) is pivotal in understanding and evaluating the health benefits associated with such compounds. The majority of research regarding the bioavailability of olive phenolics has focused on the absorption and excretion of two major components: hydroxytyrosol and tyrosol (Cicerale *et al.*, 2010). It has been recently reported the presence of metabolites for the majority of olive phenolic compounds (i.e. secoiridoids, flavonoids and phenolic alcohols) in human urine, suggesting that these compounds are metabolised and absorbed post-ingestion (García-Villalba *et al.*, 2010).

However, it has been suggested that also poorly absorbed phenolic compounds may exert local antioxidant activities in the gastrointestinal tract and this hypothesis is supported by research demonstrating the free radical scavenging capacity of olive phenolics in both the fecal matrix and intestinal epithelial cells (Selma *et al.*, 2009).

In addition, unabsorbed dietary phenolics remain in the gut where intestinal bacteria can metabolize them generating active metabolites. Indeed, germfree or antibiotic-treated animals no longer form the phenolic acid metabolites (ring-fission products) of apigenin, myricetin, hesperidin, naringin, rutin, etc. (Griffiths and Barrow, 1972).

Only a few species of intestinal bacteria responsible for phenolic metabolism have been identified, and there is scarce knowledge of the mechanisms involved. In addition, the transformation of the native phenolics into their metabolites depends on the individuals, and both metabolite "producers" and "nonproducers" have been reported (Cerdá *et al.*, 2005).

Certain gut bacteria including some already recognized as potentially health-promoting ones (i.e. some species belonging to the genera *Bifidobacterium* and *Lactobacillus*) seem to be involved in the release of bioactive hydroxycinnamic acids in the human colon. The use of dietary supplements of beneficial bacteria, which modify the colonic microbiota by increasing

the number of specific microbial strains able to transform some phenolics, could have, therefore, wide-ranging implications for the health of the host, resulting in beneficial effects.

On the other hand, unabsorbed dietary phenolics and their metabolites, may exert significant effects on the intestinal environment by modulation of the microbiota (Lee *et al.*, 2006). Several phenolics have been recognized as potential antibacterial compounds able to repress pathogenic bacteria in the human gut by bacteriostatic or bactericidal actions or also by inhibition of the adhesion of infection-causing bacteria within cells of the intestinal and urinary tract. In conclusion, a better understanding of the dietary phenolic and gut microbiota relationship should help in the prevention of intestinal diseases, such as inflammatory bowel diseases and colon cancer, as well as in improvement of human health avoiding diseases in other tissues.

IV – Biological activities and potential health benefits of dietary phenolics

Reactive oxygen species (ROS), formed as a result of oxidative stress, are known to be responsible for the development of some diseases targeting lipids, proteins and deoxyribonucleic acid (DNA) in living organisms. Diseases attributed to ROS include, for example, aging, arteriosclerosis, cancer and neurodegenerative diseases such as Parkinson's (Uttara *et al.*, 2009; Rezaie *et al.*, 2007).

Oxidative stress is imposed on the body' s cells when the level of ROS outweighs the reducing capacity of antioxidant and antioxidative stress mechanisms. While a low level of stress is always present, an increase in the amplitude and duration of stress can result in damage to cell membranes, proteins, and DNA. This in turn can set the conditions for a new disease or can exacerbate an existing condition. Inflammatory bowel disease, ischemia/reperfusion disorders, and cancer have all been linked to oxidative stress. Biomarkers of oxidative damage in diseased tissues are often disproportionately altered and in most cases, are higher than that present in healthy cells (Aw, 1999).

In eukaryotes, superoxide anion is primarily produced: when electrons leaking from the mitochondrial electron transport chain reduce oxygen, during tissue injury by xanthine oxidase, via auto-oxidation reactions in the presence of transition metal ions, during cytochrome P450 cycling, and at inflammatory sites by activated neutrophils and phagocytes via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Halliwell *et al.*, 2007).

Although endogenous antioxidants and antioxidative stress enzymes are effective in the removal of ROS, the added burden of environmental ROS and the reduction of plasma and cellular antioxidant potential during aging and mitochondrial dysfunction stretches the body antioxidant defenses to the limit (Rizvi *et al.*, 2006).

Various studies (*in vivo* and *in vitro*) have demonstrated that olive phenolic compounds beneficially alter oxidative processes and inflammation. In vitro olive oil phenolics have been found to decrease ROS production and elicit significant free-radical scavenging effects (Goya *et al.*, 2007).

Further in vitro research has shown that olive oil phenolic compounds reduce detrimental oxidative damage to red blood, renal and intestinal cells as measured by red blood cell lysis morphology, membrane-bound haemoglobin, membrane protein profile and changes to the membrane lipid fraction (Paiva-Martins *et al.*, 2009; Loru *et al.*, 2009; Deiana *et al.*, 2010).

In vivo human and animal studies have demonstrated a decrease in LDL oxidation with an increased ingestion of olive oil phenolic compounds (Gimeno *et al.*, 2007). An explanation of this effect is that olive phenolic compounds are able to bind to LDL and this may account for the increased resistance to LDL oxidation.

Research concerning DNA damage (as measured by the comet assay and 8-oxo-deoxyguanosine) shows the intake of phenol-rich olive oil (up to592 mg/kg) decreases oxidative DNA damage in vivo in humans and in animals (Machowetz *et al.*, 2007; Jacomelli *et al.*, 2010).

Additional beneficial effects on inflammation have been demonstrated by olive oil phenolics, such as: (i) inhibition of both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inflammatory enzymes in a dose-dependent manner; (ii) decrease of Tumor Necrosis Factor a and interleukin 1b expression; and (iii) decrease of the expression of several inflammatory genes including NFkb and COX-2. Some olive phenolic compounds have shown also a direct effect on cancer cell proliferation in human breast, prostate and intestinal cancer lines, on cell transformation in mouse epidermal JB6 Cl41 cells, and on the modulation of apoptosis in normal and transformed cells (Fig. 1) (Cicerale *et al.*, 2012).

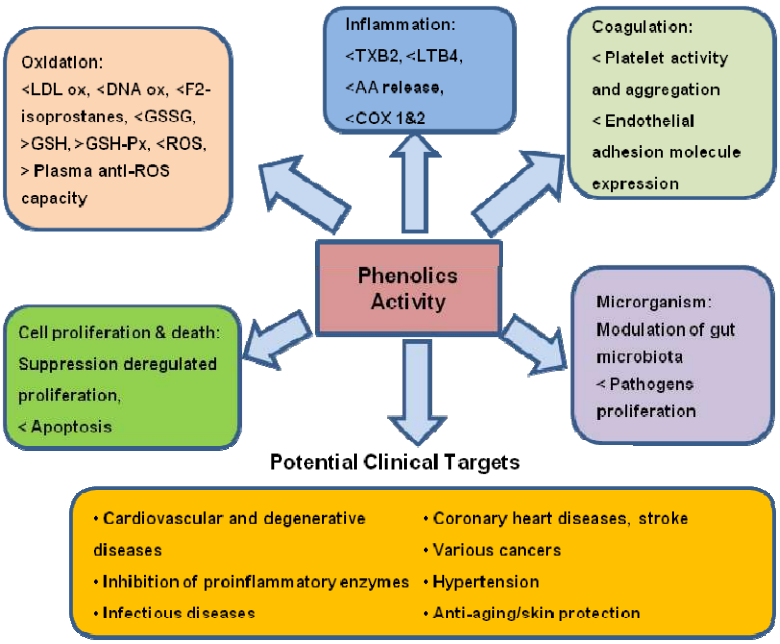

Fig. 1. Biological activities of olive phenolic compounds.

V – Anti-ageing effects of olive derivatives in the skin

Human skin is the potential anatomical barrier for pathogens and damage, which acts as an important fence between internal and external environment in the bodily defense. Continuous exposure to UV light leads to numerous complications that are correlated with various pathological consequences of the skin damage and sunburn occurs when exposure to UV light exceeds the protective capacity of an individual's melanin (Agar *et al.*, 2004).

UV radiation is a potent initiator of ROS generation in the skin. The type(s) of ROS generated, however, depends on the UV wavelength. UVB mainly stimulates the production of $^{\bullet}O_2^-$ through the activation of NADPH oxidase and respiratory chain reactions, while UVA produces $^1O_2$ through a photosensitizing reaction with internal chromophores such as riboflavin and porphyrin. UVA also generates $^{\bullet}O_2^-$ through NADPH oxidase activation and photosensitization of

advanced glycation products (Masaki, *et al.* 1995; Valencia and Kochevar, 2008). The major type of ROS produced on the skin surface is $^1O_2$, which is generated by a photosensitizing reaction with UVA and porphyrins from bacterial flora living in the skin (Ryu *et al*., 2009). $^1O_2$ is oxidized to squalene, cholesterol, and to unsaturated acyl residues in the sebum to yield lipid hydroperoxides (Fig. 2).

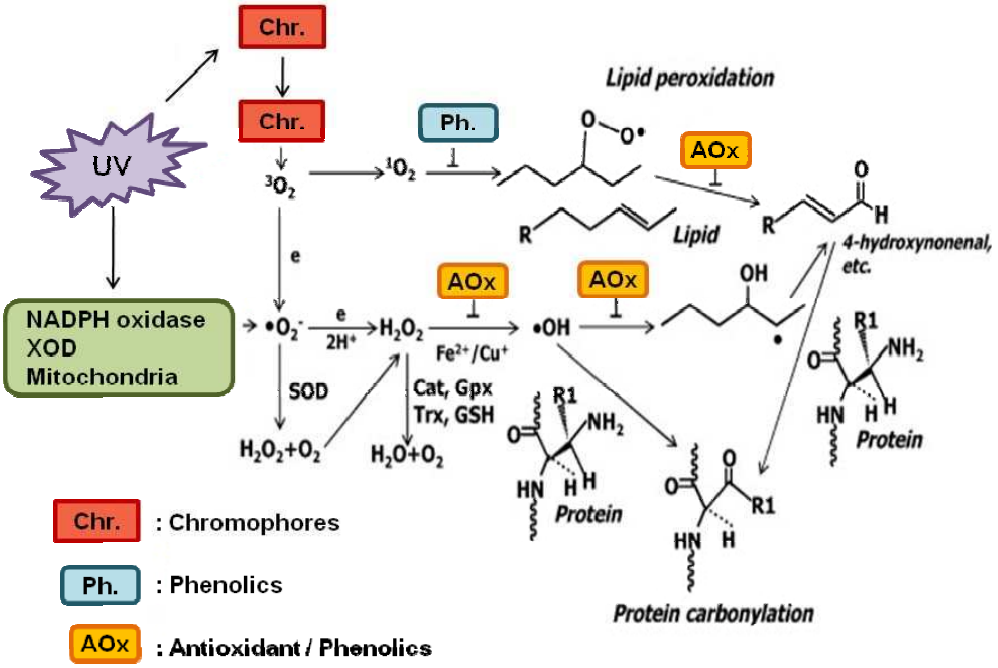

Fig. 2. ROS-initiated oxidative chain reactions and scavengers.

UV irradiation induces photo-damage of the skin, which is characterized by distinct alterations in the composition of the dermal extracellular matrix (ECM), resulting in wrinkles, laxity, coarseness, mottled pigmentation and histological changes that include increased epidermal thickness and connective tissue alteration. Breakdown of a balance of combination between the connective tissue components (biomolecules including collagens, proteoglycans and glycoproteins) leads to the detrimental effects i.e. photoaging in dermal fibroblasts. Skin contains antioxidant defenses, which nullify reactive oxygen species (ROS) including free radicals, but these defenses will be overwhelmed if the dose of UV light is high enough, and this result in free radical damage to cellular components such as proteins, lipids and DNA. ROS induced by oxidative stress can ultimately lead to apoptotic or necrotic cell death. Especially, the accumulated ROS plays a critical role in the intrinsic aging and photoaging of human skin *in vivo*, thus suggested to be responsible for various skin cancers and other cutaneous inflammatory disorders (Kawaguchi *et al*.,1996).

Several investigations have revealed that collagen gets degraded in photoaged skin due to the inhibition of collagen synthesis mediated by matrix metalloproteinases (MMPs), which are a family of secreted or transmembrane zinc endopeptidases that are capable of digesting ECM. MMPs are divided into subclasses of collagenases, gelatinases, stromelysins, matrilysins and membrane-type MMPs (MT-MMPs) according to their substrate specificity and domain structure. It is reported that ROS effects the MMP gene expression through signal transduction

pathway. Expression of MMPs is usually induced by various extracellular stimuli such as growth factors, cytokines, tumor promoters and UV. Over expression of MMPs has been observed in tissue remodeling, repair and destruction by many extracellular stimuli and among the MMPs, MMP-2 and MMP-9 degrade the ECM and they influence skin wrinkle formation and skin thickness (Inomata *et al*., 2003).

Moreover, the MMP-9 proteolytic system may also modulate active VEGF. It has been reported that inflammatory cells, including neutrophils, macrophages, and mast cells, especially those expressing MMP-9, can be accomplices to neoplastic cells during squamous carcinogenesis (Bergers *et al.,* 2000). COX-2 expression is critical for chronic UV-induced murine skin carcinogenesis (Chun and Langenbach, 2007). One of the most frequent events in carcinogenesis is the uncontrolled activation of the Ras signaling pathway and Lee *et al.* (2006) reported that H-Ras upregulated MMP-9 and COX-2 expression through the activation of extracellular signal-regulated kinase and the IκB kinase-IκBα-nuclear factor-κB signaling pathway, which may contribute to the malignant progression of WB-F344 rat liver epithelial cells. Thus, UVB-induced skin carcinogenesis and tumor growth might be closely associated with the systems of MMP, VEGF, and COX-2 expression.

Therefore, protecting the skin against UV radiation is vital. Photoprotection is a group of mechanisms that nature has developed to minimize the damages that an organism suffers, when exposed to UV radiation. These mechanisms can be controlled or organized by certain organic and inorganic compounds or substances (i.e. melanin) produced by different terrestrial and aquatic sources. In a series of studies of the effects of natural products on UV-induced skin damage, it was found that olive extract, and in particular, oleuropein, inhibited increases in skin thickness and reductions in elasticity induced by long-term exposure to UVB in hairless mice. In addition, oleuropein reduced the incidence and growth of tumors in chronically UVB-irradiated mice (Kimura and Sumiyoshi, 2009).

Recently, it was demonstrated the involvement of the antioxidant response element (ARE), a transcriptional control element that meditates a family of phase 2 enzymes and antioxidant proteins, under control of the transcription factor NRF2, in skin protection. Induction of ARE-dependent genes plays an important role in protection of cells against oxidative damage (Aleksunes and Manautou, 2007).

Again, transcription of this family of enzymes provides a powerful repair mechanism for skin against ROS, caused through UV exposure or inflammation associated with ageing. Previous work with olive phenolics has demonstrated that these compounds, being free radical scavengers, contribute positively towards skin health by preventing the oxidative damage linked with the formation of wrinkles and other such disorders, such as skin dryness and hyperproliferation (Aldini *et al*., 2006). Recently, gene expression profiling of age spots has also provided an understanding of the role of olive oil derivatives in inducing antiageing skin benefits, by upregulating the transcription of the antioxidant response element, and antihyperpigmentation benefits, by downregulating the transcription of beta adrenergic receptors (Osborne *et al*., 2012).

IV – Conclusions

In summary, the bioavailable nature of olive oil phenolic compounds supports evidence for their health promoting properties. However, in this regard, significant efforts should also be focused on the isolation and structural elucidation of olive phenolics and other bioactive derivatives. This can promote application of olive in the food, pharmaceutical and cosmetic industries and provide sound clinical basis for assessment of potential anti-atherosclerotic, anti-hypertensive, anticancer, anti-platelet aggregation and immune-modulatory functionalities of olive bioactives.

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