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Influence of polyphenolic compounds on the oxidative stability of virgin olive oils from selected autochthonous varieties

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Abstract

The evaluation of the influence of olive oil phenols on the stability of extra virgin olive oils by the determination of the oxidative stability index, the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical test, and the quali-quantitative analysis of the phenolic compounds, plays a key role in the assessment of the overall olive oil quality. In this study virgin olive oils from selected autochthonous Croatian varieties were investigated in order to evaluate the contribution of their phenolic compounds on the oxidative stability. Two year researches were conducted on the monovarietal olive oils (Oblica, Lastovka, Levantinka, Drobnica, Mastrinka) obtained by the same technological processing system, centrifugal extraction into two phases. The olive fruits came from different groves in Dalmatia region, a central part of Croatian Adriatic coast. The polar extracts of olive oil samples were submitted to spectrophotometric analysis of total phenols and to liquid chromatographic determination of their quali-quantitative profile (HPLC-DAD/MSD). To obtain a complete description of oil samples, fatty acid composition, ultraviolet indices (K232, K270 and ΔK), free acidity degree and peroxide value were also determined according to the European Union methods stated in Regulation 2568/91. Beside analyses mentioned above, researches were amplified by oxidative stability determination by Rancimat method, evaluation of antioxidant activity by DPPH and ABTS methods as well as antioxidant capacity (AOP). The evolution of the analytical parameters studied showed that certain groups of phenolic compounds (secoiridoides, flavones and lignans) have a higher antioxidant activity and antioxidant capacity than the total phenols. The results obtained in this study will contribute to better understanding of peculiar characteristics of local varieties of olive oil and set the basis for their characterization and evaluation.

Key words: Antioxidant activity, fatty acid composition, monovarietal virgin olive oils, oxidative stability, polyphenols.

Introduction

Oxidative stability is one of the basic parameters for assessing the quality of virgin olive oil (VOO) mainly because it provides a reliable assessment of the vulnerability of oil oxidation changes, which are the main cause of its deterioration [15,19]. Although unavoidable, the oxidation process can be delayed by natural antioxidant compounds present in VOO that prevent the propagation of lipid peroxidation or remove free radicals [42]. The main antioxidants in VOO are carotenes and phenolic compounds, including lipophilic and hydrophilic phenols [8]. Lipophilic phenols such as tocopherols are also found in the composition of other vegetable oils, while some hydrophilic phenols in olive oil are not present in other oils and fats [3, 9, 14, 39]. Phenolic compounds (phenols or polyphenols) act as primary antioxidants (AH) and prevent oxidation of VOO [31].

VOO has good oxidation stability because of the specific fatty acid composition and the presence of nonglyceridic components with antioxidant properties. Good stability of olive oil at elevated temperatures is due to the high content of monounsaturated fatty acids and low content of polyunsaturated fatty acids [11]. Many authors have studied the relationship between the share of natural antioxidants in olive oil and its oxidative stability [1, 2, 5, 6, 10, 12, 17, 18, 23, 27, 28, 34, 36, 39, 40]. The common conclusion of the researches is that the enhancement of the share of antioxidants in the oil increases its oxidative stability. The largest contribution to the total oxidation stability, greater than 50% of virgin olive oil phenolic compounds, gives a hydrophilic character [4]. Different methods have been introduced for antioxidant activity examination of VOO [19, 21]. Most of them investigated the ability of oil to scavenge free radical [21], but only one method is based on electrochemical properties [19].

The aim of this paper was to evaluate the antioxidant activity of certain phenolic groups in monovarietal VOOs from selected autochthonous Croatian cultivars and to compare the results with oxidative stability of oils. Accelerated deterioration tests at elevated temperature (Rancimat method) were applied to record induction time. Dependence of the phenol and fatty acid profile of the VOOs was also defined. The antioxidant activity of prepared phenolic extracts as free radicals scavengers was compared using the ABTS and DPPH methods and antioxidant capacity by the electrochemical method [19].

Materials and Methods

Sample description: Olive fruits from five autochtonous cultivars were selected from several locations in Dalmatia region (coastal part of Croatia including some islands) and harvested during two consecutive crop years (2006/7, 2007/8) at different ripening stage. All fruits were immediately processed in oil equally by centrifugal system with two phases. All oil samples were stored in dark glass bottles at +4°C until the analyses were carried out.

Reagents and standards: The standard used for electrochemical determination of the antioxidant power (quercetin) and for the evaluation of antioxidant capacity of phenolic extracts (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox) were produced by Sigma-Aldrich Inc. (St. Louis, MO, USA). ABTS (2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid diammonium salt) was purchased from Sigma-Aldrich. DPPH (2,2-diphenyl-1-picryl-hydrazyl), Folin- Ciocalteu reagents and gallic acid were acquired from Sigma Chemicals Co. (St. Louis, MO, USA). All solvents used were analytical grade (Merck & Co. Inc., Darmstadt, Germany).

D etermination of quality indices: Basic quality parameters (free acidity FA, peroxide value POV and UV spectrophotometric indices at 232 and 270 nm) were determined according to EC method [20]. All parameters were determined in triplicate for each sample.

Fatty acids methyl ester analysis by CGC analysis: Fatty acid methyl esters (FAME) from the oil samples were obtained by alkaline treatment with 1M KOH in methanol, with the conditions described in Table 1, according to ISO method [26]. The gas chromatographic analyses were carried out according to ISO method [25]. The average was calculated from four replications for each sample.

Table 1. GC conditions for fatty acids methyl ester analysis.

Column	CP Sil-8;, film 0.25µm; 50 m x 0.25 mm, (Superchrom S.r.l., Milano, Italy)
Split	1:20, v/v
Column temperature (programmed)	170°C to 230°C, 2.5°C min^{-1} 170°C, 2 min 230°C, 8 min
Injector temperature	220
Detector temperature	230
Carrier gas	Helium, flow rate: 1 ml min^{-1}
Sample	0.5 µl

Liquid-liquid extraction of phenolic fraction: Extraction was performed following the protocol described by Pirisi *et al.* [33] and modified according to Rotondi *et al.* [37]. The sample was dissolved in 1 ml of n-hexane and 2 ml of solvent methanol/water (60:40, v/v), vigorously stirred up and centrifuged for three min at a speed of 3000 r min^{-1}. Aqueous-alcoholic phase was collected and the rest re-extracted twice with 2 ml of solvent methanol/water (60:40, v/v). The hydro-alcoholic phase was washed with 2 ml of n-hexane in order to remove any residual oil. The extract was concentrated and dried in a vacuum at a temperature of 35°C. Dry extracts were redissolved in 0.5 ml of a methanol/water (50:50, v/v) solution and filtered through 0.2 µm nylon filter (Whatman Inc., Clifton, NJ, USA) before being analyzed by HPLC-DAD/MSD. Unless otherwise stated, extractions were performed in three replicates (n = 3). Extracts were stored at -18°C before use.

Total phenol content determination: The total phenol content in VOO samples were determined according to Gutfinger's method [22], using Folin-Ciocalteu reagent by spectrophotometer (Shimadzu, UV-VIS 1204 (Kyoto, Japan) at 750 nm. The total phenols were expressed as mg gallic acid kg^{-1} oil (calibration curve with r^2 = 0.996). The spectrophotometric measurements were repeated three times for each extract.

Phenol composition determination by HPLC – DAD/MSD: HPLC analyses were performed according to Rotondi *et al.* [37]. Measurement was carried out on the HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with two detectors (Diode-Array UV-VIS detector DAD and mass spetrometry detector MSD) with the conditions presented in Tables 2 and 3. All solvents used were previously filtered through a 0.45 µm mesh nylon filter (Lida Manufacturing Corp., Kenosha, WI, USA). The analyzes were conducted in reverse phase by column C_{18} Luna™ (5 µm, 25 cm x 3.00 mm, Phenomenex, Torrance, CA, USA) (Table 3). All analyzes were carried out at room temperature, at a wavelength of 280 nm. The average was calculated by four replications for each sample.

Table 2. HPLC-DAD / MSD conditions for phenol composition determination.

Cartridges	C18 Luna (5 µm, 25cm x 3.00 mm; Phenomenex, Torrance, CA, USA)
Sample	10 µl
Eluent	Gradient (Table 3.)
Flow rate	0.5 ml min^{-1}
Temperature	25 °C
Detector	HP Diode-Array UV-VIS detector (DAD) and HP mass spectrometry detector (MSD)

Table 3. HPLC gradient for phenol composition determination (A – H_2O / formic acid (99.5: 0.5, v/v); B – acetonitrile).

Time (min)	A (%)	B (%)	Time (min)	A (%)	B(%)
0	95	5	40	70	30
5	93	7	42	69	31
10	91	9	45	68	32
15	88	12	48	66	34
18	85	15	50	65	35
20	84	16	55	60	40
25	82	18	60	50	50
30	80	20	70	5	95
32	78	22	75	95	5
35	75	25	80		
38	72	28			

Radical scavenging activity of phenolic extracts by ABTS$^{\bullet+}$ assay: The antioxidant activity determination by ABTS$^{\bullet+}$ assay was performed according to Re *et al.* [35]. ABTS was dissolved in H_2O at a concentration of 7 mM. The radical cation of ABTS was obtained by reaction with 2.45 mM potassium persulfate (final concentration) and allowing the stock solution to stand in the dark at room temperature for at least 12 h. Before use, ABTS$^{\bullet+}$ solution was diluted with EtOH to an absorbance of 0.70 ± 0.02 at 734 nm at 30°C (using a UV-Vis 1204 Shimadzu spectrophotometer, Kyoto, Japan). Next, 1 ml of ABTS$^{\bullet+}$ solution was added to 0.01 ml of extract and the decrease in absorbance was recorded for 10 min. Absorbance values were corrected for radical decay using blank solution (0.01 ml of 50% aq MeOH). Measurements were made in triplicate and the antioxidant activity was calculated as the Trolox equivalent antioxidant capacity (TEAC) (r^2 = 0.9739).

Radical scavenging activity of phenolic extracts by DPPH method: Determination of antioxidant activity by free DPPH• radical was conducted according to Brand-Williams *et al.* [13]. Measurement of radical scavengers (RSA) was performed on the spectrophotometer Perkin-Elmer Lambda Bio 20th. The method is based on the reduction of the stable radical DPPH• (2,2-diphenyl-1-picrylhydrazyl). Ethanolic solution of DPPH• (2.9 ml concentration of 15 M) was mixed with 0.1 ml of phenolic extract. After 30 min of incubation at 25°C temperature the absorbance at 515 nm was measured and compared with a control sample that was prepared adding 0.1 ml of methanol/water (1:1, v / v) mixture. Each phenolic extract was prepared by six dilutions with methanol (1:2, 1:5, 1:10, 1:15, 1:20, 1:40, 1:50, 1:80 and 1:100, v / v). Radical scavenging activity was expressed as TEAC (Trolox equivalent antioxidant capacity) using the calibration chart ($r^2 = 0.9974$).

Antioxidant power (AOP) evaluation: An amperometric flow injection method (FIA) was used for the evaluation of the antioxidant power of the phenolic extracts of the samples according to Del Carlo *et al.* [19]. The apparatus consisted of a Minipuls II peristaltic pump (Gilson, France), a high-pressure injection valve model 7125 (Rheodyne, Rohnert Park, CA), equipped with a 20 µl loop, an electrochemical cell model UniJet (BAS, West Lafayette, IN) using a glassy carbon working electrode, and an amperometric detector AMEL 559 HPLC detector (AMEL, Milan, Italy) linked to a chart recorder RC 102 (Pharmacia, Sweden). Injections of three extract samples were performed at the potential 0 mV versus Ag/AgCl, and the flow rate was 150 µl min^{-1}. The current produced in the electrochemical oxidation of the phenolic compounds was recorded. The method was calibrated using quercetin, one of the easiest electrochemically oxidizable phenols in the concentration range of 0.1- 25.0 µM. Samples were appropriately diluted to obtain current signals within the linear range of the applicable standard molecule.

Oxidative stability: Oxidative stability was determined using Rancimat apparatus (Metrohm 743 Ltd., Herisau, Switzerland) in order to evaluate accelerated oxidation at high temperature. Olive oil samples were tested at a temperature of 120°C ($\Delta t = 1.4$°C) with the constant air flow of 20 litres h^{-1}. In this way, the oxidation changes or the presence of volatile compounds produced by oxidation were determined. Conductivity was measured conductometrically as a function of time. The obtained result is presented as induction time which is known as a sustainability index of oil or fat at certain temperature [23].

Statistical analyses: The statistical analysis used SPSS r.11.0.0. statistical software (SPSS Inc., Chicago, IL, USA). Significant differences at the 5% level between mean values were determined using one-way ANOVA analysis and non-parametric tests (Kruskal-Wallis test and post-hoc (Bonferroni) tests).The correlation of antioxidant DPPH, ABTS, AOP and OSI with groups of phenols and the most important fatty acids were analyzed. Each physical and chemical determination was replicated three times and averages and standard errors were calculated.

Results and Discussion

Quality of VOO is defined by basic qualitative chemical parameters whose limits are prescribed for certain categories by international regulation EC [15] . The results in Table 4 from both years studied show that each oil sample during study period were produced from healthy good quality olives.

Data for FA showed that all samples had the specified value less than 0.8% expressed as oleic acid, making them the international and domestic legislation classified as extra virgin olive oil. Peroxide values (POV) indicate formation of primary products of oxidative spoilage (peroxides and hydroperoxides). In all oil samples the values for POV were below 10 mmol O_2 kg^{-1}, which is a limit value for the VOO in all categories for edible oils prescribed in the EC regulations [20].

Numerous authors [4, 7, 24] examined the relationship of oleic and linoleic acid (18:1 / 18:2) and oxidative stability of VOO. The results show that the ratio of monounsaturated and polyunsaturated fatty acids is one of the major factors responsible for evaluating the oxidative stability of VOO and it's also used as a parameter for the oil characterization [41]. In this paper all analyzed oils showed a stable ratio of oleic and linoleic acid (18:1 / 18:2) with values close to or higher than 7, which is shown in Table 4, with the exception of Mastrinka oil because of the fairly high proportion of linoleic acid.

Phenols are part of nonglycerine components in olive oil consisting mainly compounds with functional significance, whether biological or nutritional. Biological characteristics of the olive oil are the result of the presence of nonglycerine ingredients, such as squalene and phytosterols, including phenols and tocopherol that are the most important antioxidant compounds [32].

Environmental stress, such as UV radiation and relatively high temperatures that are typical of the Mediterranean area, stimulate secondary metabolism in fruits and vegetables, including olives. As a consequence of this process is the increased formation of phenolic compounds with protective (antioxidant) activity. Phenolic molecules are typical of unrefined oils, and this group of antioxidant compounds are present in VOO [3]. The literature states that the share of phenolic compounds in olive oil varies in the range of 40 to more than 1000 mg kg^{-1} [39].

Examinations of VOOs from different Mediterranean countries [30] showed that there are large variations in the absolute values of hydrophilic phenols, especially dialdeidic form of elenolic acid associated with 3,4-dihydroxyphenyl ethanol (3,4-DHPEA-EDA) and oleuropein aglycone (3,4-DHPEA-EA). Comparing the identified phenolic compounds in the oil samples it's evident that the share of higher phenolic alcohols in oils from selected local varieties is significantly higher, while the share of secoiridoids is smaller.

Cerretani *et al.* [16] studied different olive oils from Sardinia (Italy) and Corsica (France) that were processed by different types of continuous mills. Total tocopherol content of the samples can be considered as medium - high (within 212.3 – 377.5 mg of tocopherols kg^{-1} of oil) in accordance with previous reports. However, the α-tocopherol content did not appear to be dependent on the varietal origin of the oils.

During this survey the largest amount of simple phenols was detected in Drobnica oil (172.28 mg kg^{-1} of oil) and Lastovka oil (165.76 mg kg^{-1} of oil), as shown in Table 5. This amount can be considered as medium comparing with the VOOs in research carried out in Italy [16].

Lastovka oil had the largest share of secoiridoids derivatives measured 441.03 mg kg^{-1} of oil and Mastrinka oil 320.11 mg kg^{-1} of

Table 4. Chemical and qualitative parameters in olive oil samples.

Season	2006/07						2007/08				
Sample	Free acidity (%)	POV (mmol O_2/kg)	K_{232}	K_{270}	ΔK	C18:1/ C18:2	Free acidity (%)	POV (mmol O_2/kg)	K_{232}	K_{270}	ΔK	C18:1/ C18:2
OB1	0.20±0.06	2.45±0.04	1.85±0.07	0.17±0.01	0.005±0.001	6.34	0.24±0.11	5.45±0.14	1.63±0.01	0.16±0.09	0.001±0.000	6.00
OB2	0.20±0.03	1.50±0.09	1.64±0.04	0.14±0.05	0.005±0.001	6.98	0.39±0.06	3.95±0.16	1.72±0.09	0.13±0.06	-0.002±0.001	6.80
OB3	0.18±0.07	2.25±0.06	1.56±0.04	0.13±0.04	0	6.72	0.13±0.04	2.90±0.15	1.76±0.07	0.12±0.07	0.001±0.000	6.30
OB4	0.19±0.06	3.70±0.13	1.65±0.07	0.15±0.08	-0.004±0.001	12.39	0.21±0.05	3.85±0.06	1.90±0.17	0.12±0.02	-0.002±0.001	7.10
OB5	0.33±0.07	3.00±0.22	1.38±0.07	0.13±0.07	-0.001±0.001	5.60	0.18±0.02	4.05±0.05	1.65±0.07	0.11±0.04	-0.002±0.001	6.70
OB6	0.16±0.04	4.30±0.09	1.73±0.07	0.16±0.05	0.001±0.000	6.40	0.12±0.02	1.90±0.12	1.28±0.06	0.13±0.08	0.005±0.001	9.50
LA1	0.41±0.05	3.30±0.14	1.76±0.07	0.16±0.08	0.002±0.002	8.38	0.44±0.05	2.65±0.14	1.75±0.06	0.13±0.03	0.004±0.001	7.20
LA2	0.44±0.07	2.35±0.09	1.85±0.08	0.18±0.09	0.005±0.001	6.59	0.21±0.04	3.05±0.06	1.76±0.12	0.12±0.06	0.005±0.001	6.30
LA3	0.21±0.04	3.15±0.13	1.88±0.06	0.15±0.04	0.002±0.000	6.40	0.32±0.05	2.95±0.14	1.57±0.08	0.12±0.05	0.005±0.001	7.09
LE1	0.12±0.02	2.15±0.20	1.58±0.07	0.13±0.05	0.005±0.001	8.32	0.26±0.12	3.15±0.06	1.82±0.03	0.16±0.07	0.002±0.001	7.26
LE2	0.22±0.08	2.45±0.18	1.65±0.09	0.14±0.06	0.005±0.001	7.80	0.30±0.05	3.00±0.13	1.67±0.05	0.15±0.07	-0.001±0.001	7.90
LE3	0.16±0.04	7.30±0.48	1.81±0.02	0.15±0.04	-0.014±0.003	10.00	0.19±0.02	3.75±0.17	1.75±0.04	0.16±0.07	0.002±0.001	8.67
DR1	0.22±0.05	3.20±0.13	1.56±0.08	0.15±0.05	0.0025±0.001	9.80	0.17±0.05	1.95±0.07	1.54±0.09	0.12±0.05	0.002±0.001	9.18
DR2	0.24±0.06	2.85±0.12	1.62±0.09	0.14±0.07	0.001±0.001	10.48	0.19±0.07	2.15±0.08	1.60±0.10	0.14±0.06	0.001±0.000	9.32
DR3	0.21±0.06	2.75±0.38	1.50±0.09	0.12±0.05	0.001±0.001	9.84	0.15±0.05	2.20±0.20	1.65±0.07	0.11±0.07	0.002±0.001	9.05
MA1	0.30±0.06	3.25±0.20	1.85±0.07	0.16±0.07	0.005±0.001	7.14	0.54±0.05	4.20±0.10	1.73±0.05	0.12±0.06	0.005±0.001	5.90

Abbreviations: Olive oil samples were produced by different olive cultivars: OB1 and OB5 were from Oblica (grown on island Hvar); OB2 and OB6 were from Oblica (grown on island Murter); OB4 were Oblica (grown in Kaštel Stari); OB3 is from Oblica (grown on island Bra); LA1 and LA3 were from Lastovka (grown on island Hvar); LA2 is from Lastovka (grown in Kaštel Stari); LE1 and LE3 were from Levantinka (grown on island Hvar); LE2 is from Levantinka (grown in Kaštel Stari); DR1 and DR3 were from Drobnica (grown on island Hvar); DR2 is from Drobnica (grown in Kaštel Stari) and finally MA1 is from Mastrinka (olives grown in Kaštela).

oil (Table 5). Lignan derivates were measured in largest content in Drobnica oil (248.78 mg kg^{-1} of oil) followed by Levantinka and Lastovka oils. Oil from Lastovka had the largest proportion of flavones (114.47 mg kg^{-1} of oil).

Antioxidant activity (ABTS, DPPH) and antioxidant capacity determined electrochemically (AOP) were investigated on the phenolic extracts of varietal olive oils. The values for the AOP were in a strict interval 14.0 to 24.6 QE0. Also the values for ABTS were concentrated in a strict interval, since 78% of the samples had ABTS values within 0.75 to 1.5. This confirms that the oil samples were fresh and well preserved.

There were statistically significant correlation coefficients of parameters DPPH and ABTS with simple phenols ($r = 0.32$, $p < 0.05$ and $r = 0.39$, $p < 0.01$) (Table 6), indicating that between them there is a positive correlation of low magnitude, and at the lesser extent, the parameter values of DPPH and ABTS vary in the same direction as the value of simple phenols. The same relationship was found for ABTS and AOP and secoiridoid derivates, with correlation coefficients moderately high ($r = 0.65$, $p < 0.001$ and $r = 0.61$, $p < 0.001$), as in the case of connection of ABTS with lignans derivates ($r = 0.53$, $p < 001$) and AOP with flavones ($r = 0.58$, $p < 0.001$).

Spanish group of authors [14] detected new phenolic compounds pinoresinol and acetoxypinoresinol that were unknown. Carrasco-Pancorbo *et al.* [15] isolated the individual phenolic compounds in VOO and measured their antioxidant activity by DPPH method, oxidation stability test OSI accelerated decay and antioxidant capacity by the electrochemical method. Results showed the strongest antioxidant activity of hydroxytyrosol, deacetoxy oleuropein aglycone and oleuropein aglycone. These compounds had the highest OSI values (the highest oxidative stability), while others showed a pro-oxidant effect of phenols ((+)-pinoresinol > tyrosol > ligstroside aglycon > (+)-1- acetoxypinoresinol> elenolic acid).

Cerretani *et al.* [16] studied the influence of the phenolic composition on the sensory properties and the oxidative stability in the Italian varietal oils obtained by different ways of processing. The considerations were carried out in relation to the group of phenolic compounds and low correlation was observed between the OSI index and total phenols ($r = 0.56$, $p < 0.05$), but a positive correlation was detected between the OSI index and the simple phenols (hydroxytyrosol and tyrosol) ($r = 0.90$, $p < 0.001$).

Group of Spanish scientists [38] comparatively used four different methods of measuring antioxidant capacity of Spanish varietal olive oils in relation to the content of total phenols. At the end of research the ABTS radical method showed high reproducibility and acceptable coefficients of variation and it is featured as the most acceptable method. In another paper [37] the properties of Italian varietal oils and influence of the fruit maturity degree on the oxidative stability of the oil were investigated. DPPH method was listed as most suitable for the determination of antioxidant activity. There was a positive correlation between OSI values and group of secoiridoid derivates ($r^2 = 0.95$). Some authors [29] even claim that the antioxidant activity determined by DPPH method can be used as a parameter to distinguish extra virgin olive oil. Gorinstein *et al.* [21] stated that method with β-carotene was the most favorable (correlation with total phenols $r^2 = 0.9958$).

In this study the influence of the phenolic composition on oxidative stability of Croatian varietal VOOs from Dalmatia region shows low correlation with another phenols except simple phenols

Table 5. Descriptive statistics for groups of phenols (mg kg^{-1}) (2006/07 and 2007/08).

		M	SD	95% CI		Min	Max
Simple phenols	Oblica	67.91	30.06	54.92	80.91	34.10	142.09
	Lastovka	165.76	58.69	128.48	203.05	102.25	282.60
	Levantinka	58.15	20.76	44.97	71.34	30.87	85.16
	Drobnica	172.28	58.79	136.75	207.80	36.11	285.45
	Mastrinka	98.46	11.35	80.39	116.52	88.43	110.45
	Total	107.54	64.89	91.33	123.75	30.87	285.45
Secoiridoids derivatives	Oblica	238.26	175.39	162.41	314.10	45.01	647.36
	Lastovka	442.03	222.61	300.59	583.48	229.29	873.60
	Levantinka	225.42	105.25	158.55	292.29	51.98	365.41
	Drobnica	224.64	50.29	190.86	258.43	91.58	267.57
	Mastrinka	320.11	22.55	284.22	355.99	296.80	349.04
	Total	278.08	171.84	234.44	321.72	45.01	873.60
Lignan derivatives	Oblica	191.93	65.63	163.55	220.31	103.41	321.72
	Lastovka	201.65	43.32	174.12	229.17	166.46	282.76
	Levantinka	202.58	35.98	179.72	225.44	153.88	285.42
	Drobnica	248.78	65.38	209.27	288.29	38.53	292.58
	Mastrinka	184.05	19.72	152.67	215.43	164.56	203.85
	Total	206.80	58.11	192.29	221.32	38.53	321.72
Flavones	Oblica	72.45	27.55	60.54	84.37	45.01	152.77
	Lastovka	114.47	22.71	100.04	128.90	78.81	140.49
	Levantinka	64.89	15.40	55.10	74.67	38.67	99.30
	Drobnica	66.41	16.59	56.38	76.43	30.04	99.30
	Mastrinka	64.89	5.77	55.71	74.07	60.16	73.16
	Total	77.21	28.04	70.21	84.21	30.04	152.77

Abbreviations: Simple phenols (hydroxytyrosol and tyrosol), Secoiridoids derivates (dialdehydic form of deacetoxy-oleuropein aglycon, oleuropein aglycon and ligstroside aglycon), Lignan derivates ((+)-pinoresinol and (+)-1-acetoxypinoresinol), Flavones (luteolin, apigenine); M - mean, SD - standard deviation, 95% CI - 95-percent confidence interval of the mean estimate of the population.

Table 6. Correlations of DPPH, ABTS, AOP and OSI by phenol groups.

		Simple phenols	Secoiridoids derivatives	Lignan derivatives	Flavones
DPPH	r	0.32	-0.08	0.22	-0.16
	P	**0.010**	0.552	0.082	0.215
	n	64	62	64	64
ABTS	r	0.39	0.65	0.53	0.16
	P	**0.002**	**<0.001**	**<0.001**	0.194
	n	64	62	64	64
AOP	r	0.20	0.61	0.24	0.58
	P	0.266	**<0.001**	0.181	**0.001**
	n	32	31	32	32
OSI (h)	r	-0.18	-0.19	0.13	-0.38
	P	0.163	0.135	0.322	**0.002**
	N	64	62	64	64

Abbreviations: r - coefficient Pearson correlation, P - statistical significance, and probability of type I error (α), n - number of samples.

($r = 0.32$, $p = 0.01$) if we use DPPH method (Table 6). When ABTS method was used, a positive correlation with simple phenols ($r = 0.39$, $p = 0.002$) was found, as well as with group of secoiridoid derivatives ($r = 0.65$, $p < 0.001$) and lignan derivates ($r = 53$, $p < 0.001$). Considering AOP, the larger influence has secoiridoids composition ($r = 0.61$, $p < 0.001$) than flavones ($r = 0.58$, $p = 0.001$).

Conclusions

All results presented in this paper proof that phenolic compounds in general have significative influence on VOO stability and antioxidant properties. Different phenolic groups have demonstrated their responsibility for antioxidant properties, depending also on the method applied. The values of antioxidant activity (DPPH, ABTS) and antioxidant capacity (AOP) showed statistically significant differences among varieties, which means that the observed varieties differ in their population of Central Dalmatia by these indicators. A significant positive correlation between total phenolics and ABTS and DPPH values was noticed. Certain groups of phenolic compounds (secoiridoides, flavones, and lignanes) showed a higher antioxidant activity and antioxidant capacity than the total phenols. Observing the results obtained in this study as significant association between content of each phenolic groups and antioxidant activity, demonstrated with various methods it is neccesary to continue with deeper research on varietal Croatian oils. These methods should be used for characterization of specific properties of certain indigenous varieties of olive oil in the region of Dalmatia.

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