Phytochemicals and Human Health

Pharmacological and Molecular Aspects

A Tribute to Late Professor Bimal Kumar Bachhawat

Akhlaq A Farooqui and Tahira farooqui have brought together an international group of authors from 9 countries to discuss the recent developments in the field of phytotherapeutics. The main objective of this book is to present graduate students, postdoctoral fellows, and senior scientists with cutting edge information on the beneficial effects of phytochemicals on signal transduction processes associated with the modulation of various body functions. It is hoped that our attempts to integrate information on molecular aspects of phytotherapeutics would jumpstart more studies on the beneficial effects of phytochemicals in acute and chronic human diseases.

Akhlaq A. Farooqui - Tahira Farooqui
Editors

About the Editors

Dr. Akhlaq A. Farooqui is a biochemist, who has been interested on the effects of phytochemicals in acute neural trauma and neurodegenerative diseases. Akhlaq A Farooqui has published 6 monographs (Glycerophospholipids in Brain: Phospholipase A2 in Neurological Disorders (2007), Neurochemical Aspects of Excitotoxicity (2008), Metabolism and Functions of Bioactive Ether Lipids in Brain (2008), Hot Topics in Neural Membrane Lipidology (2009), Beneficial Effects of Fish Oil in Human Brain (2009); and Neurochemical Aspects of Neurotraumatic and Neurodegenerative Diseases (2010) in press). All monographs are published by Springer, New York. In addition, Akhlaq A. Farooqui has edited 2 books (Biogenic Amines: Pharmacological, Neurochemical and Molecular Aspects in the CNS (2010) and Molecular Aspects of Neurodegeneration and Neuroprotection, Bentham Science Publishers Ltd (2011).

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Chapter IX

Health Effects, Antioxidant Activity, and Sensory Properties of Virgin Olive Oil

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Abstract

Virgin olive oil (VOO) is a natural product obtained directly from olives without any further refining process. If extracted from fresh, healthy olives and properly processed and stored, it is characterized by unique sensory characteristics that are markedly different from those of other edible fats and oils. Many studies have been carried out to clarify the relationships between the sensory attributes in a VOO and its volatile and phenolic profiles, which are responsible for aroma and taste, respectively. Moreover, several lines of evidence suggest that VOO, a basic ingredient of the Mediterranean diet, can play an important role in the prevention of several immune and inflammatory and degenerative diseases. The health value of VOO has been attributed to its high levels of oleic acid and other minor components such as phytosterols, carotenoids, tocopherols, and in particular hydrophilic phenolic molecules. These natural antioxidants, both lipophilic (carotenoids and tocopherols) and hydrophilic (phenol and polyphenols) may exert an effective antiradical activity that is able to limit lipid oxidation. Its health value and pleasant flavour have contributed to an increase in VOO consumption, also outside the traditional olive-oil-producing regions of the Mediterranean basin. In this chapter, a survey on the main positive properties of the VOO will be made. Since hydrophilic

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phenolic molecules have been the subject of intense investigation in recent years, this review will focus on their specific health, antioxidant and sensory effects.

Abbreviations

ABTS	2,2'-azinobis(3-ethylbenzothiaziline-6-sulfonate)
AOM	Active Oxygen Method
COX	Cyclooxygenase
CUPRAC	Copper Reduction Assay
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
$LO^{\bullet}$	Alkoxyl Radical
$LOO^{\bullet}$	Peroxyl Radical
LOX	Lipoxygenase
MUFA	Monounsaturated Fat
OSI	Oxidative Stability Instrument
PAI-1	Plasminogen Activator Inhibitor Type I
PH	Phenolic Compounds
TBAR	Thiobarbituric Acid Reactive Substance
VOO	Virgin Olive Oil

1. Health Effects of Virgin Olive Oil

In 2004, an important international conference concerning the beneficial effects of virgin olive oil (VOO) was organized in Spain. The primary aim of this scientific meeting, which brought together the majority of researchers working on these aspects, was to summarize new findings in VOO, including fat and non-fat components. The results published one year later [1] support that a Mediterranean diet based in VOO is a good dietary model to achieve healthy aging and to prevent some of the most important causes of morbidity and mortality. Some of the most interesting points were as follows:

- A Mediterranean diet, rich in VOO, improves protection against major risk factors for cardiovascular disease, such as the lipoprotein profile, blood pressure, glucose metabolism and antithrombotic profile. Endothelial function, inflammation and oxidative stress are also positively modulated. Some of these effects are attributed to minor components of VOO. Therefore, the definition of the Mediterranean diet should include VOO.
- Several observational studies in humans have shown that the intake of monounsaturated fat may be protective against age-related cognitive decline and Alzheimer's disease.

- Microconstituents from VOO are bioavailable in humans, and have shown antioxidant properties and capacity to improve endothelial function. Furthermore, they are able to modify the haemostasis, showing antithrombotic properties.

1.A. Some Health Benefits Related to Monounsaturated Fatty Acids and Triterpenoid Compounds

Olive oil, which is particularly rich in monounsaturated fat (MUFA) due to a high percentage of oleic acid, improves postprandial lipoprotein metabolism inducing lower levels of postprandial triacylglycerols and higher HDL (high density lipoprotein) cholesterol. Low density lipoprotein (LDL) particles from individuals consuming a favourable MUFA pattern are protected from oxidative modification compared with individuals consuming diets rich in high polyunsaturated fatty acids (PUFA). A rich MUFA diet elicits a less prothrombotic environment by modifying different haemostatic components, such as platelet aggregation, fibrinogen, Von Willebrand factor, total plasma factor VII, tissue factor and PAI-1 (plasminogen activator inhibitor type I) plasma levels. The postprandial increase in activated factor VII is reduced by the intake of VOO in comparison with saturated fats [2].

Oleic acid is not exclusively accountable for the healthful properties of olive oil. In fact, it is also noteworthy that some seed oils obtained from genetic selection, such as high-oleic sunflower oil, can express a similar percentage of this monounsaturated fatty acid. Several investigations indicate that the minor components, either characteristic or particularly abundant in VOOs, such as phenols and squalene, may play a key role in atherosclerosis protection [3-4].

Squalene is a triterpenic hydrocarbon molecule that is an intermediate in sterol biosynthesis. Several studies have shown that squalene is able to contribute to the anti-tumoral activity of VOO, and also modulates cholesterol, triglyceride, glucose and leptin plasmatic levels [5-7]. In particular, Bellosta and co-workers have shown that squalene strongly inhibits the activity of beta-hydroxy-beta-methylglutaryl-CoA reductase, a key enzyme in cholesterol synthesis. Squalene has been also found to inhibit chemically-induced tumors in animal studies [8-9].

Farina and co-workers [10] focused on the interesting anti-ulcer properties of pentacyclic triterpenoids as the derivatives of oleanolic and ursolic acids, proposing the synthesis of new molecules practically devoid of mineralcorticoid activity and potentially non-toxic.

1.B. Some Health Benefits Related to Polar Phenolic Compounds

Pathologies such as cardiovascular disease and cancer have been linked to excessive free radical formation, and thus a higher intake of antioxidants appears to have beneficial effects on human health. Several scientific studies have suggested that phenol-rich olive oil could be particularly useful in contributing to the dietary intake of biologically active compounds [3]. The biological effects of VOO phenolics have been well detailed in a recent review [11].

It must be remembered that only VOO (according to the current regulations) contains a significant amount of polar phenolic compounds, as other oils have almost totally lost them as a consequence of the chemical-physical procedures employed during refining.

1.B.1. Phenolic Bioavailability

Important studies have been carried out to demonstrate the dose-dependent absorption of olive oil phenolics in humans and their urinary excretion as *O*-glucuronide conjugates [12-13] Phenolic aglycones, such as secoiridoids, are subject to time-dependent hydrolysis in the acidic gastric environment, leading to an approximate 5-fold and 3-fold increase, respectively, in the amount of free hydroxytyrosol and tyrosol after only 30 min following assumption [14]. The major site for its absorption is the small intestine. Following ingestion of VOO, the levels of hydroxytyrosol and tyrosol increase rapidly achieving a peak concentration at approximately 1 h in plasma and 2 h in urine [15]. Studies have confirmed that hydroxytyrosol is absorbed by passive diffusion from the gut, through a pathway independent of chylomicron formation [16]; during the post-prandial phase this molecule is incorporated into human lipoproteins, thereby increasing plasma antioxidant activity and decreasing LDL oxidation, thus exerting a significant antioxidant effect *in vivo* [17]. Recent investigations have also demonstrated that secoiridoids, which do not appear to be absorbed in the small intestine, undergo bacterial catabolism in the large intestine with oleuropein undergoing rapid degradation by the colonic microflora producing hydroxytyrosol as the major end product [18].

It is noteworthy that hydroxytyrosol exists in the brain as an endogenous catabolite of cathecholic neurotransmitters such as dopamine and norepinephrine that are formed via monoamino oxidase-catalyzed deamination and subsequent reduction. Therefore, it is plausible that hydroxytyrosol is recognized and metabolized by catecholamine enzymatic systems (catechol-*O*-methyltransferase). In this regard, the formation of homovanillyl alcohol in Caco-2 cells has been reported [13]. Trials conduced on human volunteers who ingested olive oil supplemented with increasing amounts of phenolics suggested that the catechol-*O*-methyltransferase derived metabolite of hydroxytyrosol (i.e. homovanillyl alcohol) enters into cellular compartments where it exerts its antioxidant activity [19].

1.B.2. Enzyme Activity Modulation

The protective effects of hydroxytyrosol and oleuropein (this latter molecule contains hydroxytyrosol in its structure) have been demonstrated through both *in-vitro* and *in-vivo* tests. The activities of olive oil phenolics on enzymes have been tested on macrophages, leukocytes and platelets: several studies [20-22] have demonstrated the capacity of hydroxytyrosol to inhibit chemically induced *in-vitro* platelet aggregation, accumulation of thromboxane in human serum, production of pro-inflammatory leukotrienes and inhibition of arachidonate lipoxygenase.

1.B.3. Antioxidant Activity

The mutagenic properties of oxidatively-damaged DNA suggest that antioxidants might have a protective activity toward cancer formation. The orthodihydroxy (catecholic) substitution, as for hydroxytyrosol and oleuropein, confers high antioxidant capacity as shown by several *in-vitro* tests. For example, both hydroxytyrosol and oleuropein were able to scavenge superoxide anion generated by the xanthine/xanthine oxidase system and by human polymorphonuclear cells, and to block potent oxidant species produced in vivo in the site of inflammation by activated neutrophilis [23].

During inflammation events, macrophages react to the endotoxin by increasing nitric oxide production, which in turn inhibits platelet aggregation and exerts a protective role in preventing oxidative LDL modification that may occur at the site of inflammation. Visioli and co-authors [23] verified that oleuropein significantly increases the production of nitric oxide as a functional response of the immunocompetent cells.

A scavenging effect of hydroxytyrosol and oleuropein has also been demonstrated with respect to hypochlorous acid [24], a potent oxidant produced in vivo by neutrophil myeloperoxidase at the site of inflammation.

A potent and dose-dependent reduced formation of various markers of LDL oxidation has been observed, indicating protection of the apoprotein layer [25] and confirming their metal chelator and radical scavenger activities [24]. Data obtained on human oxidized LDL [26] demonstrated that phenols present in the hydroalcoholic extract of VOO inhibit the formation of oxysterols in a dose-dependent manner. Moreover, protection against the oxidative modifications of apoproteins may play an important role in preventing the accumulation of lipids in the arterial wall.

Recently, Visioli and co-workers [27] showed a significant increase in total plasma glutathione levels, both in the reduced and oxidized forms, in 98 healthy subjects after ingestion of 2 ml of a phenolic extract obtained from olive mill waste waters that were particularly rich in hydroxytyrosol. It was suggested that the observed effects might be governed by the antioxidant response element mediated increase in phase II enzyme expression, including that of γ-glutamylcysteine ligase and glutathione synthetase.

1.B.4. Anti-Inflammatory Activity

One VOO phenolic molecule of particular interest due to its putative health-benefiting properties is decarboxymethyl ligstroside aglycone (also named oleocanthal), the main component responsible for the throat irritation associated with VOO consumption (codified as pungent positive taste). Beauchamp and colleagues [28] demonstrated that oleocanthal possesses anti-inflammatory activity due to its dose-dependent ability to inhibit the same cyclooxygenase (COX) enzymes that are involved in the same prostaglandin biosynthesis (inflammatory) pathway as ibuprofen. It has also been demonstrated that the dose-dependent anti-inflammatory properties exhibited in vitro are mimicked by its dose-dependent irritation in the oral cavity.

Interestingly, in 2009 Cicerale and co-authors [29] demonstrated a minimal decrease in oleocanthal upon heat treatment, but a significant decrease in the biological activity of this compound with extended heating (up to 31%). The authors hypothesized that the reduction in biological activity after heating may be due to the formation of an oleocanthal antagonist, which could mask the anti-inflammatory activity.

1.B.5. Anti-Tumoral Activity

The effects of VOO phenols on colorectal cancer have gained particular attention, since it is the second most common cause of cancer mortality in Western countries. Their ability to inhibit colon cancer development has been demonstrated in cancer cell models [30], animals [31] and humans [32]. In spite of their direct antioxidant effects, VOO phenolics may exert chemopreventive effects via their interactions with cellular signalling pathways that are important in controlling the growth, differentiation and metastasis of cancer cells [33-36]. The anti-cancer properties in the gut seem to be mediated at least in part by the phenolics through

modulation of MAPK kinases and down-regulation of the expression of COX-2 and Bcl-2 proteins, which play a crucial role in colorectal carcinogenesis.

2. Antioxidant Activity of Phenolics in Virgin Olive Oil

2.A. General Concepts Relating to Antioxidant Activity of Phenolic Molecules

Phenolic compounds can slow lipid oxidation, inhibiting initiation and propagation steps, by inactivating or scavenging free radicals. By acting as free radical scavengers or chain-breaking antioxidants, phenolic compounds (PH) in VOO are able to accept a radical from oxidizing lipids species such as peroxyl ($LOO^{\bullet}$) and alkoxyl ($LO^{\bullet}$) radicals through the following reaction:

$$LOO^{\bullet} / LO^{\bullet} + PH \rightarrow LOOH / LOH + P^{\bullet}$$

Hydrogen donation generally occurs through the hydroxyl group of phenols, and the subsequently formed radical is stabilized by resonance delocalization throughout the phenolic ring structure.

A second hydroxyl group, especially at the *ortho* position, stabilizes the phenoxyl radical through an intramolecular hydrogen bond; thus phenols with a catechol structure, known as *o*-diphenols, such as hydroxytyrosol, decarboxymethyl oleuropein aglycon and oleuropein aglycon, are the most potent antioxidants in VOO [37].

The antioxidant efficiency of phenols in VOO is dependent on the ability of the antioxidant molecule to donate hydrogen to the free radical, such that the transfer of the hydrogen to the free radical is more rapid and energetically favourable as the hydrogen bond energy of the phenols decreases. A phenol (e.g. catechol $E°'$=530 mV) having a reduction potential lower than the reduction potential of a radical (e.g. peroxyl radical $E°'$=1000 mV) is capable to react, by donating a hydrogen to it, with a feasible reaction from a kinetic point of view [38].

A phenolic molecule can lose antioxidant activity if present in a lipid substrate at concentrations that are too high, and also behave as pro-oxidants by involvement in initiation reactions [39].

Another general concept to keep in mind, which has important implications for antioxidant activity, is related to the distribution in lipidic substrates of different molecules. In fact, natural antioxidants exhibit complex properties between air-oil and oil-water interfaces that significantly affect their relative activities in different lipidic systems. The presence of hydrophilic phenolic compounds in VOO, and their high antioxidant activity, can be explained by the so-called "polar paradox" [40-41]. According to this, "polar antioxidants are more effective in non-polar lipids, whereas non-polar antioxidants are more active in polar lipid emulsions". The polar paradox is based on the fact that in a bulk oil system hydrophilic antioxidants, such as polar phenols, are oriented in the air-oil interface (a low quantity of air

is always trapped in the oil) and become more protective against oxidation than the lipophilic antioxidants, such as tocopherols, which remain in solution in the oil.

2.B. General Concepts On Direct and Indirect Methods to Determine Antioxidant Activity of Phenols in Virgin Olive Oil

There are several approaches to determine the antioxidant activity of the phenols present in VOO. For simplicity, these can be divided into two groups: indirect and direct methods [42]. Whereas the former measure the ability of phenols to block free radicals that are not linked to oxidative degradation, the latter are closely associated with the study of the effect of phenols with respect of lipid peroxidation. Generally, indirect methods are used more frequently than direct methods, and each type of method has its advantages and disadvantages. Direct methods are more satisfactory in principle, and are generally more sensitive; on the other hand, they are more time-consuming and more difficult experimentally.

2.B.1. Indirect Methods

Indirect methods commonly provide information on the ability of phenols to scavenge stable free radicals as 2,2-diphenyl-1-picrylhydrazyl (DPPH test) and 2,2'-azinobis(3-ethylbenzothiaziline-6-sulfonate) (ABTS assay) or to reduce a metal, such as FRAP (ferric reducing antioxidant power) and CUPRAC (copper reduction assay) tests [43] One widely applied indirect method is the Folin–Ciocalteu test, which allows estimation of the reductive capacity of phenolic molecules (previously extracted by VOO) [44]. The Folin–Ciocalteu test is a well-standardized indirect method that is useful for routine estimation of antioxidant activity of phenols in VOO. It is also possible to use methods based on chemiluminescence to assess the antioxidant capacity of phenols [45].

2.B.2. Direct Methods

The first important choice concerns the lipidic substrate in which the antioxidant activity of phenolic compounds is tested. Preference should be given to individual lipids such as methyl linoleate, linoleic acid or trilinolein since their commercially accessibility provides reproducibility [46]. Moreover, these compounds are relatively inexpensive, and their oxidation is representative of the most essential features of lipid peroxidation. Another possibility is the use of a VOO in which its antioxidants and pro-oxidant components were eliminated through washing with appropriate solvent mixtures.

The initiation of lipid peroxidation can be start by using an initiator, such as a thermolabile azo-compound (i.e. water soluble AAPH or lipid-soluble AMVN), or a transition metal (i.e. Fe^{3+} or Cu^{2+}) [47].

Monitoring of lipid peroxidation is possible through two means: discontinuous, via collection of several aliquots, or continuous. This latter is preferable as it allows both a higher throughput and more detailed observation of the process.

The most popular determinations are based on the quantification of the primary products of oxidation (i.e. through the measure of peroxides and of conjugated dienes), of secondary products (i.e. through the TBARS assay or of specific volatile aldehydes as hexanal or nonanal) or of oxygen consumption. Measurement via oxygen consumption seems to be more representative of the oxidation development and is also quite sensitive.

Studies on lipid matrices enriched in phenolic compounds or VOO can be performed under normal storage conditions or under accelerated oxidation such as active oxygen method (AOM), Schaal oven test, oxygen uptake/absorption, or by using a fully automated oxidative stability instrument (Oxidative Stability Instrument called OSI or Rancimat apparatus) [48-49]. Automatic assays of lipid oxidation in bulk using enzymatic sensors to record the consumption of phenolic components have been demonstrated [50-51].

2.C. Evaluation of Antioxidant Activity in Virgin Olive Oil

Various investigations have studied the importance of the total or individual phenol contents with regards to VOO stability. The active phenols in VOO are mainly *o*-diphenols such as hydroxytyrosol and its oleosidic forms. However, monophenol tyrosol and its oleosidic and derivative forms show less antioxidant activity. In 1999, Aparicio *et al.* [49] used Rancimat (set to 100°C) to study the relative contribution of several chemical compounds (phenols, carotenoids, tocopherols, chlorophylls) to the oxidative stability of 79 VOOs. It was observed that the main components protecting VOO against oxidation are *o*-diphenols, and that other purported antioxidants do not contribute significantly to VOO stability in the presence of phenols.

In 2003, Mateos and co-authors [52] spiked a purified olive oil with several single phenols and tocopherols (with concentration ranges similar to those found in VOO) to evaluate their antioxidant or pro-oxidant activities under accelerated oxidation in a Rancimat apparatus at 100°C. The data were in agreement with the fact that antioxidant activity is correlated with the number of phenolic hydroxyls and the *o*-disubstitution in the molecule. In fact, hydroxytyrosol, hydroxytyrosyl acetate and oleuropein aglycon showed similar antioxidant activities per mmol of substance, whereas the activity of α-tocopherol was significantly lower than that of similar concentrations of these phenols. There was only a slight contribution of tyrosol to oil stability. The flavone luteolin showed an antioxidant activity similar to that of hydroxytyrosol, whereas apigenin did not show any effects.

In investigations carried out by Carrasco-Pancorbo and co-authors [46], the antioxidant activity of several single phenolic compounds of VOO (hydroxytyrosol, tyrosol, elenolic acid, decarboxymethyl oleuropein aglycon, (+)-pinoresinol, (+)-1-acetoxypinoresinol, oleuropein aglycon and ligstroside aglycon) was evaluated by three different chemical approaches: radical assay (DPPH), accelerated oxidation in a lipid model system by OSI and an electrochemical method (flow injection analysis FIA amperometry and cyclic voltammetry). These authors verified that, as is generally assumed, the presence of a single hydroxyl group on a benzene ring conferred only limited antioxidant activity. On the other hand, the presence of a catechol structure enhances the ability of phenolic compounds to act as antioxidants. In fact, the results obtained with all three different approaches showed that *o*-diphenols of VOO (hydroxytyrosol, decarboxymethyl oleuropein aglycon and oleuropein aglycon) were the strongest in terms of antioxidant power. Moreover, by OSI the authors demonstrated a pro-oxidant effect of elenolic acid, (+)-pinoresinol, tyrosol, ligstroside aglycon and (+)-1-acetoxypinoresinol.

In 1999, Mannino *et al.* [53] proposed a procedure to evaluate the antioxidant capacity of VOO based on electrochemical properties by direct injection of the samples in an FIA system with an electrochemical detector operating at a potential of +500 mV (vs. Ag/AgCl). The

results from this procedure were compared with those by Rancimat and the $ABTS^{\bullet+}$ assay (after dilution of samples with *n*-hexane). In a previous work by the same authors [54], it was shown that molecules with an oxidation potential lower than +600 mV (vs. Ag/AgCl) possess good antioxidant activity, whereas molecules with higher values show no or moderate antioxidant activity. Through the study of the hydrodynamic voltammetric profiles of VOO compounds, it was demonstrated that only a few compounds in VOO are oxidizable at potentials lower than +600 mV (i.e. 3,4-dihydroxyphenylacetic acid, caffeic acid, gallic acid, α-tocopherol, oleuropein, protocatechuic acid), and no compounds can be detected at potentials lower than +400 mV. There was good agreement between the results of the proposed electrochemical method in several VOO and those of the $ABTS^{\bullet+}$ decolouration assay (r=0.988), whereas a lower linear correlation was observed with data by Rancimat (r=0.893).

In 2004, Del Carlo and co-workers [55] applied the hydrodynamic voltammetry in flow injection analysis (FIA) for the antioxidant power evaluation of 22 samples of VOO. They also analysed the total phenol content and the antioxidant activity by Folin-Ciocalteu and DPPH, respectively. The antioxidant activity of phenolic extracts determined by DPPH and hydrodynamic voltammetry in flow injection analysis was positively correlated with the oxidative stability measured with Rancimat (r=0.810 and r=0.808, respectively).

3. Sensory Properties of Virgin Olive Oil

VOO is a natural oil obtained directly from olives without any further refining process. Its flavour (combination of odors and tastes) is characteristic and highly appreciated by consumers and is markedly different from those of other edible fats and oils. Many studies have been performed to clarify the relationships between the sensory attributes in a VOO as perceived by assessors and its volatile and phenol profiles, which are responsible for aroma and taste, respectively [37, 56].

Some phenols mainly elicit the tasting perception of bitterness; however, other phenolic molecules can stimulate the free endings of the trigeminal nerve located in the palate and in the gustative buds giving rise to the chemesthetic perceptions of pungency, astringency and metallic attributes.

Some researchers have suggested that the secoiridoid derivatives of hydroxytyrosol are the main contributors to olive oil bitterness: Andrewes et al. [57] assessed the relationship between polyphenols and olive oil pungency. The decarboxymethyl ligstroside aglycone or oleocanthal (an ester between tyrosol and elenolic acid) was the key source of the burning sensation found in many VOO. In contrast, the decarboxymethyl oleuropein aglycone (an ester between hydroxytyrosol and elenolic acid), tasted at an equivalent concentration, produced very little burning sensation. This is a clear example of the different sensory properties of a secoiridoid derivative of tyrosol and hydroxytyrosol. In 2003, Gutierrez-Rosales and co-authors [58] isolated the major peaks found in the phenolic profile of VOO using preparative HPLC; after dissolving in water, these molecules purified were then tasted to evaluate the intensity of bitterness. It was concluded that the peaks corresponding to the decarboxymethyl oleuropein aglycone, oleuropein aglycone and decarboxymethyl ligstroside aglycone were those mainly responsible for the bitter taste of VOO. As previously reported,

Mateos et al. [59] verified the better correlation between the aldehydic form of oleuropein aglycon and bitterness.

In 2005, Beauchamp and co-authors [28] measured the pungent intensity of oleocanthal isolated from different VOO, confirming that this molecule is the principal agent responsible for throat irritation.

As above mentioned, volatile compounds are responsible for the aroma of VOO. The C6 and C5 compounds are enzymatically produced from polyunsaturated fatty acids through the so-called lipoxygenase (LOX) pathway. Quantitatively, linear C6 unsaturated and saturated aldehydes are the most important fraction of volatile compounds in high quality VOO [60], but several other compounds belonging to different classes (e.g. alcohols, esters, ketones, acids, hydrocarbures, terpenes) are also present. The quali-quantitative profiles of these volatile compounds depend on the level and activity of each enzyme involved in the LOX pathway [61]. It should be noted that although the *E*-2-hexenal content, which gives the typical "green note" to VOO, is by far the major C6 aldehyde in all fresh oils, hexanal seems to contribute more to the green odour than *E*-2-hexenal because of its lower odour threshold [62-65].

In addition to fruity, the "green" sensation reminiscent of freshly-cut grass, leaf, tomato, artichoke, walnut husk, apple or other fruits generally contributes to the aroma of high quality oil. "Green" notes include *Z*-3-hexenal, and *Z*-3-hexenyl acetate, whereas alcohols such as *E*-2-hexen-1-ol, *Z*-3-hexen-1-ol and hexan-1-ol have less sensory significance than aldehydes due to their higher odour threshold values. Their sensory descriptions are associated with ripe fruity, soft green and aromatic sensory notes [66].

Esters are compounds associated with fruity nuances [64]. Hexyl acetate and *Z*-3-hexenyl acetate are present in the aroma of all fresh VOOs, but are minor components compared with aldehydes or alcohols. Different authors [62, 64] indicate that *Z*-3-hexenyl acetate is linked to the pleasant green and banana notes. Among the C5 compounds, 1-penten-3-one has been mostly associated with fruity, sweet and pleasant attributes such as tomato and strawberry [64]. It has a very low odour threshold (0.7-50 μg kg^{-1}) and so its contribution to the total aroma can be considered important.

When a low quality of olives or incorrect agricultural and technological practices is used, VOO can be characterized by unpleasant odours coded as defects. The molecules responsible for these negative attributes originate from sugar fermentation (e.g. ethanol, ethyl acetate and acetic acid), degradation reactions of amino acids (e.g. 3-methyl-1-butanol), mould enzyme action (e.g. octen-1-ol) or fatty acid auto-oxidation (i.g. *E*-2-heptenal) [67].

Conclusion

In spite of their low amount, phenolic molecules are very important compounds that are characteristic of VOO. Several experimental lines of evidence have shown that the amount of phenols, particularly those with a catecholic structure, is related to several healthy attributes that include reduction of risk factors for coronary heart disease, prevention of several types of cancer and modification of immune and inflammatory responses. It is also of interest to highlight that phenolic molecules are responsible for bitterness, pungency, astringency taste sensations and for the antioxidant activities of the polar fraction.

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