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Virgin Olive Oil Phenolic Compounds: Insights on Their Occurrence, Health-Promoting Properties and Bioavailability

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Abstract

Virgin olive oil is a highly appreciated edible oil, considered as a relevant component of the Mediterranean diet. The spread of this foodstuff all over the world is making, to a certain extent, that new markets and consumers are getting used to this “Mediterranean’s golden treasure”. Currently, there is great momentum in research relating virgin olive oil intake to healthiness, which has been mainly associated with its phenolics content. Phenolics are considered health-promoting compounds due to their multifaceted biochemical actions that can potentially reduce the risk of various health problems. Yet, since the health-promoting effects of various phenolic compounds have been widely attributed to their metabolic products rather than the naturally occurring forms, the assessment of virgin olive oil phenolics bioavailability is still gaining immense attention and considered a great hot topic among researchers. In the first section of this contribution, the main groups of phenolic compounds identified in virgin olive oil are described, their qualitative and quantitative variability is discussed while analytical approaches applied for their determination are highlighted. The second section reports the beneficial health properties of virgin olive oil consumption related to its phenolics content paying special attention to their bioavailability.

Keywords: Virgin olive oil, phenolic compounds, bioavailability, health-promoting properties, Mediterranean diet

1. Introduction

For centuries, virgin olive oil represented the foremost source of lipid in the daily cuisine of most populations around the Mediterranean Basin owing to its unique sensory characteristics that naturally flavour dishes. Nowadays, even this vegetable oil is still being extensively appreciated for its organoleptic attributes, being consumed either fresh or as flavouring ingredient in prepared foods; nevertheless, its consumption has gained enormous significance worldwide due to its well-established health benefits. Indeed, over the last few decades, the phytochemical profile and health-promoting properties of virgin olive oil have been extensively

explored. There are several studies in which scientists suggest that virgin olive oil intake contributes to improve human's health and well-being by providing protective effects against a plethora of chronic and cardiovascular diseases, neurodegenerative and ageing-related degenerative disorders. Within the frame of virgin olive oil's healthy properties investigation, over the past decades, two main research lines have been proposed. In one of them, the stress has been placed on evaluating and proving biological features and bioavailability of several of its bioactive compounds, particularly, phenolics [1]. In the second one, several epidemiological studies and clinical trials (such as EUROLIVE, Predimed and European Prospective Investigation into Cancer and Nutrition (EPIC)) have brought to the fore the effects of regular intake of virgin olive oil on health [2–4]. Further detailed information about this matter can be found in remarkably interesting manuscripts, which give a deep insight into recent clinical studies, showing the effects of dietary virgin olive oil intake on the human health [5, 6]. Likewise, very stimulating information about the main bioactive compounds which naturally occur in virgin olive oil and their health effects are well detailed in various relevant publications [7, 8].

Even though the nutritional value of virgin olive oil has been attributed to its overall composition involving various chemical substances such as fatty acids, aliphatic and triterpenic alcohols, phytosterols, tocopherols and phenolic compounds, the latter are widely accepted to be the main contributors. Particularly, the bioactivity exerted by these compounds has been associated with their antioxidant activities as indicated by several studies that identified, through *in vitro* and *in vivo* experiments, a strong relationship between the level of virgin olive oil phenolics content and oxygen radical absorbance capacity, free radical scavenging and ferric reducing ability [9]. This propriety is mainly due to the presence in their chemical structure of one or multiple hydroxyl groups able to donate electrons or hydrogen atoms neutralising, in this manner, free radicals and other reactive oxygen species which allows them to act as reducing agents and singlet oxygen quenchers [10].

Moreover, whereas the phenolic profile of virgin olive oil depends widely on the cultivated variety, orchard geographical location (edaphoclimatic conditions), olive growing system and cultivation practices, harvesting time, processing technologies and conservation conditions; however, data accrued from both clinical and experimental studies have conclusively shown that the health advantages of these bioactive compounds are based on their bioavailability considered, thereby, as the main precondition for their efficient biological effect [11, 12]. Additionally, since the most abundant phenolic compounds in this foodstuff are not necessarily those who exert the highest biological effects, understanding the bioavailability of each one of these metabolites is thereby of utmost importance to establish convincing evidence for their efficiency in health improvement and disease prevention. Having that in mind, it is clear that collecting as much as possible data about the bioavailability of the ingested virgin olive oil phenolic compounds is essential to understand their real biological effects.

This chapter brings new insights on the phenolic compounds as one of the main virgin olive oil bioactive components. Major attention has been paid on phenolic fraction composition, the genotypic and agro-environmental and technological factors implicated, analytical approaches for its determination, and their potential health benefits and bioavailability.

2. Phenolics: virgin olive oil bioactive fraction's key compounds

The positively and highly correlated relationship between virgin olive oil intake and health has increased consumer's demand for more information related particularly to its content on bioactive metabolites such as phenolic compounds. Virgin olive oil is

characterised by a wide diversity of phenolics and high total phenolic content ranging from 110 to 900 mg caffeic acid equivalents per kg depending on varietal and geographical origins among other factors [13]. Likewise, it is widely recognised that the phenolics content of virgin olive oil strongly depends on the initial concentration of these compounds in olive fruit. It was established that these metabolites are present in concentration ranges between 1% and 3% of the weight of the fresh pulp [10]. However, given their hydrophilic nature and their partition coefficient between aqueous and oil phases during oil processing, a large amount of these antioxidants is lost with the olive by-products (olive mill wastewater and pomace). Consequently, only 1–2% of the phenolic amount naturally present in the olive fruit could be found in virgin olive oil [14]. Another key characteristic of these metabolites lies in their chemical structures largely different from those identified in corresponding olive fruit. Indeed, the latter shows a complex and highly diversified phenolic composition where glycosylated forms of oleuropein and ligstroside are the most abundant [15]. They are believed to be the precursors of the main secoiridoids compounds found in virgin olive oil [16]. In this regard, during the mechanical extraction of virgin olive oil (particularly the crushing and malaxation steps), various enzymatic and nonenzymatic hydrolysis and oxidation reactions take place inducing several transformative changes in the chemical structure of olive fruit native phenols resulting on the generation of new phenol derivatives [17, 18]. For instance, during the crushing and malaxation steps some enzymatic reactions occur when enzymes (such as polyphenol oxidase, peroxidase, and lipoxygenase) and glycosylated forms of oleuropein and ligstroside meet. Among these enzymes, β -glucosidase seems to play a significant role in the transformation of these compounds into various secoiridoids derivatives such as dialdehydic forms of decarboxymethyl elenolic acid esterified with tyrosol and hydroxytyrosol (oleocanthal (*p*-hydroxyphenyl-ethanol linked to dialdehydic form of elenolic acid (*p*-HPEA-EDA)), and oleacein (3,4-dihydroxyphenyl-ethanol linked to dialdehydic form of elenolic acid (3,4-DHPEA-EDA)) [19]. This fact may explain the rapid decrease of oleuropein and ligstroside concentrations when passing from olive fruit to the corresponding oil [20]. Likewise, hydrolytic mechanisms are known to be involved in the release of hydroxytyrosol and tyrosol in virgin olive oil during storage from complex secoiridoids [21]. Totally, we conclude that phenolic compounds in olive fruits are predominantly found in their glycosylated form whereas conjugate-free compounds, known as aglycones, are the most abundant ones in the corresponding virgin olive oil.

Keeping in mind the above-mentioned, we can claim that virgin olive oil phenolic fraction encompasses those metabolites originally present in olive fruit and those generated during processing.

2.1 Classification of virgin olive oil phenolic compounds

Being abundant in the *Oleaceae* family, which includes *Olea europaea* L., phenolic compounds are a large group of secondary metabolites composed of an aromatic heterocyclic ring bearing one or more hydroxyl groups. The presence of aromatic ring and hydrogen atom of phenolic hydroxyl group makes them as weak acids. To date, more than 32 phenolic compounds have been isolated and identified from virgin olive oil samples using various analytical approaches [22]. For instance, the key phenolic compounds in this vegetable oil can be classified into different chemical classes according to their chemical structures considering mainly the number of aromatic rings, the elements that bind the rings with each other, and the substituents linked to the rings. Accordingly, six chemical families have been reported present in virgin olive oil; namely: simple phenols, secoiridoids, phenolic acids, lignans, flavonoids and hydroxy-isocromans. A representative structure of the main phenolic compounds identified in this product are illustrated in **Figure 1**.

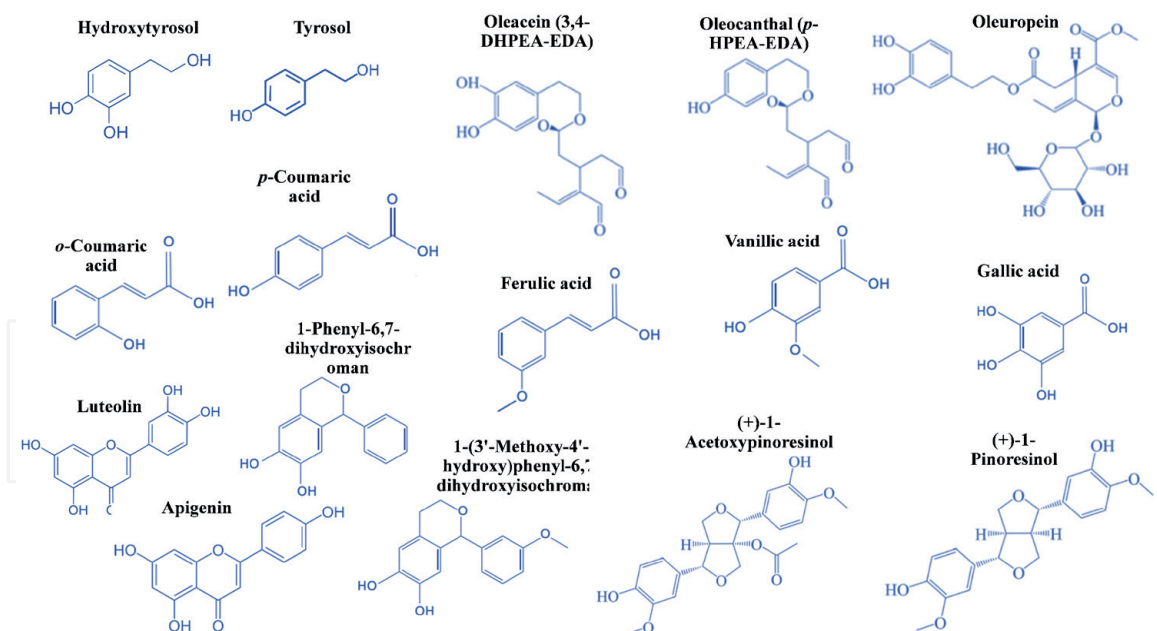


Figure 1.
Chemical structure of the main phenolic compounds found in virgin olive oil.

In a previous metabolomic study of phenolic fraction of virgin olive oil samples obtained from various Mediterranean varieties cultivated in Morocco, a comprehensive analysis was performed by liquid chromatography coupled to mass spectrometry (LC–MS) suggesting that even quantitative differences were observed, the phenolic profiles did not show a significant qualitative differences between studied cultivars [22]. Furthermore, results revealed that secoiridoids prevailed in all analysed samples. A similar result about the phenolic composition of the main cultivated varieties worldwide has also been reported by various authors [23].

2.1.1 Simple phenols

The main phenolic alcohols identified in virgin olive oil are hydroxytyrosol (3,4-dihydroxyphenyl-ethanol (3,4-DHPEA)) and tyrosol (*p*-hydroxyphenyl-ethanol (*p*-HPEA)). Their concentrations are usually low in fresh samples but increases with advanced storage time in a proportional manner due to the hydrolysis of secoiridoids (oleuropein and ligstroside aglycones) [24]. Whilst tyrosol, which has one single hydroxyl group substitution, has been related to a weak antioxidant ability, hydroxytyrosol displays a great radical-scavenging power leading to a better prevention from cardiovascular diseases and to plethora of health effects including anti-inflammatory, anti-cancer and anti-age activities [25]. Thus, in view of the health-promoting effects of this compound and its derivatives, recently, European Food Safety Authority (EFSA) approved a health claim stating that the dietary intake of olive oil phenolic compounds could be able to prevent low density lipoprotein (LDL) oxidation. The exact wording of the claim is olive oil polyphenols contribute to the protection of blood lipids from oxidative stress. The EU restricts the use of this claim to olive oils which contains at least 5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil [26].

2.1.2 Secoiridoids

In virgin olive oil, secoiridoids derivatives are the major group of phenolic compounds. Oleocanthal, and oleacein are among the most abundant ones [16]. These secoiridoids together with the aglycon forms of oleuropein

(3,4-dihydroxyphenyl-ethanol linked to elenolic acid (3,4-DHPEA-EA)) and ligstroside (*p*-hydroxyphenyl-ethanol linked to elenolic acid (*p*-HPEA-EA)) have been associated with some remarkable health effects of virgin olive oil intake [27]. Other derivatives can also be found in lower amount, mainly the aldehydic forms of oleuropein and ligstroside aglycon [28]. Virgin olive oil samples with elevated levels of secoiridoids exhibits greater resistance to oxidation (higher oxidative stability) and higher bitterness intensity.

2.1.3 Phenolic acids

Typically, the term “phenolic acids” refers to the phenolic compounds having one carboxylic acid group. They are mainly divided into two sub-classes: hydroxybenzoic acids (derived from benzoic acid) and hydroxycinnamic acids (derived from cinnamic acid). The most commonly phenolic acids detected in virgin olive oil are: protocatechuic, *p*- and *o*-coumaric, *p*-hydroxybenzoic, caffeic, gallic, cinnamic, vanillic, syringic, and ferulic acids [28, 29]. As compared to other chemical classes, phenolic acids are generally found in lower concentrations in virgin olive oil. Nevertheless, these compounds are acknowledged as strong natural antioxidants displaying a significant role in wide range of biological properties and sensory features of virgin olive oil [29].

2.1.4 Flavonoids

Virgin olive oil flavonoids compounds are widely appreciated for their beneficial health-related effects. They possess two aromatic rings linked by a linear three carbon chain. The main identified flavonoids in this product are luteolin, apigenin and also, even at very low concentration, methoxyluteolin [24].

2.1.5 Lignans

The chemical structure of these metabolites is generally formed by the combination of two units of phenylpropane (carbon 6- carbon 3). (+)-1-acetoxypinoresinol and (+)-1-pinoresinol are the main lignans found in virgin olive oil [30].

2.1.6 Hydroxy-Isochromans

They are present at low concentrations, being mainly formed during the malaxation step through the interaction of hydroxytyrosol and aromatic aldehydes [29, 31]. Mainly two hydroxy-Isochroman compounds were detected in virgin olive oil: 1-phenyl-6,7-dihydroxyisochroman and 1-(3'-methoxy-4'-hydroxy)phenyl-6,7-dihydroxyisochroman [31].

2.2 Major factors affecting virgin olive oil phenolic fraction

Recent attempts to boost virgin olive oil consumption are focusing on the promotion of large range of its quality traits with a specific focus on sensory features and health value. Interestingly, there is huge interest, among producers, in improving organoleptic and nutritional characteristics to meet consumer's demand for healthy premium quality virgin olive oil. These features are acknowledged as the result of complex interactions between biotic and abiotic factors that regulate the biosynthesis and the amounts of key aroma and bioactive compounds in this matrix. Following that logic, seems to make sense that a first steps towards the development of holistic promising approach to produce high organoleptic quality

and healthy virgin olive oil, should start with a deep investigation of the metabolites involved, focusing particularly on their qualitative and quantitative variability in response to endogenous and external factors. Doing so, it could provide crucial information for a better understanding of the mechanisms underlying the content of virgin olive oil on these compounds and, by the way, the impact of various agro-technological factors on virgin olive oil sensory quality and health value.

In this line, for their presumed roles in determining the main virgin olive oil nutritional characteristics along with their implication on enhancing the oxidative stability of this product as well as their great influence on its overall sensory quality (phenolic compounds in combination with volatile constituents are mainly responsible of virgin olive oil's astringency and bitterness), great attention has been paid to understanding those factors responsible of their qualitative and quantitative variations. Several works have been carried out in this respect with a final goal to modulate the impact of these factors to promote the production of a virgin olive oil with high phenolics content. Undoubtedly these compounds are among the most investigated virgin olive oil constituents during the last two decades.

As mentioned above, precursors of virgin olive oil phenolic compounds are biosynthesised during fruit development, resulting from many interacting fruit's growth processes and metabolic and enzymatic activities, which are regulated by internal and external factors such as cultivar, edaphoclimatic growing conditions, cultivation techniques, and ripening process. Once harvested, post-harvest processing (crushing, malaxation, centrifugation, filtration, and storage conditions) factors induce the production and/or the loss of different types of phenolic compounds.

2.2.1 Genetic factors

A large diversity of olive cultivars is used worldwide to produce virgin olive oils with distinct characteristics. Genotype has been pointed as one of the most important factors which significantly influence the phenolic composition of virgin olive oil [32]. As such, the effect of cultivar on this fraction has been attributed to differences in genetic characteristics. Phenolic profiles of virgin olive oil from different varieties and countries obtained by using different agronomic and technological tools have been extensively studied in the literature. In a recent three years study realised by Miho et al. [33] on mono-varietal virgin olive oil obtained from 44 cultivars, the results showed a great qualitative variability in the phenolic composition among the studied cultivars. The genotype was responsible of the highest proportion of variance (66.79%) while the inter-annual parameter explained only 3.67% of variance. Besides, although it is generally assumed that qualitatively, the phenolic composition of virgin olive oils is the same regardless the variety used, however, the existence of quantitative variation of phenolics among olive cultivars has been cited [22, 23]. These differences are outlined as critical information when dealing with the prediction of virgin olive oil stability against oxidation and its organoleptic features. In this sense, cultivars with high phenolic content are expected to be more bitter taste (high intensity of bitterness and pungency) and show higher shelf life and health value [34]. In this sense, Beltran and co-workers [35] suggest a classification scale of virgin olive oils based on their total phenols content expressed as milligramme of caffeic acid per kilogramme of oil. Accordingly, virgin olive oil with phenols level lower than 220 mg/kg is considered as non-bitter oil or with imperceptible bitterness; a slight bitter oil shows a phenolics content ranging from 220 to 340 mg/kg; bitter oils are characterised by a total phenols content varying between 340 and 410 mg/kg; whereas in bitter or very bitter virgin olive oil, richness on phenolics exceeds 410 mg/kg.

Table 1 contains data on the total phenolic contents and antioxidant activities of virgin olive oils obtained from the major olive cultivars growing in the main

Country	Cultivar	Total phenolic content	ABTS assay	DPPH assay	FRAP assay	References
Spain	Picual	419-671 ^a	3.46 ^c	0.40 ^c	1.15 ^c	[36, 37]
	Cornicabra	317 ^a	2.62 ^c	0.60 ^c	0.94 ^c	[36]
	Arbequina	104-302 ^a	0.2–2.09 ^c	0.44–1.58 ^c	0.54–2.22 ^c	[36, 38, 39]
Italy	Coratina	112-532 ^b	21.37 ^d	35.82 ^d	82.32 ^d	[40, 41]
			0.27–0.57 ^e	NA	0.32–0.63 ^e	
	Frantoio	94.6-256 ^b	17.9–36.4 ⁱ	56.5 - 106 ^d	91.3 – 156 ^f	[42, 43]
Greece	Koroneiki	116–373.3 ^b	0.25–0.44 ^e	NA	0.30–0.54 ^e	[41, 44]
Tunisia	Chemlali	4.3–11.52 ^h	0.7–3.03 ⁱ	2.69–25.87 ^d	NA	[45, 46]
		158 ^b	0.6 ^c	37.23 ^g		
	Chétoui	3.46–9.24 ^h	0.25–2.2 ⁱ	10.23–184.12 ^d	NA	[45, 46]
		395 ^b	2.42 ^c	78,56 ^g		
Portugal	Galega	118-137 ^b	0.33 ^e	NA	0.42 ^e	[41, 47]
			0.35 ^c	NA	NA	
Turkey	Memecik	296.24–407.13 ^a	1.18–1.86 ^c	0.50–0.81 ^c	NA	[48, 49]
			95.86 ^e			
	Gemlik	150.92–245.40 ^a	0.76–1.24 ^c	0.32–0.44 ^c	NA	[48, 49]
			42.17 ^c			
Ayvalik	93.19 ^e	1.34–2.26 ^c	0.53–1.05 ^c	NA	[41, 48]	
		91-130 ^b	0.16–0.28 ^e	NA	0.2–0.3 ^e	
Morocco	Picholine	216.83–668.67 ^a	NA	NA	NA	[50, 51]
	Marocaine	112-390 ^b				

Results are expressed as: ^a: mg caffeic acid/kg oil; ^b: mg gallic acid/kg oil; ^c: mmol de Trolox /kg oil; ^d: IC 50 µg/mL; ^e: mg/kg oil; ^f: µM Fe (II)/g; ^g: percentage; ^h: g/kg of fresh olive fruit; ⁱ: mmol/L TEAC; NA: not analysed/not reported. Abbreviations: ABTS: 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing ability of plasma.

Table 1.
 Total phenolic contents and antioxidant properties of virgin olive oil.

virgin olive oil producing countries. Data is taken from selected scientific papers published between 2010 and 2020. To quantify total phenolic contents, all researchers use Folin–Ciocalteu reagent and except a few report, total phenolic contents are reported as gallic acid equivalents (GAE)/kg.

As can be seen from this Table, the levels of phenolics are highly variable. “Picual”, “Koroneiki” and “Picholine Marocaine” are among the main olive varieties with the highest phenolic content compared to the lowest levels recorded in “Arbequina” virgin olive oil. It has been hypothesised that the differences in phenolic content between olive varieties may be attributed to the differences in the expression of genes encoding for enzymes involved in these metabolites biosynthesis’s pathway during olive growth and ripening, and in the activity of these enzymes during oil processing. Thereby, some monovarietal virgin olive oils present peculiar and specific taste induced by their phenolic fraction deriving from olive fruit or generated during processing. As such, phenolic profiles start to be used as effective varietal markers to classify virgin olive oils according to their varietal origin [52, 53]. Moreover, in the current globalised and ever more fiercely competitive global virgin olive oil market, competitiveness can mainly be maintained by investing in differentiation strategies. In this regard,

producing monovarietal virgin olive oil with high phenolics content and specific sensory properties is a common differentiation strategy used worldwide. These peculiar characteristics of this kind of virgin olive oil are the major driving forces for their high economic value. However, high priced monovarietal virgin olive oils are frequently subjected to fraud and mislabelling practices, therefore, recently there has been a growing interest in developing analytical approaches to determine the varietal origin of virgin olive oil. To this end, varietal authentication based on phenolic profiling and/or fingerprinting is one of the reliable tools used nowadays [52, 53].

2.2.2 Geographical origin

The term “geographical origin” must be taken to include both grown region edaphoclimatic conditions and olive growing agronomic practices. In this respect, it has been elucidated that virgin olive oils obtained from the same cultivar, growing in different geographical locations, show distinctive phenolic profiles. Consequently, various phenolic profiling and fingerprinting approaches were successfully applied to authenticate the geographical origin of this product [54, 55].

Environmental factors may influence widely the phenolic profile of olive fruits before harvesting. Indeed, when it undergoes stressful environmental conditions, olive tree responds by increasing the biosynthesis of antioxidant secondary metabolites such as phenolic compounds to combat oxidative damage induced by stress. It is also proposed that abiotic stress induces profound changes in the expression of endogenous olive fruit enzymes activities which in turn will affect the amount phenolics in the obtained oils. Thus, when dealing with the impact of environmental factors on virgin olive oil phenolic fraction, studies have mainly focused on the effect of edaphoclimatic conditions such as soil type and salinity, altitude, and climatic conditions. Among these factors, water availability has been gaining prominence in the literature due to its influence on olive fruit growth and development, especially in Mediterranean regions where rainfalls and water resources are limited. In this regard, there is ample scientific evidence about the noticeable increase of virgin olive oil total phenols content under drought stress. In this sense, Romero and Motilva [56] reported high total phenols content in oils extracted from olive fruits that have undergone a summer drought stress. Similarly, high phenolics concentration could be expected in virgin olive oil produced in semi-arid and arid region characterised by extremely hot climates during the summer period [57].

Furthermore, cultivation conditions have been shown to influence greatly the levels of virgin olive oil phenolic compounds. Water deficit generates a stress situation that induces the production of phenolics. For instance, by applying a water deficit irrigation strategy (46 to 48% of water requirements calculated from reference evapotranspiration using a crop coefficient of 0.55), Caruso and co-workers [58] reported higher contents of total phenolics and secoiridoids derivatives when compared to oils obtained from fruits of fully irrigated trees [58]. These findings explain why virgin olive oils obtained from fully irrigated trees tend to be less bitter and pungent than those obtained from rainfed olive orchards if bearing in mind the strong correlation between these organoleptic features and phenolics amounts in virgin olive oil.

In addition, by investigating the effect of geographic origin latitude on the phenolic content in Tunisian virgin olive oil, Issaoui and co-authors [59] reported that oils obtained from olive cultivars growing in high altitudes had three times higher phenolic content than oils from the same cultivars grown at low altitudes.

Fertilisation is another cultural practice affecting the biosynthesis of phenolics in olive fruits. The effect of fertiliser application on virgin olive oil's phenolic fraction

has been cited, being strongly depending on the application period, and nutrients type and doses. For instance, previous scientific findings reported a significant decrease in the total phenolics content in virgin olive oil with an increased amount of nitrogen fertilisation supplied even by foliar application during specific fruit development stages or *via* irrigation water throughout the crop season [60, 61]. Some authors suggest a protein-phenol competition to explain this significant decrease of total phenols induced by nitrogen fertilisation excess [61]. Hence the importance of a balanced controlled nitrogen fertilisation to obtain virgin olive oils with a high total phenolics content.

2.2.3 Harvest time

Besides varietal and geographic origin dependent variations, significant differences in virgin olive oil phenolics content have been observed according to the corresponding olive fruit ripening stage at harvest. In this context, it is important to remember that the predominant phenolic compounds detected in virgin olive oil, i.e. the secoiridoids derivatives, result from the enzymatic hydrolysis of the glycosylated form of oleuropein, demethyloleuropein and ligstroside compounds naturally present in olive fruits. Overall, it can be concluded that the phenolic amount of the olive fruits considerably affects the concentrations and proportions of various phenols of the oil that will be obtained after mechanical extraction [62]. Therefore, olive harvesting at the most appropriate stage of ripening is crucial to maximise the phenolic content of virgin olive oil. Extensive research has been conducted to investigate the evolution of phenolics content and composition in olive fruits and corresponding oils during ripeness [63]. Although many of these previous studies have shown that phenolics concentration decrease sharply during ripening (oils obtained from olive fruits harvested at earlier maturity stages show the highest contents of phenolic antioxidants when compared with those derived from fully ripe olives), contrasting data to such a pattern have been also cited [64]. In this sense, several authors found that the influence of olive ripening stage on virgin olive oil phenolics content is cultivar dependent [65].

2.2.4 Oil processing

The phenolic profile of virgin olive oil depends not only on olive fruits composition at harvest but also on the changes occurring during oil processing. Virgin olive oil extraction mainly involves preliminary operations (fruits reception and cleaning); paste preparation by means of breaking the fruit structure (crushing); the liberation of the oil from the cells and the formation of solid and liquid phases (malaxation); separation of the solid (pomace) and liquid phases (oil and/or wastewater) by pressing or horizontal centrifugation, and separation of the liquid phases (oil and wastewater) by decantation or vertical centrifugation. There are many reports in the literature related to the impact of each one of these steps on virgin olive oil phenolic profile suggesting that the latter is strongly determined by some processing conditions such as crushing method (stone mill or hammer or disk crusher) and conditions (grid holes diameter and rotation speed), the malaxation time and temperature and the presence or absence of oxygen during malaxation. Recent and exhaustive reviews are available in literature for getting more comprehensive detailed information about the impact of processing conditions on virgin olive oil phenolic compounds [66, 67]. It is worth underlining that some of these variables have been studied with the aim of controlling and/or modulating enzymatic process affecting the phenolic fraction, mainly the action of the β -glucosidase, phenoloxidases and esterases, which hydrolyse precursors of

oleuropein and ligstroside to produce secoiridoids derivatives, the main phenolic group in virgin olive oil, and therefore the phenolic concentration and the sensory characteristics associated with them. For instance, Antonini et al. [68] revealed that centrifugation by means of a two-phases decanter system produced oils with higher concentrations of oleacein, oleocanthal, oleuropein aglycone, lignans, (+)-pinoselinol and (+)-1-acetoxypinoselinol when compared with three-phases decanters. Similarly, the contents of oleacein and oleocanthal raised linearly with crushing speed [69]. Regarding malaxation conditions, shorter malaxation time (30 to 45 min) was widely associated with higher contents of phenolic compounds regardless of the cultivar [70]. In contrast, rising the malaxation temperature from 27 to 47°C induced higher concentrations of hydroxytyrosol, tyrosol, pinoselinol and *p*-coumaric acid [71].

2.2.5 Oil storage conditions

Once virgin olive oil is extracted, storage conditions are the next critical points to be considered for preserving its phenolic fraction. In fact, virgin olive oil shelf life is quite related to its phenolic content being exposed to a significant decline if this product is stored under inappropriate conditions. Lolis and co-workers [72] recently approved the fact that dark glass containers and low temperature would maintain virgin olive oil phenolic composition for up to 9 months while a temperature of 37°C was associated to a rapid deterioration of its quality after only 3 months. The same trend was reported by Li et al. [73] who revealed that cold storage conditions induced a better preservation of hydroxytyrosol, tyrosol and oleuropein contents when compared with optimal temperature (25°C).

To conclude this section, it should be underlined that, even though virgin olive oil phenolic fraction is very complex due to its origin and the large number of factors that influence its amount in this product, it remains one of the most determining factors of health value and organoleptic quality of virgin olive oil. Thereby, understanding the quantitative and qualitative variability of this fraction is of paramount importance when dealing with putting in place an agro-technological strategy to produce a virgin olive oil which phenolic content fulfils the EFSA health claim requirements. Nevertheless, accurate measurement of virgin olive oil phenolics content remains a challenging task since no official analytical method has been yet considered by any regulatory body. To fill this gap, tremendous effort has been put during the last decades and a great variety of analytical methodologies have been developed and implemented so far. Most common methods and recent advancements are succinctly described in the following section. An in-depth discussion of analytical methodologies applied to virgin olive oil phenolic fraction characterisation is beyond the scope of this chapter. We suggest the reading of some interesting reviews book chapters and research articles previously published, where the authors give an exhaustive overview on this topic [9, 74, 75].

2.3 Advancement in analytical techniques for the characterisation of virgin olive oil phenolic fraction

The need for highly sensitive and selective analytical methods for the determination of phenolics and checking their content in commercialised virgin olive oils has emerged the development of several analytical methodologies from targeted analysis to metabolomics-based approaches. Thus, substantial developments in research focused on the extraction, separation, identification, and quantification of virgin olive oil phenolic compounds have occurred over the last decades. To date, there is no universal workflow described for all analytical approaches dealing

with the characterisation of phenolics in virgin olive oil. However, regardless of the applied approach, a typical analytical method consists of several steps such as sampling, sample preparation, separation, detection, and data analysis.

A carefully samples preparation and extraction of phenolics from virgin olive oil samples is a critical step to avoid the loss of these analytes which can lead to significant errors. Before the extraction and isolation of these components, samples must be collected, preserved, and properly prepared. Commonly, phenolic compounds extraction prior to their identification and quantification is necessary. Currently, the main extraction methods used to this end are liquid–liquid extraction (LLE) and solid-phase extraction (SPE). Even though the first technique is an easy-operating method, it remains quite inefficient and time-consuming. In contrast, SPE is a less solvent-consuming and effective method but considered as labor-intensive. Selecting the most pertinent method depends on the scope of the analysis, the expected concentrations, and the analytical platform to be used.

The recent progress in the so-called “Metabolomics” and the continuous effort towards the development of new high-throughput technologies induced a great advancement in analytical techniques developed for the characterisation of virgin olive oil phenolic fraction. Thus, although the traditional colorimetric assay (also known as Folin–Ciocalteu assay) remains the most widely used technique for the quantitative determination of the total phenolics content in this matrix, a deep characterisation of its phenolic profile requires a prior separation, usually done by reversed phase LC coupled to diode array detector (DAD). For identification purposes, the use of MS is becoming increasingly popular [74]. Other alternatives have also emerged such as ultra-high-performance liquid chromatography (UHPLC) and gaz chromatography (GC) coupled to MS (GC–MS), nuclear magnetic resonance (NMR) and other spectroscopic techniques [75, 76].

Folin–Ciocalteu method is mainly based on the interaction between the functional hydroxyl groups of phenolic compounds and the Folin–Ciocalteu reagent, which consists of a mixture of phosphotungstate and phosphomolybdate. This colorimetric method is simple and offers a great reproducibility and repeatability. However, low specificity is considered as its main drawback [77]. Din et al. [78] recently developed a robust methodology combining SPE and the traditional Folin–Ciocalteu method for the quantification of total phenolic content in virgin olive oil. Phenolic compounds recovery was higher than 95% and the quantification through the colorimetric assay was linear, repeatable, reproducible and precise in 100–500 ppm measuring range.

Otherwise, to overcome these drawbacks, a variety of analytical methodologies have been proposed and successfully applied taking advantage of recent developments in LC and GC instrumentation coupled to different detection systems which in turn allowed increasing the selectivity and sensitivity of virgin olive oil phenolics analysis. In this sense, various MS-based strategies, NMR and vibrational spectroscopy approaches have emerged. NMR spectroscopy is continuously gaining interests as a powerful and robust analytical platform. It offers the ability for a rapid and high screening of the phenolic profile leading to a better detection of all possible changes occurring in response to various factors such as storage, packaging, environmental, agronomic, genetic and processing conditions, etc. Interesting examples to illustrate the application of NMR spectroscopy in virgin olive oil phenolics analysis were reported in a review paper by Dais et al. [79]. Nevertheless, despite its high accuracy, non-destructive nature and limited sample preparation needs, NMR remains an underused technique in virgin olive oil analysis due to its low sensitivity and the need for highly skilled scientists for spectral interpretation [80].

MS based metabolomic approaches (using LC–MS, GC–MS or capillary electrophoresis (CE-MS)) have been largely applied for the quantitative and qualitative

analysis of virgin olive oil phenolic fraction [74, 76]. CE offers high resolution, fast analysis speed and relatively low operating cost [81]. However, poor sensitivity and low reproducibility are its main disadvantages, precluding its use for the analysis of phenolic compounds at very low concentrations [82]. In contrast, GC is believed to be one of the most sensitive and reproducible separation techniques used in virgin olive oil analysis [76]. GC-MS approaches are widely used for the accurate analysis of volatile and non-volatile organic metabolites. Yet, the need for a derivatisation step is mandatory for non-volatile compounds, including phenolic compounds, which is obviously the major drawback of this analytical platform [76]. This fact has boosted the extensive use of other alternative separation methods mainly LC. This technique is considered as “the workhorse” tool for phenolic compounds separation due to their non-volatile character. Researchers in the field of virgin olive oil analysis seem to steadily adopt LC methodologies owing to their simplicity, rapidity, and high sensitivity [76]. Combined with highly sensitive and selective MS detection, it could provide the simultaneous separation, identification and quantification of different phenolic compounds occurring in virgin olive oil. Thus, various LC-MS phenolic profiling or fingerprinting approaches were developed and successfully applied for quality control and authentication purposes as well as to assess the bioavailability of this product [74].

3. Virgin olive oil phenolic compounds: health-promoting effects and bioavailability

Phenolics are believed to confer a wide range of benefits to virgin olive oil (Figure 2) due to their powerful antioxidant activity (Table 1). Thus, since both oxidative stress and inflammation are considered as major contributing factors to neurodegenerative and cardiovascular diseases, virgin olive oil phenolics are thought to exhibit a strong anti-inflammatory effect and directly participate to the redox balance of human cells [28]. For instance, hydroxytyrosol, oleuropein and their derivatives may exert a protective effect against the amyloid plaque generation [83]. Similarly, oleuropein aglycone and oleocanthal have been shown to interact with A β aggregation states leading to a better protection against Alzheimer disease [84]. As revealed in PREDIMED trials, a regular consumption of virgin olive oil induces a significant decrease in risk parameters of developing cardiovascular diseases, namely inflammatory cytokine, the vascular cell adhesion molecule and intercellular adhesion molecule, as well as a rise in high-density lipoprotein (HDL) levels and reduced LDL levels [85]. In particular, the phenolic compounds present in this vegetable oil are related to the inhibition of lipid peroxidation induced by free radicals [86]. It has also been noted that hydroxytyrosol can improve the levels of circulating lipids and repairing oxidative damage which is responsible for numerous cardiovascular issues [87].

Consumption of virgin olive oil with a high content on phenolic antioxidants may also be effective in cancer prevention. Oleuropein glucosides, hydroxytyrosol, and to a lesser degree tyrosol, were highlighted in an interesting review paper by Casaburi et al. [88] as the most promising chemopreventive agents among olive oil phenols against cancer, mainly for their role in preventing DNA oxidative damage in various human cell types. The same researchers revealed the fact that olive oil phenolic compounds induce anti-tumour effects against various tumour types including leukaemia, colorectal and breast cancer due to their ability to inhibit proliferation and enhance apoptosis.

Another potential therapeutic effect of virgin olive oil phenolics includes the prevention and treatment of type 2 diabetes. This was explained by the capability of these compounds to decrease insulin resistance *via* the inhibition or reduction of

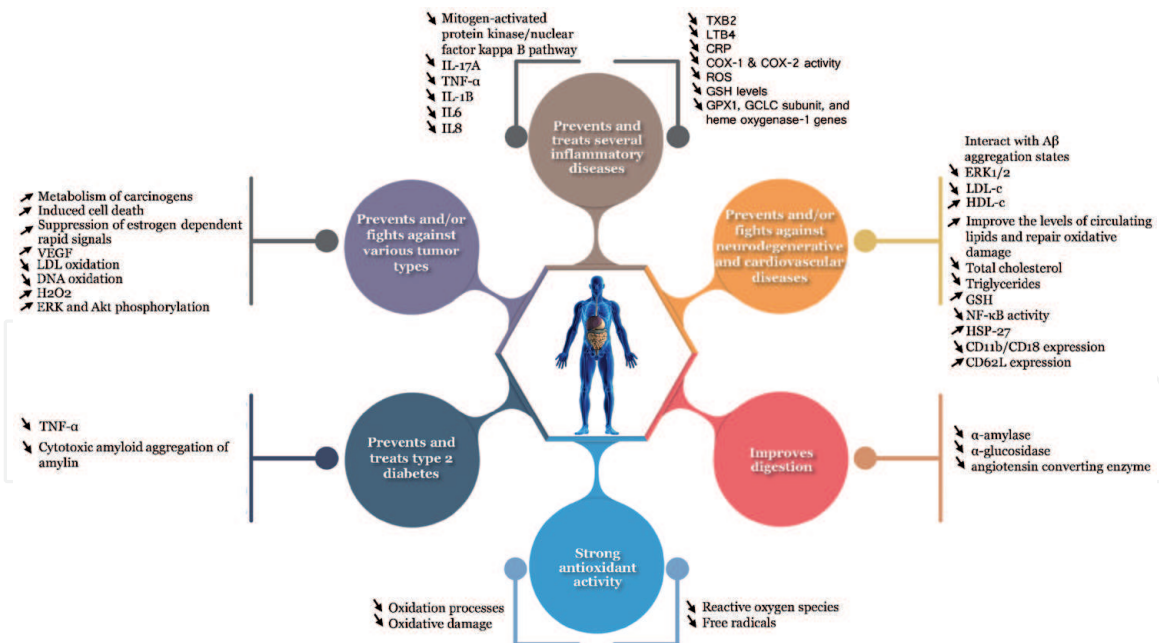


Figure 2.
 Main mechanisms involved in the health properties of virgin olive oil phenolic compounds.

pro-inflammatory molecules such as TNF- α [89]. Besides, hydroxytyrosol and oleuropein have been shown to exhibit an antimicrobial effect against several bacterial strains. Indeed, although they were more effective against ATCC bacterial strains including *Hemophilus influenzae* ATCC 9006, *Moraxella catarrhalis* ATCC 8176, *Salmonella typhi* ATCC 6539 and *Staphylococcus aureus* ATCC 25923, these molecules induced a promising cytotoxic activity against a great number of bacterial strains such as *Salmonella* spp., *Vibrio alginolyticus* and *Vibrio cholerae* [90].

Nevertheless, even virgin olive oil functional value is strongly determined by its phenolic content, however, only a percentage of this content can be biologically active in the body, as it must be absorbed through the gastrointestinal tract and reach the bloodstream, which is known as bioavailability. The latter may simply be defined as the fraction that reach the organism where it can pursue its biological effect, may be enhanced or inhibited depending on various factors such as chemical structure, concentration, interaction with other compounds, enzymes activity, host's age and physical condition, etc. [91].

However, in the case of virgin olive oil most investigations have focused on some specific phenolic compounds such as oleuropein, tyrosol, hydroxytyrosol, and their derivatives as they were associated with the highest bioactivity. Outcomes of selective recent studies of the bioavailability of these compounds are presented in **Table 2**.

Thus, after intake, virgin olive oil forms a micellar solution in the gastrointestinal tract. Most olive oil phenolic compounds pass through the mouth and stomach to reach the small intestine and colon without any modification [89]. Hydroxytyrosol and tyrosol have been demonstrated to be the most absorbed phenolics in the intestinal tract (absorption rate is 40 to 95% approximately) [107]. Also, Corona et al. [108] suggested that the amounts of hydroxytyrosol and tyrosol that reach the small intestine following incubation and passage through the acidic conditions of the stomach are considerably higher than those initially present in ingested virgin olive oil. The recovery of these two phenolic compounds is also widely related to the biological matrix in which they were administered. Indeed, urinary recovery of hydroxytyrosol was greater after virgin olive oil intake than after the addition of hydroxytyrosol to a yoghurt or to a refined olive oil [109]. The urinary hydroxytyrosol and tyrosol are absorbed from a moderate and sustained dose of virgin olive oil

Phenolic compound	Health effect	Dose	Cell type and/or animal model.	Mechanism	Reference
Hydroxy-tyrosol	Protection against breast, prostate and colon cancers.	100 μ M.	Breast: MDA and MCF-7; Prostate: LNCap and PC3; Colon: SW480 and HCT116 cancer cells.	\uparrow H ₂ O ₂ .	[92]
	Prevention of neurodegenerative diseases.	50 μ M.	N2a neuroblastoma cells.	\downarrow NF- κ B activity. \uparrow GSH (at 100 μ M).	[93]
	Prevention and treatment of metabolic syndrome.	10 and 50 mg/kg/day.	C57BL/6J mice.	\downarrow SREBP-1c/FAS pathway. \uparrow Antioxidant enzyme activities. Normalise expression of mitochondrial complex subunits and mitochondrial fission marker Drp1.	[94]
	Prevention of cardiovascular diseases.	0.1–10 μ g/ml.	H9c2 cells.	\downarrow Phosphorylation of a transcriptional target c-Jun. \uparrow Phosphorylation of extracellular signal-regulated kinase 1/2. \uparrow Heat shock proteins (HSP)-27.	[95]
Tyrosol	Prevention of neurodegenerative diseases.	50 μ M.	N2a neuroblastoma cells.	\downarrow NF- κ B activity.	[93]
	Anti-inflammatory effect.	100 μ M (<i>in vitro</i> study) and 0.1–0.5 mg/kg (<i>in vivo</i> study).	Human umbilical vein endothelial cells, a human monocytic cell line THP-1 and male Swiss albino mice.	\downarrow ROS. \downarrow GSH levels. \downarrow GPX1, GCLC subunit, and heme oxygenase-1 genes.	[96]
Oleuropein	Prevention of cardiovascular diseases.	100 μ M.	Vascular smooth muscle cells.	\downarrow ERK1/2.	[97]
	Anti-cancer effect.	125 mg of oleuropein / kg of diet.	MCF-7 cells xenograft and female nu/nu athymic 7–8 week old mice.	Unknown.	[98]
	Prevention of neurodegenerative diseases.	Unknown	—	\downarrow Senile plaque formation through amyloid beta peptide (A β) aggregation.	[99]
	Treatment of skin diseases and wounds.	50 mg of oleuropein / kg/day.	Male Balb/c mice.	\uparrow VEGF.	[100]

Phenolic compound	Health effect	Dose	Cell type and/or animal model.	Mechanism	Reference
Oleacein	Anti-cancer effect.	1–100 μ M.	Human epidermoid carcinoma cell line A431 and human immortalised keratinocytes.	↓ Erk and Akt phosphorylation. Suppression of B-Raf expression.	[101]
	Anti-inflammatory effect.	50–100 μ M.	Human monocytes.	↓ Cox-2. ↓ Superoxide anions production.	[102]
	Prevention of cardiovascular diseases.	50–100 μ M.	Human isolated neutrophils.	↓ Neutral endopeptidase activity, elastase, metalloproteinase 9 and IL 8 (100 μ M). ↓ CD11b/CD18 expression (50 μ M). ↑ CD62L expression (50 μ M).	[103]
Oleocanthal	Prevention of neurodegenerative diseases.	Ranging between 0.1 and 25 μ M.	—	Inducing stable conformational modifications of tau-441 protein secondary structure and interfering with tau aggregation.	[104]
	Anti-cancer effect.	Between 5 and 80 μ M for the <i>in vitro</i> study and up to 10 mg/kg for the <i>in vivo</i> study.	Human hepatocellular carcinoma cells and Male BALB/c athymic nude mice.	↓ Epithelial-mesenchymal transition. ↓ Transcription factor STAT3 nuclear translocation. ↓ DNA binding activity.	[105]
		1–100 μ M.	Human epidermoid carcinoma cell line A431 and human immortalised keratinocytes.	↓ Erk and Akt phosphorylation. Suppression of B-Raf expression.	[101]
	Anti-inflammatory effect.	50 μ M.	ATDC5 murine chondrogenic cells and murine macrophages J774.	↓ MIP-1 α inflammatory mediator at the protein and mRNA level. ↓ IL-6 inflammatory mediator at the protein and mRNA level.	[106]

Abbreviations. Cox-2: cyclooxygenase2; ERK1/2: extracellular signal-regulated kinase 1/2; GCLC: glutamate-cysteine ligase catalytic; GPX1: glutathione peroxidase 1; GSH: glutathione; IL 8: interleukin 8; NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; ROS: reactive oxygen species; VEGF: vascular endothelial growth factor; ↓: decreasing trend; ↑: increasing trend.

Table 2.
Selective studies on bioactivities and potential health benefits of main virgin olive oil phenolic compounds.

which is similar to that consumed daily in a typical Mediterranean diet [110]. These simple phenols were largely identified in both urine and plasma mainly in glucuronides and sulphate conjugates, while their free forms were not detected in plasma samples. In fact, aglycones and glycosides forms of tyrosol and hydroxytyrosol undergo a prompt hydrolysis phenomenon under gastric conditions together with a substantial rise in the contents of tyrosol and hydroxytyrosol free forms penetrating the small intestine [108]. The intestinal transport of hydroxytyrosol occurs through a bidirectional passive diffusion mechanism as demonstrated in an *in vitro* study conducted by Manna and co-workers [111]. While crossing epithelial cells of the gastrointestinal tract, hydroxytyrosol is usually transformed through enzymatic reactions into homovanillyl alcohol and its glucuronide forms [108].

Regarding secoiridoids, they remain highly stable in the mouth but suffer significant losses in the gastric, duodenal, and colonic regions, with a recovery rate at the duodenal level ranging between 7% and 34%. Glycosylation and cleavage of glycosidic linkages take part in the secoiridoids absorption, and it is thought that some of them, such as oleacein, are absorbed in the small intestine by passive diffusion through the membrane of intestinal cells [89]. In the case of oleuropein, the mechanism of absorption is still confusing and remains unclear. However, several studies showed that it exerts its biological effects *via* its conversion into hydroxytyrosol [112]. This was mainly explained by the fact that the content of oleuropein was in the mass range of few nanograms in plasma while hydroxytyrosol was detected in high concentrations after the intake of great doses of oleuropein in both rats and humans trials [113, 114]. When considering other secoiridoids, Vissers et al. [114] could not analyse oleuropein glycoside, oleuropein- or ligstroside-aglycons in urinary excretions which supports the idea that they may be hydrolysed into hydroxytyrosol and tyrosol and extensively metabolised once absorbed from the small intestine.

4. Concluding remarks

In the Mediterranean area, the largest virgin olive oil producing region in the world, new agronomic practices and processing technologies are steadily developed and adopted over the past decade. Thereby, virgin olive oil production has achieved outstanding performance both in terms of increasing oil yield and quality. Nevertheless, the future of this sector in a context of globalisation, and the consequent changes in lifestyle and consumers adherence to the Mediterranean diet, has become an important subject of attention for the producers and governmental bodies alike. For instance, in the search for new opportunities to boost virgin olive oil consumption, growing importance is attributed to promote its health value and organoleptic features among consumers, paying special attention to those compounds responsible of these characteristics. In developing such strategies, a central role should clearly be reserved for the phenolic fraction of this product if considering its health-promoting properties and its contribution to the oxidative stability and sensory quality of virgin olive oil. Undoubtedly, the recent agro-technological advancements supported by the scientific data available up till now offer promising tools to produce virgin olive oils rich on phenolics; however, the bitter taste of such oils is often not appreciated by consumers. For this reason, what still remains a challenge in this sector is the development of holistic agro-technological approaches to produce virgin olive oils with phenolics content that comply not only with institutional regulations (EFSA health claim for example) but also with consumer preferences. Furthermore, the lack of an official method of determining these compounds in compliance with health claim requirements is an obstacle to be overcome in a not-too-distant future if we want to avoid a lack of credibility of these health claims.

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