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Chapter

Olive Oil Phenols

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Abstract

Olive oils contain numerous substances that have a beneficial role in human health. Phenols are natural compounds that are present in extra-virgin olive oil (EVOO), and they are produced at the malaxation step of the olive oil production. The four most abundant phenols in EVOO are oleocanthal, oleacein, ligstroside aglycon, and oleuropein aglycon. These phenols exhibit significant biological effects in many diseases, participating in various cellular and biochemical processes. Oleocanthal can protect and prevent against the Alzheimer disease, demonstrates acute antiplatelet effects, which has a vital role against cancer, and can act like ibuprofen. Oleacein has antioxidant and anti-inflammatory activities and helps against atherosclerosis. Moreover, it acts as an antiaging factor and as a 5-lipoxygenase inhibitor. Ligstroside aglycon implicates to mechanisms against breast cancer, while oleuropein aglycon shows activities against the Alzheimer disease and breast cancer.

Keywords: EVOO, phenols, oleocanthal, biological activities, functional foods

1. Introduction

In the fourth century B.C., Hippocrates said: “Let food be thy medicine and medicine be thy food,” as he wanted to point the meaning of choosing the right human diet. Nowadays, food science has focused on this sentence by rethinking many prospects about foods and their relationship with human health. The concept of developing food to promote health and reduce the risk of disease to the people was, first, introduced in Japan in the decade of 1980. This consideration led to the birth of the term functional foods. This term is not quite specified yet, but generally as functional foods, they are considered foods that are basic in human diet, and they contain components with significant biological activities. Foods are a large deposit of natural compounds with health effects on humans, and scientists work on this base to develop foods and products based on these compounds.

2. Mediterranean diet and extra-virgin olive oil

The Mediterranean diet (MD) is referred to the food consumption habits around the Mediterranean basin. The particular kind of diet usually includes, at its traditional form at least, high, and daily consumption of vegetables, legumes, nuts, fruits, and other plant foods or plant derivatives like olive oil [1]. On the other hand, in the Mediterranean diet, there is less meat and dairy product consumption and a medium rate of fish feeding. Comparing with other types of diet, the
Mediterranean diet is characterized as unique in two basic elements. First, there is a high fat uptake as a result of extra-virgin olive oil existence in daily basis to the table and also from fishes and nuts [2]. The second uniqueness of this diet is the alcohol consumption, and particularly the red wine, upon meals [3]. But the real question is why the Mediterranean diet is so important for the people’s lives?

2.1 Healthy effects of the Mediterranean diet

The observation of long living people in the Mediterranean area, especially in various places in Greece and Southern Italy, intrigued the scientific community leading to the association with the Mediterranean diet and a general healthy way of living [4]. Moreover, the observation of the low percentage of the cardiovascular diseases in these populations strongly improved the initial image of diet-caused health of humans [5, 6]. Worldwide, studies took place setting as goal to discover the results of the Mediterranean diet in human health. Scientists found a wide range of situations that Mediterranean diet improved health, including, cardiovascular diseases [7, 8], type 2 diabetes protection and generally metabolic syndrome [9, 10], malignancies such as breast and gastric cancer [11, 12], depression [13, 14], and cognitive impairment [15, 16]. The Mediterranean diet also helps people to control their body weight and in obesity to improve the weight of the patient [17].

2.2 Extra-virgin olive oil in the Mediterranean diet

The Mediterranean diet is mostly plant-based and includes a high consumption of cereals, vegetables, and fruits, while red meat and sweets are rarely consumed. In MD, there is a daily consumption of dairy, fish, and poultry. Olives and red wine are moderately consumed, as well as with nuts and seeds. Less added salt sources are often consumed like herds, garlic, and onion. All of the above are supported on the basis of high water intake and regular physical exercise. Extra-virgin olive oil (EVOO) has a central place in Mediterranean diet as it shows in the Mediterranean diet pyramid below. Extra-virgin olive oil has a significant role in cardiovascular disease decreasing the risk in human that enclose it to their diet [1]. Moreover, anti-inflammatory and antioxidant properties have been attributed to EVOO [18]. EVOO is the main source of fat in the Mediterranean diet and especially of the unsaturated fats that are more beneficial than saturated fatty acids [19].

3. Functional foods, extra-virgin olive oil, and phenols

Plant foods can provide many basic nutrients to humans, participating in many ways to a healthy state of the human body [20]. Secondary metabolites are compounds synthesized in plants, with crucial roles such as the adaptation of plants to their environment [21]. These phytochemicals (PCs) are very essentials for the human diet but, also, exhibiting considerable biological activities [22, 23], leading to the usage of PCs as potential pharmaceuticals [24]. PCs have some serious advantages like their accessibility, the specificity of their response, and their low toxicity [25]. On the other hand, the negatives of these compounds are the low bioavailability and the fast metabolism in humans.

In the last decades, many pharmacological studies have been applied to secondary metabolites, guiding industries to the design of new drugs [26]. Many studies involving compounds of natural sources have shown that secondary metabolites have many actions in the human body, such as antitumor, antibacterial, and anti-inflammatory. A proper diet, like the Mediterranean, involves food consumption that supplies the human body with many beneficial nutrients, resulting in good
tolerance of the body’s health, and provides natural resources, with pharmaceuti-
cals properties, for health protection and improvement [27].

3.1 Phenols in EVOO: structure, chemistry, and biosynthesis

About 98% of EVOO consists of triacylglycerols (TGAs), a group of glycerol esters containing different fatty acids. Oleic acid is the major fatty acids, while there are also palmitic acid, linoleic acid, stearic acid, and palmitoleic acid [28] (Figure 1). Moreover, there are minor compounds that are lipophilic or amphiphilic like phytosterols such as

Figure 1.

Figure 2.
β-sitosterol, campesterol, and 4-methylsterols and hydrocarbons such as squalene and β-carotene. There are also fatty alcohols, triterpenic alcohols, and triterpenic acids, like erythroidol, oleanolic, and maslinic acids. There are, also, tocopherols such as α-tocopherol and pigments. Other minor components of EVOO are sterol esters, glycerolipids, phosphatides, waxes, sterol esters, and mono- and diacylglycerols [29].

Another group of molecules present in EVOO, of a high impact in diet, are the phenolic compounds or as usually are called, polyphenols, such as tyrosol and hydroxytyrosol and their derivatives [30]. The phenolic cluster of EVOO can be further divided into several subclasses [31]. There are the lignans like taxifolin, luteolin, apigenin, and other molecules [32]. EVOO contains simple phenols that include tyrosol, hydroxytyrosol, and phenolic acids. Another subgroup is the secoiridoids that are derivatives from tyrosol, hydroxytyrosol, and elenolic acid, like the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA or oleacein) and tyrosol (p-HPEA-EDA or oleocanthal). The secoiridoids subgroup includes also the oleuropein and ligstroside aglycons (3,4-DHPEA-EA, p-HPEA-EA, respectively) and their isoforms oleomissional and oleokoronal [33] (Figure 1).

Figure 3.
Production and structures of secoiridoids in EVOO, and their isoforms. 1 = Hydroxytyrosol; 2 = Tyrosol; 3 = Oleacein; 4 = Oleocanthal; 5a&5b = Closed ring monaldehydic forms of oleuropein aglycon; 6a&6b = Closed ring monaldehydic form of ligstroside aglycon; 7 = Ligstroside; 8 = Oleuropein; 9&10 = Unstable forms of oleuropein and ligstroside aglycons; 11a&11b = Open ring dialdehydic forms of oleuropein aglycon, or oleuropeindials; 12a&12b = Open ring dialdehydic forms of ligstroside aglycon, or ligstrodidials; 13 = Enolic form of the oleuropein aglycon; 14 = Enolic form of the ligstroside aglycon. The group of 13, 11a and 11b compounds is defined as oleomissional, as they exist in continuous balance. The same balance exists between compounds 14, 12a and 12b, and they are named as oleokoronal. (Picture taken from: Diamantakos et al, 2015. Oleokoronal and oleomissional: new major phenolic ingredients of extra virgin olive oil. OLIVAE No 122).
The majority of EVOO phenols belong to the secoiridoids tyrosol and hydroxytyrosol subgroup, contributing to the bitter taste and the throat burning sensation [34]. Figure 2 shows the metabolic pathway of phenolic compounds biosynthesis in plants. In plants, the production of secondary metabolites is initiated by the mevalonate (MVA) and 2-C-methyl-d-erythritol 4-phosphate (MEP) pathways. These two pathways lead to the production of sterols and terpenoids. Secoiridoids are produced by the MEP pathway but presumably derived from tyrosine proceeding via tyrosol. Arogenate decarboxylated is the precursor of tyrosine in the phenylpropanoid metabolism, while hydroxytyrosol is synthesized from tyrosine that is produced through the dopamine pathway. Flavonoids, lignans, and verbascoside are products of the phenylpropanoid pathway, while verbascoside could be a product of tyramine via dopamine or coming from tyrosol via hydroxytyrosol [35].

Phenols are organic molecules characterized by the existence of a hydroxyl group attached directly to the benzolic group of the compound. Secoiridoids are the primary phenolic compounds present in EVOO, and their molecules are based on a phenylethanoid structure, such as oleuropein and ligstroside, as shown in Figure 3 [29]. In EVOO these compounds are present as esters of hydroxytyrosol and tyrosol, respectively, as their initial forms are hydrophilic and are the most predominant phenols. The biosynthesis of all secoiridoid derivatives in EVOO has as start point these two compounds: oleuropein and ligstroside, the predominant phenols in olives [36]. During the process steps in EVOO production and in particular in the crushing and malaxation steps, these molecules are transformed due to β-glucosidase [37], into their aglycon forms, as shown in Figure 3. The aglycon forms of oleuropein and ligstroside are very unstable, and they further transform to closed-ring monoaldehydic or open-ring dialdehydic forms [33]. At the malaxation step, the dialdehydic forms undergo demethylation and decarboxylation leading to the production of oleacein and oleocanthal.

4. Phenols and their biological aspects

For many years, the phenolic cluster of EVOO focuses the interest of researchers, as it was suspected that the products of the secondary metabolism have beneficial effects in human health. To prove this, many studies took place around the world, either with EVOOs or with purified polyphenols, at the level of cellular process, animal studies, and clinical trials in humans. By time, the suspicion approved real, and this led to a widespread research of the biological aspects of the most abundant phenols [28]. The majority of the studies proved that benefits of EVOO consumption is mostly due to the phenolic alcohols and their secoiridoid derivatives, and in particular oleocanthal, oleacein, ligstroside aglycon, and oleuropein aglycon, leading to the naming of these compounds as nutraceuticals, and the EVOO and the olives that contain them are named as functional foods [38]. The Functional Food Center (FFC) defines foods as "Natural or processed foods that contain known or unknown biologically-active compounds; these foods, in defined, effective and non-toxic amounts provide a clinically proven and documented health benefit for the prevention, management or treatment of chronic diseases” [39]. These four molecules act to a wide range of biological paths, as shown in Figure 4.

It becomes obvious that polyphenols are very important for human health participating in all these biological processes, increasing life expectancy [40]. Studies have shown anticancer properties of polyphenols [41, 42], while they exhibit astonishing strong antioxidant activities [43]. One of the most important discoveries about polyphenols was the neuroprotective role [44] and particularly the effects against Alzheimer’s disease [45]. A very important issue of polyphenols is the efficacy in heart diseases [19], like atherosclerosis [46, 47]. Studies showed
that polyphenols are able to reduce LDL oxidation in vivo and in vitro [48–50]. EVOO polyphenols, also, are effective in autoimmune inflammatory situations like rheumatoid arthritis and systemic lupus erythematosus [51].

4.1 Oleocanthal

Oleocanthal (OC) is the molecule with the most studies among the four most abundant olive oil phenolic compounds, and several studies have been conducted from researchers about OC’s biological actions. One of the most important roles of OC in humans is to act as neuroprotective agent. Thus, OC quickly used in studies associated with the Alzheimer disease (AD). AD is caused mainly by the malfunction of two important processes: the amyloid-β oligomer (Aβ) concentration in the blood-brain barrier (BBB) and the tau protein fibrillization and aggregation into neurofibrillary tangles. Aβ oligomers clearance normally happens through the BBB. The amyloid-β1–42 peptide (ADDL) is a neurotoxin that causes the concentration of Aβ oligomers to the BBB, resulting to AD development. Moreover, P-glycoprotein and LDL lipoprotein receptor-related protein-1 (LRP1) are two proteins that participate in Aβ transport and clearance through BBB in normal situations. As shown in Figure 5, OC is able to affect both processes contributing to Aβ clearance of the brain through the blood-brain barrier (BBB) [52], either by altering the assembly state of soluble ADDL oligomers [53] or by increasing expression of P-gp and LRP1 proteins [54]. In comparison with control ADDLs, oligomers formed in the presence of OC (Aβ-OC) showed equivalent co-localization at synapses but exhibited greater immunofluorescence as a result of increased

Figure 4.
antibody recognition, and direct detection of fluorescently labeled ADDLs showed an overall reduction in ADDL signal in the presence of OC. On the other hand, in vitro and in vivo studies with increased P-gp and LRP1 protein expression in the brain microvessels and inhibition studies confirmed the role of upregulation of these proteins in enhancing Aβ clearance after OC treatment, which leads to Aβ degradation.

Neurodegenerative diseases can be caused by another factor, the tau protein, which is a part of the microtubule-associated protein (MAP) family, with the other two members to be the MAP1 and MAP2 proteins. In the adult’s brain, tau is the most expressed protein, and it is found in six isoforms [55]. Tau is a phosphoprotein, and its role is the assembly of tubulin from microtubules and their stabilization. Tauopathies are called the neurodegenerative diseases associated with tau malfunction are called tauopathies, where there is abnormal hyperphosphorylation and aggregation of the protein. In AD, this abnormal activity of tau protein leads to neurofibrillary tangles. OC can affect the fibrillization and lead to their stabilization, locking the tau protein to its unfolded state. OC interacts with lysine amino groups of tau protein and especially with tau441 amino acid leading to a steady secondary structure of the protein and interferes with tau aggregation [56]. The inhibitory activity of OC is due to the two aldehyde groups existing to the molecule [57]. In AD, inflammation induced by Aβo is characterized by interleukin-6 (IL-6) increase and glial fibrillary acidic protein (GFAP) upregulation. These proteins are downregulated by OC, which also affects negatively the regulation of two Aβo-induced synaptic proteins, SNAP-25 and PSD-95, in neurons and glutamine transporter (GLT1) and glucose transporter (GLUT1) in astrocytes [58].

Many years now, OC was found to have an active role in cardiovascular diseases (CDs) and inflammatory situations. OC shows interesting role in acting like ibuprofen as an anti-inflammatory agent contributing in platelet function [59]. In inflammatory situations, the phenolic cluster of EVOO acts through the cyclooxygenase (COX) pathway in humans. Although structurally dissimilar (Figure 6), both OC and ibuprofen inhibit the same cyclooxygenase enzymes in the prostaglandin biosynthesis pathway. Both enantiomers of oleocanthal exhibited a dose-dependent inhibition of COX-1 and COX-2 activities, with no effect on lipoxygenase activity, much as observed with ibuprofen, making OC a very good anti-inflammatory agent [60].
As OC was recognized as an anti-inflammatory agent, researchers turned their attention to the possible role of OC in cancer states in humans. Cancer is a state of uncontrolled cell proliferation and migration that is caused by various genetic alterations leading to altered protein functions. OC has been found to play important roles in various types of cancer in human. Various studies showed and approved that OC is a molecule that can play an important inhibitive role in cell proliferation and migration and invasion. OC can interact with various proteins, crucial for the cancer development, such as c-Met and STAT3 proteins. c-Met is a protein that is responsible for various carcinomas in humans [61]. c-Met is a receptor tyrosine kinases (RTK), which is expressed mainly in epithelial-endothelial origin cells. Normally, c-Met is responsible for downstream signaling pathways that lead to cell growth, invasion, and angiogenesis [62], and the overexpression of c-Met levels has a crucial role in cancer pathophysiology [63]. OC has been found to be a c-Met inhibitor in breast and prostate cancers [64], capable to inhibit c-Met phosphorylation and the proliferation and invasion of epithelial cells, with IC50 in the μM range (4.47 and 4.8 μM, respectively). In breast cancer, OC exerts its effects through the Hepatocyte growth factor–c-Met-mediated pathway inhibition by blocking the epithelial-to-mesenchymal transition (EMT) and affects the G1/S cell cycle control [65]. Moreover, OC can reduce the expression levels of estrogen receptors contributing to the suppression of cancer growth [66].

Signal transducer and activator of transcription 3 (STAT3) belong to the STAT protein family and has a central role on gene expression related to cell differentiation, proliferation, and apoptosis [67, 68]. In various tumors has been noticed a highly constitutive activation of STAT3 resulting in cancer development [69], setting STAT3 as a potential target in therapeutics of malignancies. OC can play a significant role in cancer by blocking activation of STAT3 [70]. In hepatocellular carcinoma, for example, OC inhibits EMT through downregulation of STAT3 pathways cascade and also reduced STAT3 nuclear translocation and DNA binding activity. OC is able to downregulate the downstream effectors of STAT3, like Cyclin D1, the antiapoptotic proteins Bcl-2 and survivin, and the invasion-related protein MMP2. Another way that OC inhibits STAT3 activation is through the JAK21/JAK2 pathway decreasing their activities, while on the other hand increases in the activity of SHP-1 overexpression of constitutively active STAT3 partly reversed the anticancer effects of oleocanthal, which inhibited STAT3 activation by decreasing the activities of

**Figure 6.** Structures of ibuprofen and oleocanthal.
JAK1 and JAK2 and increasing the activity of SHP-1, a nonreceptor protein tyrosine phosphatase and a tumor suppressor gene. Testing the effects of OC to melanoma studies showed that OC acts through the same way against STAT3 pathway and the gene expression of STAT3 targets, like Mcl-1, Bcl-xL, MMP-2, MMP-9, VEGF [71], and Bcl-2 [72]. A very important fact is that OC causes cell death to the cancer cells but not to normal ones [73]. Heat shock proteins are involved in cancer development as well. Heat shock proteins though can interact with OC, so they are possible targets for cancer treatment [74]. Another way that OC can be used, as antitumor agent, is through targeting and downregulating mTOR, a protein that was found to participate in cancer, and especially to breast cancer, with the IC50 of OC to be in the range of nM [75].

Comparative with other anti-inflammatory agents, like ibuprofen, indomethacin, and nimesulide, experiments showed OC to be more effective against cancer [76]. The anti-inflammatory properties of OC led scientists to test the molecule behavior in joint diseases, like osteoarthritis. This situation is caused by elevated nitric oxide production (NO) in cells. As OC acts like ibuprofen, experiments showed that OC decrease NO production from cells, whereas there is no significant affection of cell viability [77].

4.2 Oleacein

Oleacein is a polyphenol found in EVOO, in various concentrations. Studies about the biological function of this molecule revealed multiple fields of action in humans, such as antimicrobial, antiproliferative, and anti-inflammatory activities [78]. Oleacein is a powerful natural antioxidant agent, showing high activity against oxidation even more than oleuropein [79] and hydroxytyrosol [80]. Oleacein acts as a protective agent against the oxidative damage of erythrocytes that happens as a result of ROS-induced oxidative stress. In epithelial progenitor cells, intracellular ROS formation is decreased upon the presence of oleacein, while proliferation of cells is increased, when they are impaired by angiotensin II. This action is related to the Nrf2/heme oxygenase1 (HO1) pathway activation by oleacein [81].

Oleacein is a very potent compound acting against inflammatory situations, like atherosclerosis. Oleacein is able to enhance anti-inflammatory activity of hemoglobin/haptoglobin complexes through increasing the expression of certain receptors, such as CD163, IL10, and HO1 [82]. Moreover, oleacein targets directly 5-lipoxygenase, an enzyme that participates in inflammatory situations, by catalyzing the initial steps of the biochemical pathway of pro-inflammatory leukotrienes synthesis [83]. Another recent and interesting finding in biological action of oleacein is the attenuation of carotid plaque destabilization. This action could be useful in cases of ischemic stroke, as could reduce the risk of the disease [84].

4.3 Ligstroside aglycon

Ligstroside aglycon is the third of the fourth most important and abundant phenols, present in EVOO. There is no extensive data about studies testing the biological activity of this molecule. Mainly, ligstroside aglycon was checked about possible anticancer function. Molecules like ligstroside aglycon are excellent scaffolds for the design of agents that act against the c-Met protein in breast cancer. Ligstroside aglycon had great effect against migration, as generally approved for tyrosol esters [85]. Ligstroside aglycon can, also, interact and modulate HER2, a tyrosine kinase receptor oncoprotein, in breast cancer cells. It is able to inhibit the gene expression in a very high percentage but with very low doses, at the same time, setting ligstroside aglycon as very potent agent against malignancies [86].
4.4 Oleuropein aglycon

Oleuropein is a very antioxidant compound and has been investigated thoroughly, but its derivative, oleuropein aglycon has paid less attention about its functional purpose. Nevertheless, oleuropein aglycon seems to have crucial biological actions in human. Oleuropein aglycon was found to have a significant role in Alzheimer’s disease. This compound can reduce the levels of β-amyloid and, also, the deposition of Aβ plaques. Animal studies showed a higher level of autophagy, while cell culture experiments suggest that probably oleuropein aglycon regulates mTOR protein [87]. Recent studies confirmed the activity of oleuropein aglycon against amyloid aggregation by modification of the conformational and biophysical properties of amyloid fibrils and of the cell bilayer surface properties at the same time, remodeling the aggregation and helping the association between the protein and the membrane [88]. In AD situation, it was observed various Aβ peptides in amyloid plaques were observed, characterized by N-terminal truncation. The products of this truncation and subsequent cyclization of the N-terminal region of Aβ peptides, by two or ten amino acids, lead to the creation of shortened peptides named as pyroglutamylated peptides (pE-3Aβ and pE-11Aβ) that are more neurotoxic and aggregate faster than normal. Oleuropein aglycon is a compound that can reduce the pE-3Aβ production via lowering expression of the glutaminyl cyclase enzyme that is catalyzing the modification described above [89]. Experiments showed that, even in later stages of AD, can activate autophagy of neurons by increasing the acetylation of histones 3 and 4, improving the synaptic function.

Oleuropein aglycon, also, increases autophagy response and expression of markers associated with autophagy, setting oleuropein aglycon capable of the heart protection in cases of cardiac stress caused by autophagy dysfunction [90]. Monoamine oxidase A (MAO-A) degrades catecholamine and serotonin resulting in hydrogen peroxide production (H₂O₂), that is responsible for oxidative stress, autophagic flux blockade, and cell necrosis. Oleuropein aglycon reverses the cytotoxic effects of MAO-A by increasing autophagy and restoring the autophagic flux. In addition, studies revealed that oleuropein aglycon can interfere with amylin preventing its cytotoxicity. When oleuropein aglycon is present, amylin aggregation cannot react with the cell membrane, driving to skip the pathway that causes the formation of toxic prefibrillar aggregates [91]. Another field, where oleuropein aglycon has an active role is breast cancer [86]. The mechanism in this case is exactly the same with the one that acts in ligstroside aglycon, pointing that polyphenols are physical agents that can be used in cancer treatment.

5. Conclusions

The Mediterranean diet is beneficial for human health as it includes consumption of foods that contain biological active substances. The central position in the MD, for fat intake, is the olive oil. Extra-virgin olive oil is basic at the meals of people around the Mediterranean Sea and, especially, for Greece, Spain, and Italy. Humans around the Mediterranean Sea seem to have a long life prediction probably due their diet. EVOO contains very essential compounds of high importance for the body’s health. Besides the obvious body weight balance, it seems that daily consumption of EVOO contributes to the protection and improvement of serious diseases, such as atherosclerosis, inflammatory states, and cancer. EVOO contains some very crucial molecules named phenols or polyphenols that belong to a larger compound family, the secoiridoids. These molecules are produced during olive oil production and exhibit stunning biological effects in humans. The most abundant
phenols in EVOO are oleocanthal, oleacein, ligstroside aglycon, and oleuropein aglycon along with the aglycon isoforms. Many studies have shown their important role in neurodegenerative diseases, like Alzheimer’s disease and in metabolic syndrome. The four phenolic compounds are natural antioxidants and have very powerful anti-inflammatory and anticancer activities. Moreover, these compounds are very active agents in cardiovascular diseases and act against LDL oxidation. All these activities made the phenols potential nutraceuticals and a good matrix for drug design. EVOO is a very strong functional food that supplies the human body with phenols that are crucial for health, so product development arising from EVOO with high concentration of phenolic compounds can be used for health improvement. Pharmaceutical companies, knowing the dynamics of the natural phenols, have started to produce products based on phenols. High phenolic EVOO, in conclusion, is a powerful natural functional food, and all people should consume it daily.

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Conflict of interest

Authors declare no conflict of interest.

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