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Review

Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil

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Abstract

Hydrophilic phenols are the most abundant natural antioxidants of virgin olive oil (VOO), in which, however, tocopherols and carotenes are also present. The prevalent classes of hydrophilic phenols found in VOO are phenolic alcohols, phenolic acids, flavonoids, lignans and secoiridoids. Secoiridoids, that include aglycon derivatives of oleuropein, demethyloleuropein and ligstroside, that are present in olive fruit, are the most abundant phenolic antioxidants of VOO. The sensory and healthy proprieties of VOO hydrophilic phenols as well as the agronomic and technological parameters that affect their concentration in the oil are discussed in this paper.
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Keywords: Phenols; Secoiridoids; Virgin olive oil; Antioxidants; (3,4-Dihydroxyphenyl)ethanol; Mechanical extraction process

Contents

1. Introduction

The chemical composition of VOO shows several compounds such as hydrophilic phenols, that affecting, it's sensory and healthy proprieties, differentiate VOO from all the others vegetable oil used by humans. Chemical composition

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of VOO consists of major and minor components. The major components, that include glycerols, represent more than 98% of the total oil weight. Minor components, that are present in a very low amount (about 2% of oil weight), include more than 230 chemical compounds such as aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants. The main antioxidants of VOO are carotenes and phenolic compounds that include lipophilic and hydrophilic phenols [1]. While the lipophilic phenols, among which tocopherols can be found in other vegetable oils, some hydrophilic phenols of VOO are not generally present in other oils and fats [1,2]. Moreover, the hydrophilic phenols of VOO constitute a group of secondary plant metabolites that show peculiar sensory and healthy proprieties. An overview of the biological properties of these compounds, their quantitative and qualitative modifications in VOO according to the agronomic and technological conditions of production will follow reported.

1.1. Occurrence of hydrophilic phenols in olives and VOO

Cantarelli, more than 40 years ago, showed the occurrence of natural antioxidants in virgin olive oil obtained by pressure to explain the difference in oxidative stability between virgin and rectified olive oil. The occurrence of these compounds was confirmed, by the same author, 8 years later when the extraction and the colorimetric evaluation of total phenols was carried out in several Italian virgin olive oils extracted by pressure. The results were compared with the phenolic composition of refined olive oils and a strong discrimination in phenolic concentration between the two groups of oils was shown [3,4]. So far however only during the last 20 years systematic studies on the occurrence of specific classes of hydrophilic phenols in VOO were performed. The results published in a large quantity of works show the peculiar composition of VOO in terms of phenolic antioxidant that cannot be found in any other vegetable oils [1,5]. As reported in Table 1, in fact, VOO contains different classes of phenolic compounds such as phenolic acids, phenolic alcohols, hydroxy-isocromans, flavonoids, secoiridoids and lignans. The phenolic acids were the first group of phenolic compounds found in VOO; these compounds together with phenyl-alcohols, hydroxy-isochromans and flavonoids [6] are present in small amounts in VOO [7–9] while secoiridoids and lignans are the most concentrate phenolic compounds of oil. One of the most important aspects, related to the occurrence of hydrophilic phenols in VOO, is the definition of the biochemical mechanisms that would explain the occurrence in the oil; mechanisms, that are largely unknown. Several compounds, i.e. the secoiridoids, moreover, are derivatives of secoiridoid glucosides present in the olive fruit that are released in the oil during mechanical extraction process [6,10]. The olive drupe, in fact, contains high concentrations of phenolic compounds that can range between 1 and 3% of the fresh pulp weight [11]. The main classes of phenols in olive fruit are phenolic acids, phenolic alcohols, flavonoids and secoiridoids (Table 2). The (3,4-dihydroxyphenyl)ethanol (3,4-DHPEA) and (*p*-hydroxyphenyl)ethanol (*p*-HPEA) are the most abundant phenolic alcohols in the olive fruit [11,12]. The flavonoids include flavonol glycosides such as luteolin-7-glucoside and rutin [12] and anthocyanins, cyanidin and delphinidin glycosides, in particular [13–15]. While the phenolic acids, phenolic alcohols and flavonoids occur in many fruits and vegetables belonging to various botanical families, secoiridoids, on the contrary, are exclusively present in the family of *Oleaceae* that includes *Olea europaea* L. Olives and VOO are the main products obtained from this specie used in the human nutrition that contain secoiridoids. The phenolic compounds classified as secoiridoid are characterized by the presence of either elenolic acid or elenolic acid derivatives in their molecular structure [11]. Oleuropein, demethyloleuropein, ligstroside and nüzhenide are the most abundant secoiridoids glucoside in olive fruit [11,16]. Bourquelot and Vintilesco [17] revealed, for the first time, oleuropein in olive fruit but only 60 years later Panizzi et al. [18] assigned its chemical structure. Demethyloleuropein was isolated and characterized for the first time by Ragazzi et al. [19] in the

Table 1
Phenolic compounds in virgin olive oil (VOO)

Phenolic acids and derivatives	
Vanillic acid	[6,7]
Syringic acid	[6,7]
p-Coumaric acid	[6,7]
o-Coumaric acid	[6,7]
Gallic acid	[37]
Caffeic acid	[6,7]
Protocatechuic acid	[6,7]
p-Hydroxybenzoic acid	[6,7]
Ferulic acid	[6,7]
Cinnamic acid	[11]
4-(Acetoxyethyl)-1,2-dihydroxybenzene	[6,7]
Benzoic acid	[11]
Hydroxy-isocromans	[6]
Phenolic alcohols	
(3,4-Dihydroxyphenyl)ethanol (3,4 DHPEA)	[35]
(*p*-Hydroxyphenyl)ethanol (*p*-HPEA)	[35]
(3,4-Dihdroxyphenyl)ethanol-glucoside	[46]
Secoiridoids	
Dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA (3,4 DHPEA-EDA)	[39,40,42]
Dialdehydic form of decarboxymethyl etenolic add linked to *p*-HPEA (*p*-HPEA-EDA)	[39,40,42]
Oleuropein aglycon (3,4 DHPEA-EA)	[39,40,42]
Ligstroside aglycon	[42]
Oleuropein	[43]
p-HPEA-derivative	[39,40,42]
Dialdehydic form of oleuropein aglycon	[44]
Dialdehydic form of ligstroside aglycon	[44]
Lignans	
(+)-1-Acetoxypinoresinol	[42,49]
(+)-Pinoresinol	[42,49]
Flavones	
Apigenin	[48]
Luteolin	[48]

Table 2
The main phenolic compounds of olive fruit

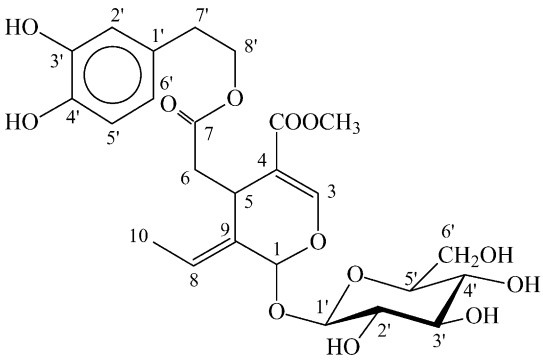
Anthocyanins	
Cyanidin-3-glucoside	[13,14]
Cyanidin-3-rutinoside	[13,14]
Cyanidin-3-caffeyglucoside	[13,14]
Cyanidin-3-caffeylaitinoside	[13,14]
Delphinidin 3-rhamosylglucoside-7-xyloside	[14,15]
Flavonols	
Quercetin-3-rutinoside	[12]
Flavones	
Luteolin-7-glucoside	[12]
Luteolin-5-glucoside	[12]
Apigenin-7-glucoside	[12]
Phenolic acids	
Chlorogenic acid	[12]
Caffeic acid	[12]
p-Hydroxybenzoic acid	[12]
Protocatechuic acid	[12]
Vaniltic acid	[12]
Syringic acid	[12]
p-Coumaric acid	[12]
o-Coumaric acid	[12]
Ferulic acid	[12]
Sinapic acid	[12]
Benzoic acid	[11]
Cinnamic acid	[11]
Gallic acid	[11]
Phenolic alcohols	
(3,4 Dihydroxyphenyl) ethanol (3,4-DHPEA)	[11,12]
(*p*-Hydroxyphenyl) ethanol (*p*-HPEA)	[11,12]
Secoiridoids	
Oleuropein	[17,18]
Demethyloteuropein	[19]
Ligstroside	[20]
Nuzhenide	[22]
Hydroxycinnamic acid derivatives	
Verbascoside	[21,22]

ripe olives while the ligstroside was isolated from olive fruit by Kubo and Matsumoto [20] (Fig. 1). A derivative of the hydroxicinnamic acid, the verbascoside, was also found in olive fruit and its chemical structure was assigned by Andary et al. [21] and confirmed by Servili et al. [22] (Fig. 2). Recently several isomeric forms of verbascoside, were identified in olive fruit by Ryan et al. [23]. Oleuropein, demethyloleuropein and verbascoside were found in all the constitutive parts of fruit such as peel, pulp and seed but mainly in the pulp, whereas nüzhenide, as characterized by Servili et al. [24] (Fig. 1), was found only in the seed. Several aglycon derivatives of oleuropein and demethyloleuropein such as the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA) an isomer of the oleuropein aglycon (3,4-DHPEA-EA), were also found in olive pulp [24–26]. These compounds are generally present in very low amount in olive fruit that show the oleuropein and demethyloleuropein as most concentrate phenolic compounds. In addition, the oleuropein concentration decreased sharply during fruit ripening [27–29]. Recently, however, Ryan et al. [26] found that the 3,4-DHPEA-EDA was the most concentrate phenolic compounds of olive pulp in a novel Australian cultivar, Hardy's Mammoth and an inverse correlation with the oleuropein concentration was also observed. The same authors show, moreover, that the 3,4-DHPEA-EDA as the most concentrate phenolic compounds of olive pulp was seasonal dependent [26,29,30]. Phenolic acids with the basic chemical structure of C6–C1 (benzoic acids) and C6–C3 (cinnamic acid) were also found in olive fruit by different authors [11]. Historically, these compounds, such as caffeic, vanillic, syringic, *p*-coumaric, *o*-coumaric protocatechuic, sinapic and *p*-hydroxybenzoic acid are also the first group of phenols observed in VOO [7,31] (Fig. 3). Several authors confirmed the occurrence of phenol acids as minor components of VOO [32–36]. Mannino et al. [37] reported that gallic acid was also present in VOO. The prevalent phenols of VOO, however, are the secoiridoids. These compounds are derivatives of oleuropein, demethyloleuropein and ligstroside. The most abundant secoiridoids of VOO are the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA or *p*-HPEA-EDA) and an isomer of the oleuropein aglycon (3,4-DHPEA-EA) (Fig. 4). These compounds were found, for the first time, by Montedoro et al. [35,38] who also assigned their chemical structures in 1993 [39], that were later confirmed by other authors [40–42]. Oleuropein and ligstroside aglycon and their dialdehydic forms were also detected, as minor hydrophilic phenols of VOO [42–44]. These compounds are intermediate structures of the biochemical transformation of secoiridoids glucosides in olive fruit such as oleuropein, demethyloleuropein and ligstroside in the final aglycon derivatives corresponding to the 3,4-DHPEA-EDA from oleuropein and demethyloleuropein and *p*-HPEA-EDA from ligstroside, respectively [44].

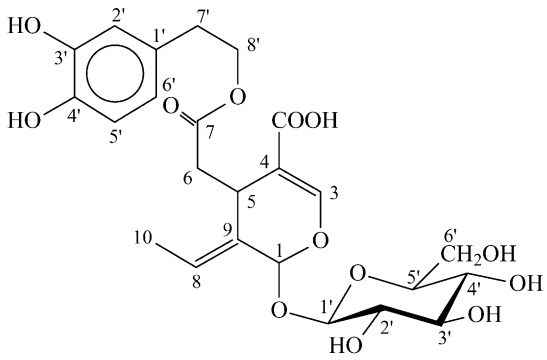
The (3,4-dihydroxyphenyl)ethanol (3,4-DHPEA) and (*p*-hydroxyphenyl)ethanol (*p*-HPEA) are the main phenolic alcohols of VOO (Fig. 4); their concentration is generally low in fresh oils but increases during oil storage [35] due to the hydrolysis of VOO secoiridoids such as 3,4-DHPEA-EDA, *p*-HPEA-EDA and 3,4-DHPEA-EA that contain 3,4-DHPEA and *p*-HPEA in their molecular structure [45]. Bianco et al. [46] identified three glucosidic forms of 3,4-DHPEA, in VOO and olive fruit, differentiated according to the hydroxyl group to which the glucose was bound. Recently, the occurrence of glucosidic forms 3,4-DHPEA, such as (4-β-D-glucosyl-3-hydroxyphenyl)ethanol has been confirmed by Romero et al. [47] in olive fruit, VOO, vegetation waters and pomaces.

Flavonoids such as luteolin and apigenin were also reported as phenolic component of VOO by Rovelli et al. [48].

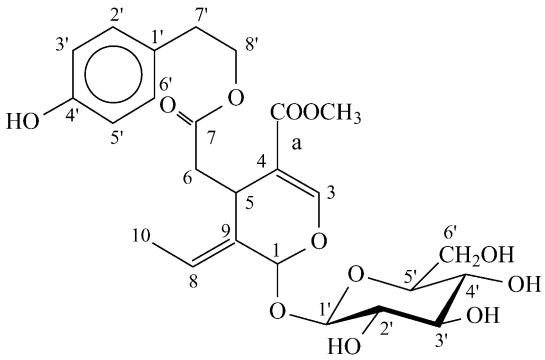
The last group of phenols found in VOO are the lignans; Owen et al. [42,49], in fact, have recently isolated and characterized (+)-1-acetoxypinoresinol and (+)-1-pinoresinol as the most concentrated lignans in VOO (Fig. 5). Brenes et al. [50] confirmed the occurrence of these compounds in Spanish VOO. The same author also reported that the lignans concentrations discriminated the oils produced from Picual to the



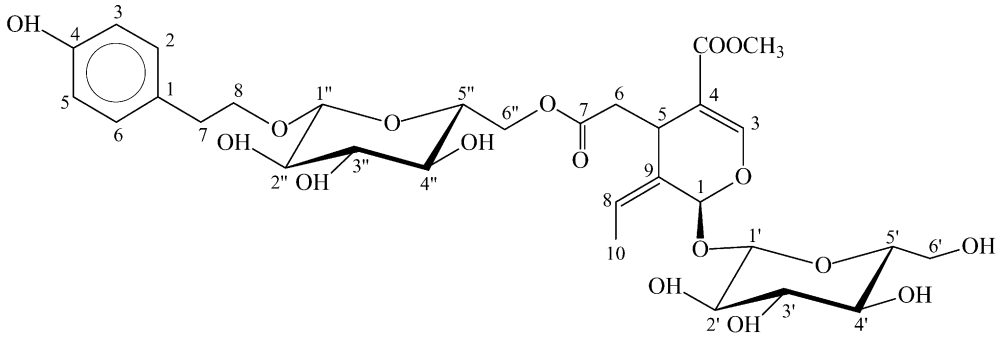
Oleuropein



Demethyloleuropein



Ligstroside



Nüzhenide

Fig. 1. Chemical structures of the secoiridoids glucosides of olive fruit.

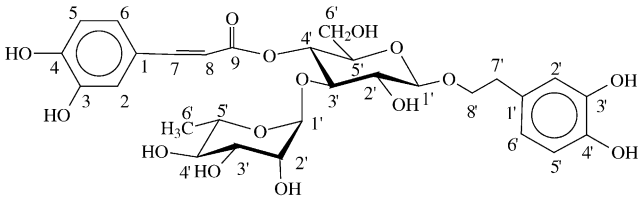


Fig. 2. Chemical structure of verbascoside.

others virgin olive oils extracted from Hojiblanca, Coricabra and Arbequina varieties [51].

Due to the agronomic and technological aspects of olive oil production, that strongly affect their occurrence, the definition of the average concentration of hydrophilic phenols in VOO is rather difficult. If evaluated colorimetrically as total phenols in the methanolic extract of oil, however, their concentration may range between 40 and 900 mg/kg. Nevertheless, higher concentrations (up to 1000 mg/kg) have also been reported in several oils [35,52].

A quantitative evaluation of several individual hydrophilic phenols of VOO was performed by HPLC and the averaged concentration, expressed as median, of prevalent secoiridoids and phenolic alcohols of VOO is reported in Table 3. These results obtained by HPLC analysis [35] of 210 VOO samples, extracted by industrial plants from different areas of Mediterranean countries, showed strong variations in the absolute values particularly for the 3,4-DHPEA-EDA and 3,4-DHPEA-EA. Lignans are also found as prevalent phenolic

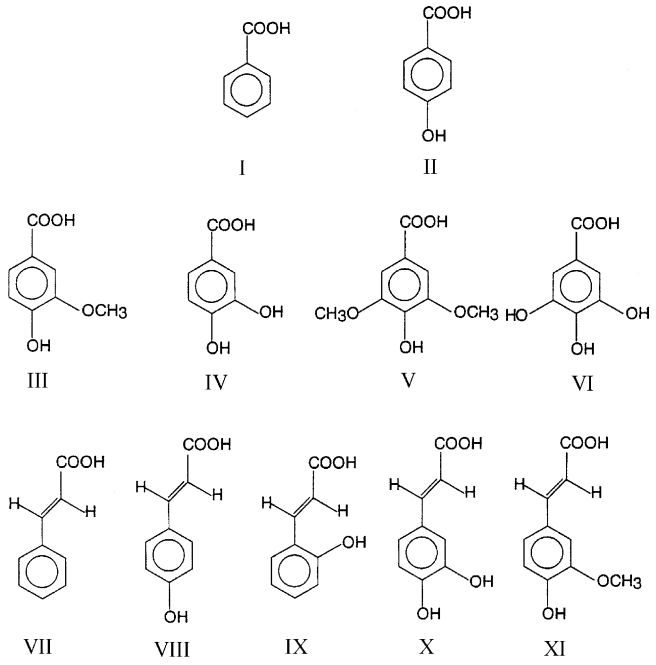


Fig. 3. Chemical structure of the main phenolic acids of VOO: benzoic acid [I], *p*-hydroxybenzoic acid [II], vanillic acid [III], protocatechuic acid [IV], syringic acid [V], gallic acid [VI], cinnamic acid [VII], *p*-coumaric acid [VIII], *o*-coumaric acid [IX], caffeic acid [X], ferulic acid [XI].

compounds in VOO. Brenes shows Spanish cultivar contains (+)-1-pinoresinol in a range of 20 and 45 mg/kg while the (+)-1-acetoxypinoresinol was found in a range of 2 and 95 mg/kg. [51]. The strong variation emphasized in the absolute value of phenolic compounds is not the only difference that can be remarked. As reported in Fig. 6, the chromatographic profiles of VOO phenolic compounds show strong differences that may be related to the agronomic and technological aspects of production. In this context, the relationships between the phenolic profile of oils and their genetic or geographic origin should be better investigated.

1.2. Analytical determination of phenolic compound in virgin olive oil

A large quantity of papers related to the evaluation of VOO phenols, published before the 1990, reported colorimetric methods that generally use the Folin–Ciocalteau reagent [3,4,8,9]. Among the chromatographic methods proposed to evaluate phenolic compounds HPLC is the most applied [31–39,42,47,51,52]. The main differences among the methods can be summarized in the separation procedure of phenols from the oily matrix and the detector choice for the HPLC evaluation.

Concerning the extraction process two main techniques are reported in literature: liquid–liquid extraction (LLE) [31–36] and solid-phase extraction (SPE) [24,37,53,54]. The most used solvent for LLE is methanol in mixture with different levels of water that range between 0 and 40% [31–36]. The second main difference in LLE methods is related to the preventive addition of a lipophylic solvent to the oil before phenolic extraction; the most used solvent is hexane, but petroleum ether and chloroform have been also proposed [31–34,36]. In this context, Montedoro et al. comparing different methods of LLE showed that the best extraction condi-

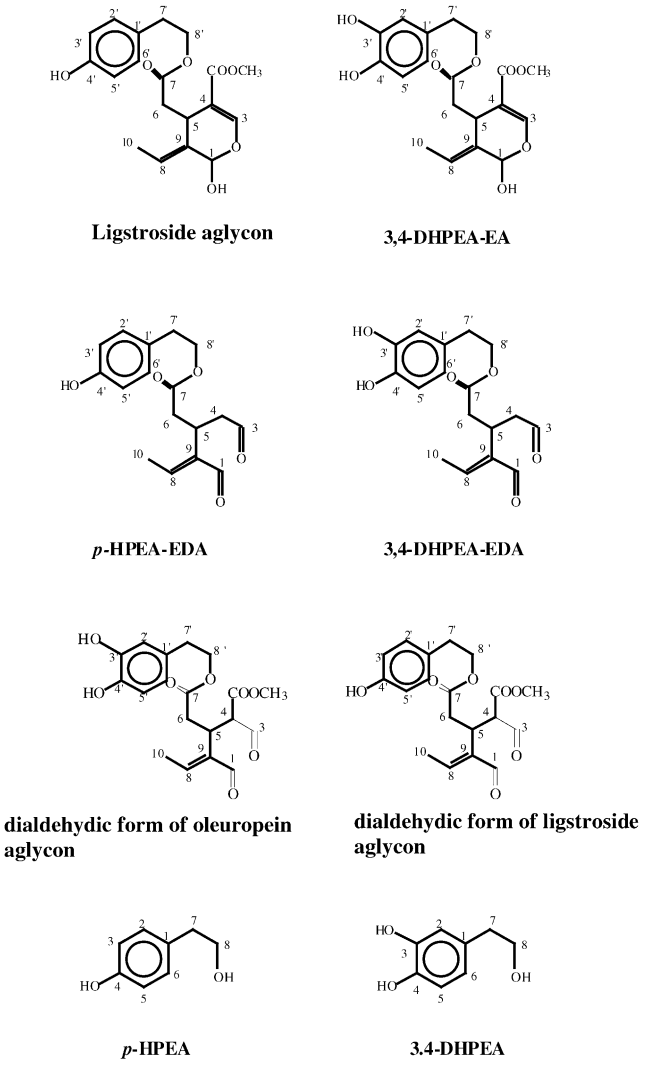


Fig. 4. Chemical structures of the secoiridoids derivatives and phenolic alcohols of VOO.

Table 3
Average values (mg/kg) of the prevalent phenolic alcohols, phenolic acids and secoiridoids of virgin olive oil calculated using 210 oil samples obtained in industrial plants[a]

	Median	Lower quintile	Upper quintile
3,4-DHPEA	1.8	1	3.6
p-HPEA	1.9	0.6	5.0
Vanitlic acid	0.2	0	0.3
Caffeic acid	0.4	0.2	0.7
3,4-DHPEA-EDA	185.7	48.2	631.1
p-HPEA-EDA	36.1	22.5	78.8
3,4-DHPEA-EA	126.3	61	231

[a] Unpublished results. The concentration of hydrophilic phenols was evaluated by HPLC as previously reported by Montedoro et al. [35].

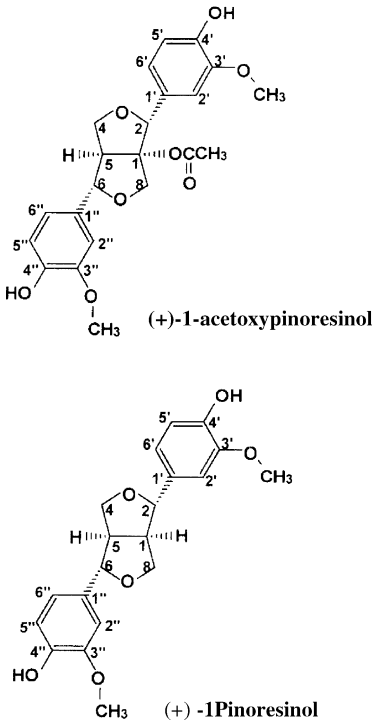


Fig. 5. Chemical structures of the lignans occurred in VOO.

tions were obtained using a mixture of methanol:water 80:20 (v/v). The addition of hexane or other organic solvents in the oil before extraction did not yield significant differences in the phenols recovery efficiency [35]. Recently the use of *N*,*N*-dimethylformamide (DMF) in LLE, proposed by Brenes et al., seem to show interesting results in terms of recovery efficiency and sample manipulation [55]. SPE was applied for the first time in VOO to separate phenols by Mannino et al. using a C18 cartridge and methanol as elution solvent [37]. However, a comparison between LLE, performed according to Montedoro et al. [35], and SPE carried out using Alltech C18 Extract-Clean High Capacity cartridges and methanol was reported by Servili et al. [22]. Results show that SPE is more efficient than LLE to separate simple phenols while on the contrary the recovery of secoiridoid derivatives using LLE was higher. In contrast to these results Pirisi et al. [54], compared SPE using a C8 cartridge and acetonitrile as elution solvent and LLE, using methanol:water 60:40 (v/v) in VOO preventively dissolved in hexane, did not show significant differences in the phenols recovery [54]. Many papers were published on the use of HPLC coupled to UV detection for the qualitative and quantitative evaluation of VOO phenols [32–36,42,53,54,56], however electrochemical and fluorometric detectors were also proposed [36,37,51,55]. About UV detector the diode array is the most used, for a routine analysis, due to the possibility owned by this detector to show the UV spectra of the phenolic compounds that can be very useful to identify the specific substances separated by HPLC [35,36]. This aspect is particularly important in the analytical evaluation of VOO phenols due to the difficulty to find appropriate standards available in commerce. Only the phenolic acids can be found as commercial standard, on the contrary secoiridoid derivatives and lignans could be preventively extracted from the oil. The use of electrochemical detector (EC) was proposed for the fist time by Mannino et al. [37] to evaluate simple phenols of VOO. Tsimidou et al. [36] compared UV detector, UV diode array and EC detector and concluded that the EC detector could be very useful to evaluate phenols occurring in minor amounts due to the high sensibility of the EC detector in comparison to the UV and the UV diode array. Recently, Brenes et al. [55] proposed a rapid analytical methodology for determining phenols concentration in VOO based on coupling the use of DMF in liquid–liquid extraction and EC detector in HPLC analysis.

The use of fluorometric detector in the analysis of phenolic acids of VOO was proposed by Cartoni et al. [57]. Brenes et al. comparing the EC, UV and fluorescence detectors and GC–MS in the evaluation of several phenolic compounds of VOO concluded that fluorescence detector is very interesting in the evaluation of lignans for the routine analysis of VOO phenols because considered easier than GC–MS to

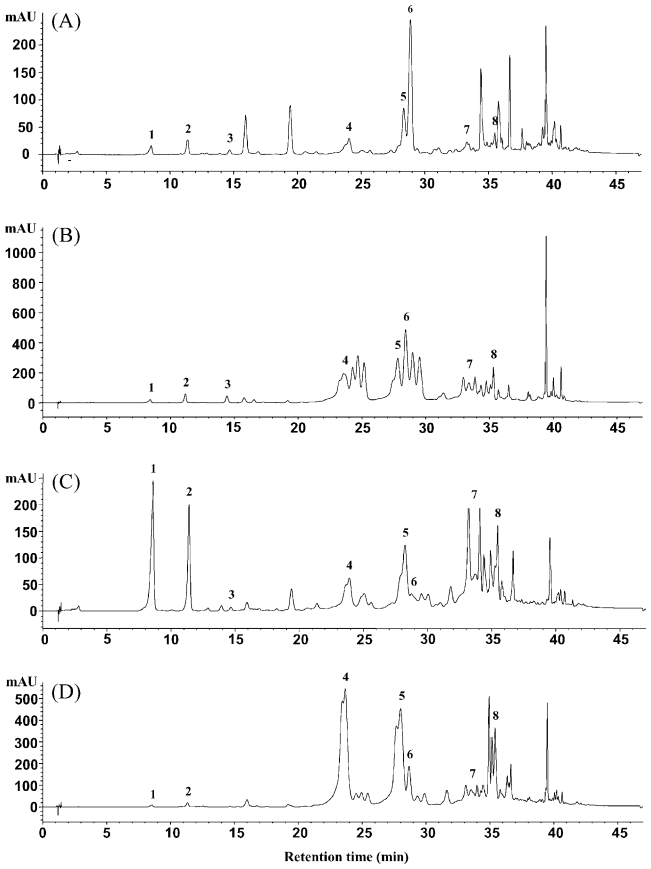


Fig. 6. Typical HPLC chromatograms of VOO characterized by different total phenols content, evaluated by colorimetric method. Unpublished results; the HPLC and colorimetric evaluations of phenolic compounds were performed as reported previously by Montedoro et al. [35]. Chromatograms (A) 43 mg/kg; (B) 626 mg/kg; (C) 262 mg/kg; (D) 551 mg/kg. (1) 3,4-DHPEA; (2) *p*-HPEA; (3) vanillic acid; (4) 3,4-DHPEA-EDA; (5) *p*-HPEA-EDA; (6) (+)-1-acetoxypinoresinol; (7) 3,4-DHPEA-EA; (8) ligstroside aglycon.

discriminate (+)-1-pinoresinol and (+)-1-acetoxypinoresinol [55]. GC–MS and LC–MS were also studied to evaluate phenolic composition of oil however LC–MS is generally used in the qualitative evaluation of chromatographic profile while the use of LC–MS as routine technique to quantify VOO phenols is unusual [44,48]. Angerosa et al. [40,41] defined a GC–MS method to evaluate phenolic compound of oil. Two main peaks at *m*/*z* 192 or at *m*/*z* 280 related to *p*-HPEA and 3,4-DHPEA, respectively, evident in the mass spectrum of secoiridoid derivatives, that can be very useful to define qualitatively and quantitatively the phenolic composition of oil were found. Owen et al. took advantage of the high sensibility of the GC–MS method in the evaluation of VOO phenols confirming the useful application of this method for all the classes of phenolic compounds occurring in the olive oil [49].

The different extraction procedures and the chromatographic methods proposed for the analysis of phenolic compounds of VOO partially explains the strong differences observed in the concentration of these compounds in the oil reported in literature [42,52,58]. But is our opinion that the most important problem to define concentration is related to the standards used to report quantitative results. Gallic acid, caffeic acidic and 3,4-DHPEA are normally used as referring standard to define quantitative results of phenolic compounds. However, these compounds, occurring in VOO in small amounts, show strong differences in terms of detector response compared to the most concentrate phenolic compounds of oil such as secoiridoid derivatives and lignans [35,42,52]. This aspect is particularly important because the quantitative relationships among the different classes of phenols, occurring in VOO, change according to their total concentration. In fact, the secoiridoid derivatives such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA are the main compounds in oils characterized by high phenolic concentration while phenolic acids and phenolic alcohols are the most important compound in oils with low phenolic amounts. In conclusion to define the real concentration of phenolic compounds in VOO the secoiridoid derivatives and the lignans could be preventively extracted from the oil matrix, purified and the pure compounds could be used to define the detector response for each specific compounds [52].

1.3. Antioxidant activity

The antioxidant activity of hydrophilic phenols of VOO has been well studied [59–68]. In fact, as reported by different authors, the concentration of phenolic compounds, evaluated colorimetrically and expressed as total phenols, was highly correlated to the shelf life of VOO, tested using accelerated methods such as AOM and Rancimat [60,61,63]. The correlation among total phenols, evaluated by colorimetric method on the methanolic extract of VOO, their antioxidant activity, expressed using the oxygen radical absorbance capacity (ORAC) test, and the shelf life of oil, evaluated by the Rancimat method, was recently confirmed [58,59,69].

The antioxidant activity of specific hydrophilic phenols of VOO such as 3,4-DHPEA, *p*-HPEA and phenolic acids (i.e. caffeic acid, *p*-coumaric acid, ferulic acid, syringic acid and vanillic acid) was studied in refined olive oil and in sunflower oil and the high antioxidant power of 3,4-DHPEA was well shown [60,62,63].

The antioxidant activity of several secoiridoid derivatives, isolated from VOO and dissolved in purified olive oil, was studied by Baldioli et al. [52] using the Rancimat test. The results show how the *o*-diphenols, such as 3,4-DHPEA, 3,4-DHPEA-EDA and 3,4-DHPEA-EA, possess a much higher antioxidant activity than *p*-HPEA and α-tocopherol [52,61] and prove that 3,4-DHPEA and the other secoiridoids containing these compounds in their molecular structure (namely 3,4-DHPEA-EDA and 3,4-DHPEA-EA) are the natural antioxidants of VOO with the highest antioxidant power [52]. A first study on the antioxidant activity of VOO lignans, was also performed by Owen et al. [42] that shows the existence of a clear correlation between the antioxidant activity of phenolic extract of VOO and the lignans concentration [42]. In contrast, other authors did not show significant correlation between the concentration of lignans and VOO oxidative stability [70].

During the last years, moreover, several simulation of virgin olive oil behaviour during frying, and others cooking process were performed. The results related to the stability of phenols during frying and microwave cooking confirm the strong effect of oleuropein derivatives such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA in the oil stability; these compounds decrease sharply during heating to preserve oil for the oxidative reactions. The *p*-HPEA, ligstroside derivatives, such as *p*-HPEA-EDA and *p*-HPEA-EA and lignans, on the contrary, show high stability during the simulation of frying and microwave cooking, thus confirming their low effect in the oil protection for the oxidative reaction during cooking process [71,72].

1.4. Health properties

The antioxidant activity of virgin olive oil components received an increasing attention in the last years since it has been related to the protection for important chronic and degenerative diseases as coronary hearth diseases (CHD), ageing neuro-degenerative diseases and tumours of different localizations. Reactive species of oxygen (ROS) responsible of oxidative stress are involved in the all above mentioned diseases through mechanisms that in part have been elucidated. ROS in fact, oxidize lipoproteins deposited on the arteries, leading to the arteriosclerosis [73] and, with regard to carcinogenic process, they are able to produce DNA oxidative damage [74]. Furthermore in the inflammatory process of bowel diseases (IBD, ulcerative colitis and chron disease) the damage of colonic mucosa can be related to the ROS overproduction by lymphocytes which pass trough the mucosa and accumulate on the surface of epithelium [75]. Moreover, ROS production is related to the modulation of

cycloxygenase (COX 2), induced by different factors (IL1, TNF-a, LPS), that is clearly involved in the inflammatory processes, IBD and cancer [76]. Oxidative stress is also related to ageing, the production of superoxide dismutase is in elderly rats is higher than in young ones [77]. In the brain of elderly rats the GSH is clearly reduced [78] and the iron homeostasis is modified [79,80]. Recently has been reported [81,82] that the DNA oxidative damage of substantia nigra increases in aged rats. Moreover, oxidative stress seems to be related to the progression of neuro-degenerative diseases such as Parkinson [83].

Natural antioxidants are therefore thought to be important to prevent severe diseases which, as it is well known, have the highest incidence in the late aged classes.

For these reasons, foods rich in natural antioxidants received an increasing attention in the past years, and, in particular, olive oil, a typical component of the Mediterranean diet, has been recognised to be protective against cancer. The studies of Martin-Moreno [84], Trichopoulou et al. [85] and La Vecchia [86] showed that olive oil intake with diet reduced the estimated relative risk for breast cancer. These results have been confirmed by Lipworth et al. [87], by Kushi and Giovannucci [88]. Similar results have been obtained for the tumors of different sites: pancreas [89], oral cavity [90], oesophagus [91], colon-rectum [92], prostate [93,94] and lung [95].

Studies carried out in the animal model showed a protective effect of olive oil against the UV induced damage of the skin [96] and its ability in preventing the colon crypts aberrant foci growth and colon carcinoma in rats [97].

Several are the evidences that the protective effects against the chronic degenerative diseases are related to the phenolic components and, in particular, to the hydroxytyrosol rather than to the unsaturated fatty acids content of the olive oil.

Among these protective effects can be underlined: the reduction of phospholipids peroxidation in liposomes [98], the protection of low density lipoprotein (LDL) oxidation [99,100], the reduced oxidative damage of the human erythrocytes by 3,4-DHPEA [101], the inhibition of platelet aggregation by 3,4-DHPEA and its involvement in the thromboxane synthesis in human cells [102], the inhibition of DNA bases change caused by peroxinitrites [103] and the reduction of free radical production in the faecal matrix [104]. Moreover, a protective effect against the inflammation has been shown in the animal model [105].

In a recent in vitro study an other interesting property of olive oil phenols concerning the ability of 3,4-DHPEA to inhibit the cellular proliferation by blocking the cell cycle in the G0/G1 phase and to induce apoptosis in tumour cell-lines (HL60), but not in lymphocites and neutrophiles freshly isolated from human peripheral blood [106], has been shown.

1.5. Sensory proprieties

Sensory proprieties of VOO are largely affected by phenolic composition. In particular, these compounds were associated to the bitter and pungent sensory notes of oil. Several study were, in fact, performed to show the relationships between the "bitter" and the "pungent" taste of VOO and the total phenols concentration [107–109]. However, the relationships between individual hydrophilic phenols of VOO and its sensory characteristics were not clearly defined. In fact, while Gonzalez-Quijano et al. [110] associated the off-flavour sensory note of "atrojado" with the occurrence in VOO of certain phenolic acids, Graciani Costante and Vasquez Roncero [111], examining the phenolic composition of several VOO, showed a strong variability in the phenolic profile evaluated by HPLC, but they did not found any correlation with the oil sensory profile. Uccella et al. [112] moreover, reported that pure phenolic acids, extracted from VOO and dissolved in lipophylic solutions did not show relationships with the bitter sensory note. During the last 10 years, moreover, several authors studied the sensory impact of VOO secoiridoid derivatives. Concerning the relationships between the secoiridoid derivatives and the bitterness of VOO, the first interest of researchers was focalised in two compounds as *p*-HPEA-EDA and 3,4-DHPEA-EDA. In this context several authors suggested that secoiridoid derivatives of oleuropein and demethyloleuropein such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA were the main contributors to the VOO bitterness [113,114]. Tovar et al. [70] moreover, show a strong correlation between the bitter and pungent sensory notes and the ligstroside derivatives as *p*-HPEA-EDA. Recently, Gutiérrez-Rosales et al. [115] found a good linear correlation between the bitter sensory notes and the oleuropein and ligstroside derivatives such as *p*-HPEA-EDA and 3,4-DHPEA-EDA. Andrewes et al. [116], moreover, analyze specifically the pungent sensory notes. These authors isolated from the VOO the secoiridoid derivatives and show that the fractions containing *p*-HPEA-EDA produced a strong burning pungent sensation; in contrast, the fraction containing 3,4-DHPEA-EDA produced a slight burning sensation perceived more on the tongue. The same authors concluded that the *p*-HPEA-EDA was the phenolic compound responsible for the majority of the burning pungent sensory notes in VOO [116].

1.6. Agronomic aspects

The qualitative and quantitative composition of VOO hydrophilic phenols is strongly affected by the agronomic and technological conditions of production.

Several agronomic parameters can modify the phenolic concentration of VOO. The most studied aspects include cultivar, fruit ripening, pedo-climatic conditions of production and some agronomic techniques such as the irrigation [70,113,117,119]. As reported by different authors, phenolic composition of fruit is qualitatively affected by the cultivar [26,51,120–122]. Thus, while the oleuropein is almost present in the drupes of all the olive cultivars, the demethyloleuropein and verbascoside, on the contrary, are cultivar-dependent and has been proposed as marker for the genetic origin of fruit [123,124]. The fruit ripening also affects the

Table 4
Phenolic concentration (mg/kg) of virgin olive oil obtained from five Italian cultivars[a]

	Coratina	Moraiolo	Frantoio	Carolea	Leccino
3,4-DHPEA[b]	1.96 ± 3.00	2.08 ± 1.79	1.38 ± 1.42	2.70 ± 2.03	7.94 ± 10.81
p-HPEA	0.89 ± 0.99	0.87 ± 0.65	0.82 ± 0.91	0.72 ± 1.11	12.3 ± 15.6
3,4-DHPEA-EDA	382.4 ± 138.2	340.0 ± 262.3	154.0 ± 260.9	268.0 ± 113.8	67.6 ± 15.5
p-HPEA-EDA	193.2 ± 65.2	99.84 ± 61.2	89.8 ± 78.8	189.6 ± 89.7	12.5 ± 6.2
3,4-DHPEA-EA	177.5 ± 92.6	157.1 ± 84.5	84.1 ± 103.0	134.5 ± 56.3	47.2 ± 15.0

[a] Unpublished results. The results are expressed as mean ± standard deviation of 10 samples.The olives were harvested at the industrial ripening stage and malaxed at 30° C for 60 min and extracted by pressure in lab scale.
[b] The concentration of hiydrophilic phenols was evaluated by HPLC as previously reported by Montedoro et al. [35].

phenolic composition of olive drupe: oleuropein decreases during maturation while demethyloleuropein increases. The concentration of both compounds, however, strongly decrease in over-ripened olives [124].

As shown in Table 4, the olive cultivar also affects the absolute concentration of the specific hydrophilic phenols of VOO, while the phenolic profile remains almost the same.

The negative effect of fruit ripening on the phenolic concentration of VOO is particularly clear. The hydrophilic phenols show the lowest concentration in oils obtained from over-ripened olives [56,117–119].

A few papers report the relationships between VOO quality and seasonal conditions of olive growing. Several results related to the relationships between water availability during olive growing and phenolic concentration of VOO show that their concentration is greatly affected by the absolute disposability and distribution of water during the vegetative cycle of olive tree [125]. These studies did not lead however to univocal conclusions. Motilva et al. [126], for instance, concluded that hydrophilic phenols in VOO increase when the olives (Arbequina cultivar) were grown under conditions of regulated deficit of irrigation whereas other authors [127] reported that the highest level of hydrophilic phenols in oil was obtained from regularly irrigated olives. During the last years results reported by Tovar et al. [70], related to young trees of Arbequina cultivar grown using a linear irrigation strategies show a negative correlation between the level of water used and the concentration of secoiridoid derivatives of VOO such as 3,4-DHPEA-EDA, 3,4-DHPEA-EA and *p*-HPEA-EDA, on the contrary the concentration of lignans was lower in the oil from the least irrigated treatment [70].

1.7. Technological aspects

Since the occurrence of hydrophilic phenols in VOO is strictly related to the activities of various endogenous enzymes of olive fruit, their concentration in the oil is strongly affected by the extraction conditions. Crushing and malaxation are the most important critical points of the oil mechanical extraction process [5,128–130]. Secoiridoid aglycons such as 3,4-DHPEA-EDA, *p*-HPEA-EDA, *p*-HPEA-EA and 3,4-DHPEA-EA are originated, during crushing, by the hydrolysis of oleuropein, demethyloleuropein and ligstroside; the reaction is catalysed by the endogenous β-glucosidases, according to the proposed mechanism reported in Fig. 6. The enzymatic hydrolysis was studied using oleuropein and demethyloleuropein as substrates by various authors in a model system [131,132]. In previous paper, the relationships between enzymatic hydrolysis of secoiridoid glucosides and the occurrence of their aglycon derivatives such as 3,4-DHPEA-EDA, *p*-HPEA-EDA and 3,4-DHPEA-EA in VOO was reported [133]. This study reported that the concentration of oleuropein and demethyloleuropein was not significantly modified in olives blanched before crushing, to inactivate endogenous glycosidases; as a consequence of the enzymatic inhibition; furthermore the aglycon derivatives such as 3,4-DHPEA-EDA, *p*-HPEA-EDA and 3,4-DHPEA-EA were not found in the olive pastes and in the corresponding VOO [133]. So far however while the production of 3,4-DHPEA-EDA as final product of the demethyloleuropein enzymatic hydrolysis is well known [131] the formation mechanism of 3,4-DHPEA-EDA and *p*-HPEA-EDA from oleuropein and ligstroside, respectively, are still unknown. Bianco et al. [132] studying the hydrolysis of oleuropein glucoside by β-glucosidase in a model system have put in evidence the formation of the dialdehydic form of oleuropein aglycon as final product of the enzymatic reaction; the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA), on the contrary, was not found. Having said this, Rovellini et al. [44] found very low amount of the dialdehydic form of oleuropein aglycon in VOO, as a potential derivative of oleuropein hydrolysis, while the main compound was the 3,4-DHPEA-EDA; these compounds, according to Lo Scalzo et al. [131], could be the final product of demethyloleuropein enzymatic hydrolysis. Anyway the concentration of demethyloleuropein in olive fruit is cultivar dependent [28,123,124], consequently the phenolic composition of olive oil could be differentiated according to the amount of demethyloleuropein and/or oleuropein in the original fruit. On the contrary, any relationships between the concentration of 3,4-DHPEA-EDA in the oil and the occurrence of the demethyloleuropein in the corresponding fruit have been found [133]. For this reason it is possible to assume the enzymatic transformation of oleuropein in the 3,4-DHPEA-EDA, that may be also include the activity of a methylesterase, according to the proposed mechanism reported in Fig. 7.

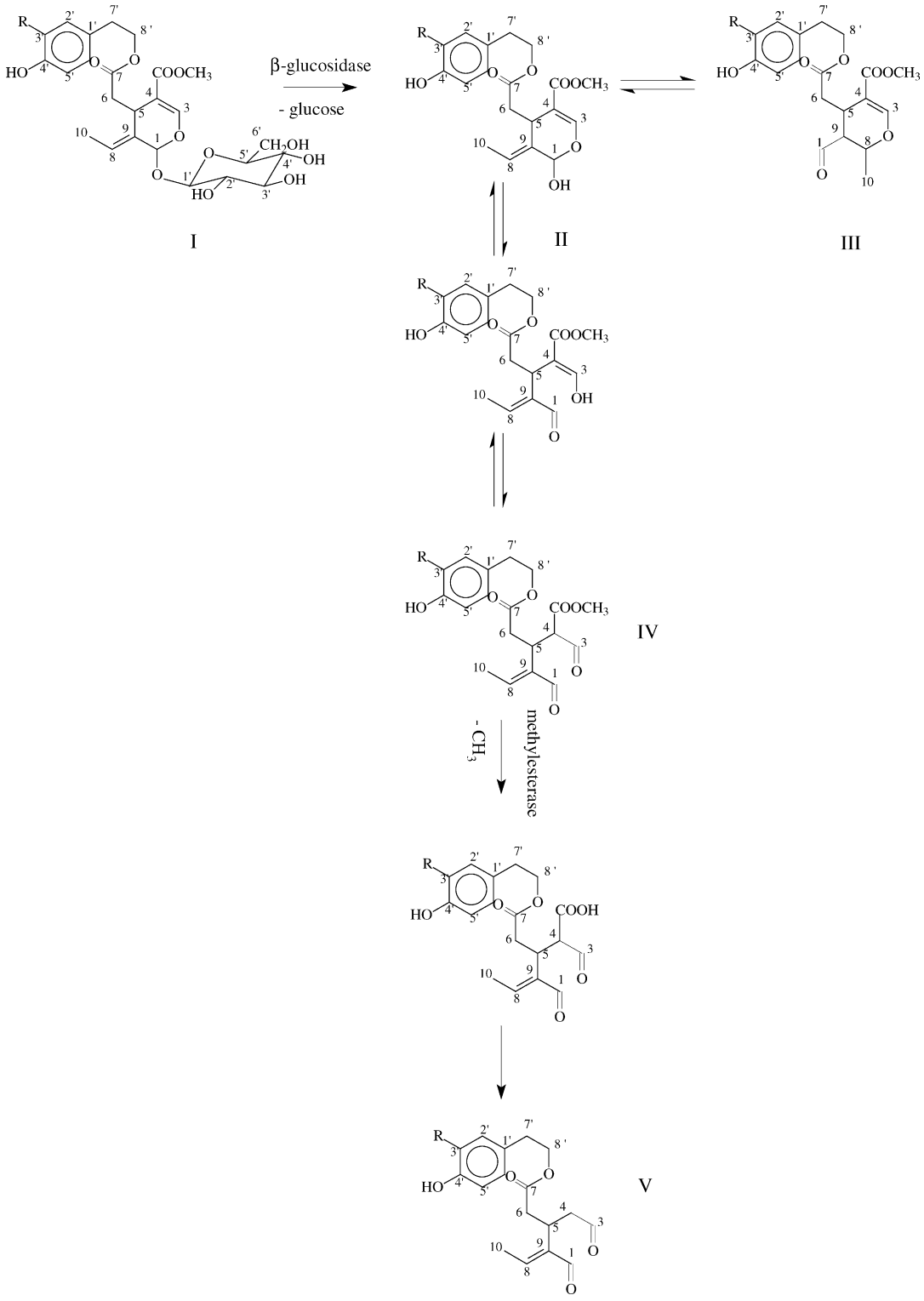


Fig. 7. Proposed biochemical mechanism of secoiridoids derivatives formation: (I) R = H: ligstroside; R = OH: oleuropein; (II) R = H: ligstroside aglycon; (III) R = OH: 3,4-DHPEA-EA; (IV) R = H: dialdehydic form of ligstroside aglycon; R = OH: dialdehydic form of oleuropein aglycon; (V) R = H: *p*-HPEA-EDA; R = OH: 3,4-DHPEA-EDA.

Table 5
Phenolic composition of virgin olive oil (mg/kg), obtained with and without air contact of the pastes during malaxation [137]

	Crushed paste blank	Malaxed paste blank	Malaxed paste under N_2 flush
3,4-DHPEA[a]	2.7 ± 0.3 a	0.7 ± 0.1 b	2.0 ± 0.2 a
p-HPEA	2.3 ± 0.4 a	1.2 ± 0.1 b	2.6 ± 0.3 a
3,4-DHPEA-EDA	515.0 ± 23 a	317.0 ± 16.0 b	504.0 ± 6.0 a
p-HPEA-EDA	24.8 ± 1.9 a	25.8 ± 1.4 b	28.4 ± 1.4 b
p-HPEA derivative	32.5 ± 1.4 a	24.2 ± 0.8 b	21.6 ± 21.6 b
3,4-DHPEA-EA	357.0 ± 13.0 a	177.0 ± 8.0 b	242.0 ± 5.0 c

[a] The phenolic content is the MEM value of three independent experiments ± standard deviation. Values in each row bearing the same superscripts are not significantly ($P < 0.05$) different from one another. The concentration of hydrophilic phenols was evaluated by HPLC as previously reported by Montedoro et al. [35].

During malaxation the concentration of secoiridoid aglycons such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA and phenolic alcohols decreased in olive pastes and oils with increasing time and temperature of processing [6,128,134,135]. The distribution of hydrophilic phenols between the oil and the water phase, as related to their solubility, is not the only mechanism involved in the reduction of the oil phenolic concentration during malaxation: oxidative reactions catalysed by endogenous oxidoreductases such as polyphenoloxidase and peroxidase can promote the phenolic oxidation during processing [6,136,137]. As shown in Table 5, the inhibition of polyphenoloxidase and peroxidase, obtained reducing the O_2 level in the paste, during malaxation, improved the concentration of hydrophilic phenols in olive paste and VOO [6,138]. As consequence, the control of O_2 concentration in the paste during processing can be use to optimize the phenolic concentration in VOO [139–141]. In this context, the time of exposure of olive pastes to the air contact (TEOPAC) was studied by Servili et al. [140] as processing parameter to regulate the averaged concentration of oxygen in the paste and as consequence the phenolic amount in the oil. As reported in Table 6, infact, the concentration of secoiridoid derivatives is strictly related to the TEOPAC [140]. The use of new technologies, such as oil mechanical extraction from destoned pastes, that can improve the oil phenolic concentration, seems to confirm the relationships between the control of oxidative reactions during processing and the

Table 6
Effect of TEOPAC on the phenolic composition of virgin olive oil evaluated a three ripening state [140]

	Time of exposure to the air contact (TEOPAC)						
	0′	10′	20′	30′	40′	50′	60′
Phenolic compounds (mg/kg)							
Pigmentation index: 2.2							
3,4-DHPEA-EA	146.0 ± 16.6	137.0 ± 6.42	117.7 ± 28.76	111.9 ± 11.31	149.3 ± 0	143.3 ± 1.23	95.8 ± 6.03
3,4-DHPEA	1.3 ± 0.21	1.1 ± 0.08	0.8 ± 0.26	0 ± 0.00	1.3 ± 0.03	1.5 ± 0.05	1.0 ± 0.40
3,4-DHPEA-EDA	804.3 ± 26.04	848.6 ± 14.47	738.1 ± 11.40	681.6 ± 20.26	622.0 ± 15.24	581.4 ± 13.23	337.6 ± 8.57
p-HPEA-EDA	29.8 ± 1.34	33.7 ± 0.69	27.5 ± 0.20	26.2 ± 0.07	40.4 ± 1.34	37.9 ± 2.50	28.6 ± 4.14
Pigmentation index: 2.6							
3,4-DHPEA-EA	354.9 ± 28.90	384.8 ± 12.56	327.7 ± 20.94	291.7 ± 21.08	290.8 ± 20.9	281.3 ± 27.56	245.9 ± 8.10
3,4-DHPEA	3.7 ± 1.11	2.7 ± 0.32	1.9 ± 0.08	2.8 ± 0.74	1.6 ± 0.08	2.4 ± 0.30	1.9 ± 0.11
3,4-DHPEA-EDA	1206.2 ± 37.79	1103.9 ± 23.83	1108.1 ± 14.13	1063.7 ± 10.02	1060.0 ± 23.14	834.3 ± 18.72	645.3 ± 6.47
p-HPEA-EDA	40.5 ± 1.41	41.4 ± 1.15	44.3 ± 1.73	49.8 ± 3.10	39.0 ± 0.26	32.5 ± 0.38	53.3 ± 0.16
Pigmentation index: 2.9							
3,4-DHPEA-EA	368.5 ± 27.83	381.7 ± 24.48	337.5 ± 15.78	310.3 ± 16.61	314.5 ± 17.03	279.6 ± 11.12	246.0 ± 16.52
3,4-DHPEA	1.9 ± 0.13	2.7 ± 0.71	2.0 ± 0.34	2 ± 0.03	1.3 ± 0.03	1.3 ± 0.21	1.2 ± 0.26
3,4-DHPEA-EDA	1003.0 ± 54.47	956.5 ± 61.10	841.0 ± 45.28	882.7 ± 26.89	665.8 ± 50.38	492.2 ± 60.93	364.6 ± 36.00
p-HPEA-EDA	34.5 ± 1.10	36.8 ± 2.22	44.9 ± 1.63	37.1 ± 1.21	29.6 ± 2.19	24.9 ± 1.41	25.4 ± 1.44

The results are expressed as mean ± standard deviation of three idependent experiments. The concentration of hydrophilic phenols was evaluated by HPLC as previously reported by Montedoro et al. [35].

Table 7
Qualitative parameters of virgin olive oils obtained from destoned and control (whole fruit) pastes evaluated at time 0 and after12 mounths of storage at room temperature (25 °C) [144]

	Oils of control olive pastes		Oils of destoned olive pastes	
	Time 0	Time 12	Time 0	Time 12
Free acidity (g oleic acid/100 g oil)	0.29	0.31	0.25	0.30
Peroxide number (meq O_2/kg oil)	6.1	25.4	5.4	21.7
K_{272}	1.922	4.000	1.826	3.250
K_{270}	0.136	0.234	0.110	0.190
Total polyphenols[a] (mg/kg)	345	150	355	195
Ortodyphenols[a] (mg/kg)	250	85	270	100

[a] Evaluated colorimetrically and expressed in mg/kg as 3,4-DHPEA equivalent.

Table 8
Phenolic composition of virgin olive oil (mg/kg) with and without enzymatic treatment of the pastes during malaxation [149]

	Crushed paste blank	Malaxed paste blank	Malaxed paste + NF12/olivex
3,4-DHPEA[a]	2.7 ± 0.3 a	0.7 ± 0.1 b	1.9 ± 0.1 c
p-HPEA	2.3 ± 0.4 a	1.2 ± 0.1 b	1.2 ± 0.1 b
3,4-DHPEA-EDA	515.0 ± 23 a	317.0 ± 16 b	439.0 ± 16 c
p-HPEA-EDA	24.8 ± 1.9 a	25.8 ± 1.4 a	29.4 ± 0.8 b
p-HPEA derivative	32.5 ± 1.4 a	24.2 ± 0.8 b	28.5 ± 0.9 c
3,4-DHPEA-EA	357.0 ± 13 a	177.0 ± 8 b	218.0 ± 8 c

[a] The phenolic content is the mean value of three independent experiments ± standard deviation. Values in each row bearing the same superscripts are not significantly ($P < 0.05$) different from one another. The concentration of hydrophflic phenols was evaluated by HPLC as previously reported by Montedoro et al. [35].

Table 9
Phenolic composition of the vegetation waters (mg/g dry weight) with and without enzymatic treatment of the pastes during malaxation [149]

	Malaxed paste blank	Malaxed paste + NF12/olivex
3,4-DHPEA[a]	0.37 ± 0.03 a	0.44 ± 0.01 a
p-HPEA	n.d. a	0.04 ± 0.01 b
Demethyioleuropein	0.47 ± 0.06 a	0.82 ± 0.01 b
Verbascoside	n.d.	n.d.
3,4-DHPEA-EDA	17.50 ± 1.7 a	31.90 ± 2.6 b
Oieuropein	0.60 ± 0.04 a	1.10 ± 0.1 b
Luteolin-7-glucoside	0.11 ± 0.01 a	0.11 ± 0.01 a
Rutin	0.06 ± 0.01a	0.10 ± 0.01 b

n.d.: Not detected.

[a] The phenolic content is the mean value of three independent experiments ± standard deviation. Values in each row bearing the same superscripts are not significantly ($P < 0.05$) different from one another. The concentration of hydrophHic phenols was evaluated by HPLC as previously reported by Montedoro et al. [35].

phenolic concentration in the oil (Table 7). In fact, because of the peroxidase is highly concentrated in the olive seed [138], the destoning process, excluding the olive seed before malaxation, partially remove the peroxidase activity in the pastes and consequently can reduce the enzymatic degradation of the hydrophilic phenols in the oils during processing thus improving their concentration and oil oxidative stability [142–144].

The oxidative reactions occurring in the pastes during malaxation can explain also the relationships between the phenolic concentration in VOO and the malaxing temperature. In several papers the negative relationships between malaxing temperature and phenolic concentration in the oil was found; so far however a few authors [135] showed that phenolic concentration improved in the oil when malaxing temperature was increased. These conflicting results may be explained in term of O_2 concentration in the pastes during processing. In fact when the activity of PPO and POD in the pastes was inhibited by a low O_2 concentration the phenolic amount in the oil increased according to the processing temperature, due to the improved solubility of these compounds in the oil phase [141].

Interactions between polysaccharides and phenolic compounds present in the olive pastes may also be involved in the loss of phenols during processing. Polysaccharides may link hydrophilic phenols in the pastes thus reducing their release in the oil during crushing and malaxation [145]. In this regard, it has been shown that the use of technical enzymatic preparations containing cell wall degrading enzymes during processing can improve the oil phenolic concentration [146–148].

Vierhuis et al. [149] showed that the addition of commercial enzyme preparations reduced the complexation of hydrophilic phenols with polysaccharides thus increasing the concentration of free phenols in the pastes and their release in the oils and the vegetation waters during processing (Tables 8 and 9).

Extraction system, such as pressure and centrifugation, plays an important role in the oil phenolic composition. In fact, in the traditional centrifugation system a large amount of water (50–100 L/100 kg of olive pastes) is added before centrifugation, to reduce the viscosity of pastes and to im-

Table 10
Phenolic composition and induction period of virgin olive oils obtained from the cultivars Coratina and Oliarola with two phases and three phases centrifugation system[a] [155]

	Coratina cultivar		Oliarola cultivar	
	Two phases	Three phases	Two phases	Three phases
3,4-DHPEA[b]	0.87 ± 0.02 a	0.58 ± 0.08 b	0.66 ± 0.11 a	0.50 ± 0.11 a
p-HPEA	3.74 ± 0.07 a	2.34 ± 0.08 b	3.30 ± 0.10 a	4.22 ± 0.10 b
Vanillic acid	0.41 ± 0.01 a	0.19 ± 0.01 b	0.26 ± 0.01 a	0.14 ± 0.05 b
Caffeic acid	0.16 ± 0.01 a	0.12 ± 0.02 b	0.09 ± 0.01 a	0.21 ± 0.03 b
3,4-DHPEA-EDA	522.2 ± 13.5 a	427.2 ± 13.8 b	30.09 ± 1.03 a	18.53 ± 0.68 b
p-HPEA-EDA	78.16 ± 0.52 a	67.26 ± 2.55 b	20.99 ± 0.82 a	22.40 ± 0.33 a
p-HPEA-ester	38.41 ± 0.10 a	35.62 ± 1.11 b	48.00 ± 3.40 a	46.72 ± 5.78 a
3,4-DHPEA-EA	351.71 ± 11.0 a	244.9 ± 13.6 b	68.01 ± 6.00 a	52.04 ± 3.11 b
Total polyphenols[c]	673 ± 4 a	585 ± 7 b	304 ± 5 a	263 ± 4 b
Induction period [h]	17.8 ± 0.1 a	15.5 ± 0.2 b	5.2 ± 0.1 a	4.6 ± 0.1 b

[a] Mean values of three independent determination. Values, in each row, bearing the same superscrits are not significantly ($P < 0.05$) different from one another.

[b] The concentration of hydrophilic phenols was evaluated by HPLC and expressed as mg/kg, as previously reported by Montedoro et al. [35].

[c] Evaluated colorimetrically and expressed in mg/kg as 3,4-DHPEA equivalent.

prove oil separation from the solid phase [142,150]. Water addition to the olive pastes, however, modifies the distribution of hydrophilic phenols between oil and water improving their release in the water phase. For this reason the oil obtained by pressure system, that does not require addition of water to the olive pastes, shows higher phenolic concentration in comparison to the one obtained by the traditional centrifugation process [142,150]. During the last 10 years, however, new centrifugation systems have been developed that require less water for the oil separation. As a consequence the decanters at present time, used to separate the oil phase from the olive pastes in the centrifugation systems, can be classified in three groups: (a) traditional three-phases decanter (50–100 L of added water per 100 kg of olive pastes); (b) three-phases decanter at low water addition (10–30 L of added water per 100 kg of olive pastes); (c) two-phases decanter that can work without water addition.

Several research works have been carried out to compare the traditional three-phases decanter with the new two-phases decanter [150–154]. Results reported in Table 10, obtained using two typical Italian cultivars such as Coratina and Oliarola, put in evidence higher concentration of hydrophilic phenols in VOO extracted using two-phases decanters as compared to the traditional three-phases centrifuges [155]. Similar results were obtained, using Spanish and Greek cultivars, by other authors [151,152]. An increased concentration of phenols was also observed in oils extracted by three-phases decanters at low water addition as compared to the traditional three-phases centrifuges [143].

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