PRELIMINARY CHARACTERISATION OF MONOVARIETAL EXTRA-VIRGIN OLIVE OILS OBTAINED FROM DIFFERENT CULTIVARS IN CROATIA

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Abstract

Chemical characterisation was carried out on 22 virgin olive oil samples obtained from 5 olive cultivars (Oblica, Lastovka, Levantinka, Drobnica, Mastrinka cvs.). The olives, that came from different groves in Croatia, were processed by centrifugation system in two phases. Several qualitative parameters were evaluated (free acidity, peroxide value and UV spectrophotometric indices) and analyses of major (fatty acids) and minor components (phenolic fraction and their antioxidant power evaluated by $ABTS^{\bullet+}$ and electrochemical test) was also carried out. The results for all samples have shown belonging to category of extra virgin olive oil, depending on analysed analytical parameters. Moreover, the oils had very good storage capacity, depending on fatty acid composition, and also very good phenols content. Particularly, among the most representative varieties, the tendency to higher antioxidant activity had oils produced from Lastovka variety, followed by oils from Oblica and Levantinka.

Riassunto

In questo lavoro è stato effettuato uno studio di caratterizzazione di oli vergini di oliva monovarietali prodotti da 5 cultivars (Oblica, Lastovka, Levantinka, Drobnica, Mastrinka cvs.) di olive allevate in Croazia e rappresentate

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da 22 campioni. Le olive provenivano da differenti areali di produzione della Croazia, e sono state trasformate nelle condizioni reali in diversi frantoi industriali mediante il sistema continuo con decanter a 2 fasi. Per i diversi campioni sono stati valutati alcuni parametri qualitativi (acidità libera, numero di perossidi e indici spettrofotometrici UV) ed è stata svolta l'analisi della componente maggioritaria (composizione in acidi grassi) e minoritaria (frazione fenolica analizzata in funzione del potere antiossidante valutato tramite i test $ABTS^{\bullet+}$ ed elettrochimico). I risultati hanno mostrato per tutti i campioni l'appartenenza alla categoria extravergine, relativamente ai parametri analitici considerati. Gli oli hanno mostrato inoltre una buona conservabilità dipendente dalla composizione in acidi grassi e da una buona dotazione fenolica. In particolare tra le varietà più rappresentate, hanno mostrato una tendenza alla maggiore attività antiossidante gli oli prodotti dalla varietà Lastovka seguiti da Oblica e Levantinka.

Keywords: virgin olive oil; olive cultivar; phenols; fatty acid composition; minor components.

Introduction

The marketing of extra virgin olive oils is increasingly directed towards the differentiation and characterisation of products from different geographical areas, as has already happened in the oenological field. Now days 96 extra virgin olive oils have received the European Protected Origin Denomination (POD) mark, as a result of their organoleptic characteristics and particular chemical composition. Denomination of origin is however closely related to the varietal composition of oils, since their recognized uniqueness is the final result of climatic conditions, agricultural practices and olive varietal characteristics. Varietal characterisation has been carried out by analyzing several minor compounds (1), which are thought to depend on agronomical (1-7), varietal (1-7) and technological conditions (1, 7-10) and differentiate the extra virgin olive oils from other edible vegetable oils.

Production of monovarietal olive oils has increased during the last few years, due to their favourable chemical and sensorial characteristics (1-7, 11).

Olive growing and olive oil production in Croatia have a long tradition, along Adriatic coast and on the Croatian islands (Figure 1).
Nowadays there are about 27000 ha of olive growing area. There are about

6 million olive trees in Croatia and most of them are autochthonous olive varieties. About 75% of all olive trees in Croatia are Oblica variety, that can be used both for table olives and olive oil production. Predominant autochtonous olive varieties that are present in the middle Dalmatia region are Oblica, Levantinka and Lastovka, mostly grown on rocky soils, difficult for mechanical cultivation. Their main use is for olive oil production. There are more than 120 olive mills in Croatia, most of them equipped with modern centrifugal sistems with two or three phases. Small number of oil mills use traditional processing system with hydraulic pressure. Average annual olive oil production is about 4-5 million liters. Autochtonous monovarietal olive oils are poorly investigated in Croatia, particulary dalmatian oils (12-14). Considering the description above, we carried out a preliminary characterisation of virgin oils produced in Croatia obtained from native olive cultivars.

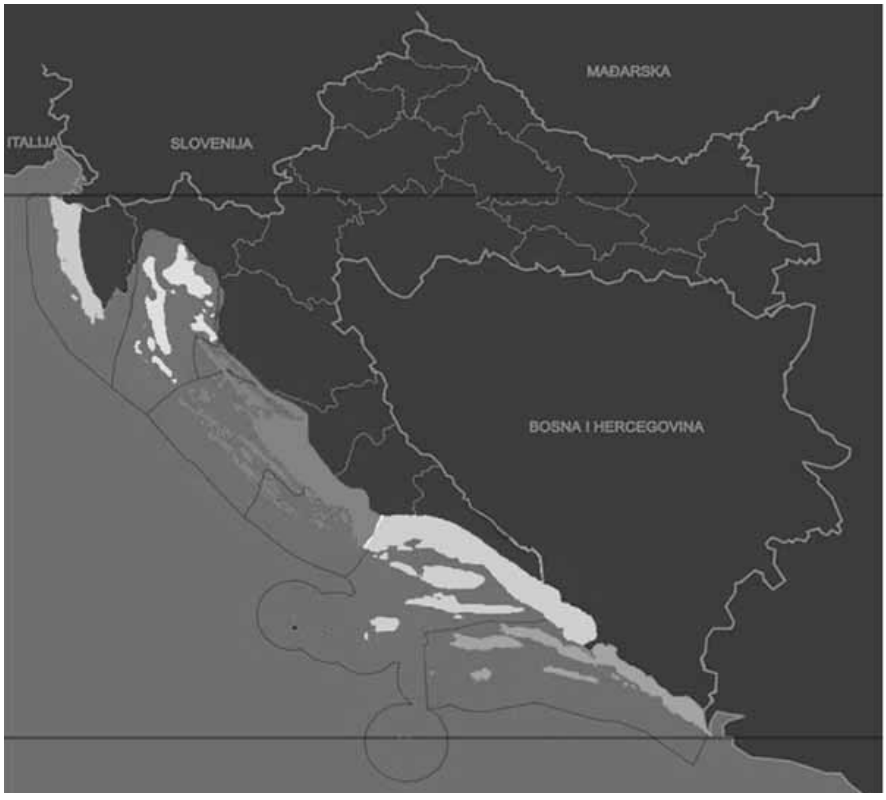

Fig. 1 - Olive growing Croatian sub-regions.

Investigation was carried out on virgin olive oils from varieties: Oblica, Levantinka, Lastovka, Drobnica and Mastrinka. Oblica is the most represented cultivar in Croatia. It is spread in all olive growing Croatian sub regions. It is very tolerant to low temperatures and to drought; it can be used for a double purpose (both for oil production and conservation as table olive). Fruit size is middle to big, with very high oil content (up to 21%). It highly inclines to alternation bearing (15). Levantinka is cultivated in the Middle and South Dalmatia, particularly on island of Šolta. It is used only for oil production. Oil content in the fruit is about 19%. This variety is very regular in bearing and has high percentage of auto-fertility. It needs deep and fertile soils. Levantinka has good susceptibility to drought and low temperatures (15). Lastovka is cultivated mainly in South and Middle Dalmatia, particularly on island of Korcula. It is used for oil production because of its high oil content (about 23%) in the fruit. Lastovka is highly sensitive to olive knot, but very susceptible to low temperatures. Drobnica is the olive variety mainly represented in old olive orchards. It has a small fruit, with good oil content (about 20%). It has good and regular bearing (15). Mastrinka is very old autochthonous olive variety in Croatia, mostly spread in south Dalmatia region. It's very similar to wild olive tree. Fruit is quite small with modest oil content. It is very useful as a good rootstock and has high fertilisation capacity. It has very high and constant bearing. There is a big olive tree of this cultivar near Split (Kaštela area) that is protected as a monument of nature by UNESCO because it is about 1500 years old.

Experimental

Virgin olive oil samples

Table 1 reports the list of samples, their growing area and location of technological system used for VOO production. All samples have been produced by continuous technological plant provided by centrifugal system with two phases. All chemical analyses were carried out contemporarily; samples were stored in dark bottles without headspace at room temperature.

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, diammo-

nium salt) was purchased from Sigma-Aldrich. All solvents used were analytical grade (Merck & Co. Inc., Darmstadt, Germany).

Analytical indices

Free acidity, peroxide value, and ultraviolet spectrophotometric indices (K232, K270, K) were evaluated according to the official methods described in the EEC Regulation n. 2568/91 (16) of the Commission of the European Union. All parameters were determined in triplicate for each sample.

Liquid-liquid extraction of phenolic compounds from olive oils

TABLE 1

SAMPLES DESCRIPTION

Sample code	**Cultivar**	Harvest date	growing area	Company name and location of olive oil mills
OB1	OBLICA	23/10/2006	BRAÈ	ARNERIÆ - BRAÈ
OB2	OBLICA	26/10/2006	KAŠTELA	NOVAK - KAŠTELA
OB3	OBLICA	28/10/2006	ŠOLTA	OLYNTHIA - ŠOLTA
OB4	OBLICA	22/10/2006	BRAÈ	SELÈANKA - BRAÈ
OB5	OBLICA	15/12/2006	BRAÈ	SELÈANKA - BRAÈ
OB6	OBLICA	03/11/2006	MURTER	RAOL -MURTER
OB7	OBLICA	30/10/2006	HVAR	BO□IÆ - HVAR
OB8	OBLICA	28/11/2006	HVAR	BO□IÆ - HVAR
OB9	OBLICA	07/11/2006	HVAR	ZASTRA□IŠÆE - HVAR
OB10	OBLICA	18/11/2006	HVAR	ZASTRA□IŠÆE -HVAR
OB11	OBLICA	17/112006	MURTER	RAOL - MURTER
OB12	OBLICA	20/11/2006	HVAR	BO□IÆ - HVAR
LA1	LASTOVKA	29/11/2006	KORÈULA	BLATO - KORÈULA
LA2	LASTOVKA	03/11/2006	KORÈULA	BLATO - KORÈULA
LA3	LASTOVKA	01/12/2006	KORÈULA	BLATO - KORÈULA
LA4	LASTOVKA	26/10/2006	HVAR	ZASTRA□IŠÆE - HVAR
LA5	LASTOVKA	28/10/2006	HVAR	ZASTRA□IŠÆE - HVAR
LE1	LEVANTINKA	03/11/2006	ŠOLTA	OLYNTHIA - ŠOLTA
LE2	LEVANTINKA	16/11/2006	HVAR	ZASTRA□IŠÆE - HVAR
LE3	LEVANTINKA	2005	ŠOLTA	OLYNTHIA - ŠOLTA
DR1	DROBNICA	18/11/2006	HVAR	ZASTRA□IŠÆE - HVAR
MA1	MASTRINKA	29/11/2006	KAŠTELA	NOVAK - KAŠTELA

Extraction was performed following the protocol described by Pirisi et al. (17), modified according to Rotondi et al. (4). Unless otherwise stated, extractions were performed in three replicates (n=3). Extracts were stored at -18°C before use.

Radical scavenging activity of phenolic extracts by ABTS$^{\bullet+}$ essay

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 spectrophometer, Kyoto, Japan).

Next, 1 mL of this 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) (18).

Antioxidant power (AOP) evaluation using a FIA-amperometric method.

An amperometric flow injection method was used for the evaluation of the antioxidant power of the phenolic extracts of the samples.

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.

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 0.1-25.0 µM. Samples were appropriately diluted to obtain current signals within the linear range of the applicable standard molecule.

Fatty acid methyl ester analysis by CGC

Fatty acid methyl esters (FAME) from the oil samples were obtained by alkaline treatment with 1M KOH in methanol (19). Gas chromatographic analyses were carried out according to Cerretani et al. (8). The average was calculated by four replications for each sample.

Results and Discussion

Chemical qualitative parameters found in olive oil samples

Table 1 shows data about harvesting date for different oils. It is evident that all olive fruits were harvested in the same time during maturation period of 40 days, from 22 October to 1 December 2006, with exception of sample LE3 which is produced in 2005. The same table shows that 12 monovarietal oils were produced from variety Oblica, 5 oils derived from Latovka, 3 oil samples from Levantinka, one oil sample from Drobnica and one from Mastrinka.

This oil sample selection is due to the fact that Oblica is the most spread autochthonous olive variety in costal part of Croatia, and with Lastovka and Levantinka is the most known and most planted domestic variety in middle and south Dalmatia olive orchards that have been chosen for this investigation. Last two varieties Drobnica and Mastrinka are included in this study as referent samples because of their particular characteristics and good oil quality. Without doubt, those two varieties will be of great interest in future works. As reported in Table 2, free acidity in all 22 olive oil samples always had values inside the range of the extra virgin olive oil category, according to European regulation (20). Particularly, it is possible to confirm that the lowest free acidity value was obtained for Oblica and Levantinka oil samples, with average of 0.2% for both varieties. Slightly higher values for free acidity were detected in Lastovka oil samples with average of 0.4%. The peroxide value for all analysed samples was inside the limits for extra virgin olive oils, according to European regulation (20). Although sample L3, produced in the last year, had a peroxide value (14.6 meqO_2/kg of oil) higher than the average (6.7 meqO_2/kg of oil), it was inside the limit provided for by the European regulation (20 meqO_2/kg of oil).

All results for K232, K270 and ΔK were widely inside the limits set by European regulation (20) for extra virgin olive oil. This is a proof of proper storage conditions which guarantees for the freshness of oil.

TABLE 2

CHEMICAL AND QUALITATIVE PARAMETERS FOUND IN MONOVARIETAL EXTRA VIRGIN OLIVE OIL SAMPLES

Samples	Free acidity (%)	POV ($meqO_2/kg$)	K232	K270	ΔK
OB1	0.18	4.9	1.85	0.17	0.005
OB2	0.18	3.0	1.64	0.14	0.005
OB3	0.20	4.5	1.56	0.13	0.000
OB4	0.16	7.4	1.65	0.15	-0.004
OB5	0.25	10.9	1.63	0.16	0.0005
OB6	0.37	7.9	1.72	0.13	-0.002
OB7	0.13	5.8	1.76	0.12	0.001
OB8	0.16	7.7	1.90	0.12	-0.002
OB9	0.40	6.0	1.38	0.13	-0.001
OB10	0.17	8.6	1.73	0.16	0.001
OB11	0.22	8.1	1.65	0.11	-0.002
OB12	0.13	3.8	1.28	0.13	0.005
LA1	0.43	6.6	1.76	0.16	0.0025
LA2	0.52	4.7	1.85	0.18	0.005
LA3	0.52	5.3	1.75	0.13	0.004
LA4	0.18	6.1	1.76	0.12	0.005
LA5	0.21	6.3	1.88	0.15	0.0025
LE1	0.14	4.3	1.58	0.13	0.005
LE2	0.25	6.0	1.67	0.15	-0.001
LE3	0.20	14.6	1.81	0.15	-0.014
DR1	0.18	6.4	1.56	0.15	0.0025
MA1	0.58	8.4	1.73	0.12	0.005

POV, peroxide values; K232, K270 and ΔK, UV spectrophotometric indices.

Fatty acid composition of the studied olive oils

The fatty acid composition of the analyzed oils obtained from different cultivars showed some differences between oils derived from different varieties (Table 3). All cultivars show a stable index expressed by the ratio of oleic acid to linoleic acid (C18:1/C18:2) with values near or higher than 7, with an exception for variety Mastrinka that had value equal to 5,

TABLE 3

FATTY ACID COMPOSITION (%) OF MONOVARIETAL EXTRA VIRGIN OLIVE OIL SAMPLES.

	C 16:0	C 16:1	C 17:0	C 17:1	C 18:0	C18:1	C 18:2	C 18:3	C 20:0	C 20:1	C 20:3	C 22:0	C 24:0	SFA %	MUFA %	PUFA %	C18:1
OB1	12.43	0.79	0.05	0.07	2.32	71.10	11.29	0.74	0.53	0.37	n.d.	0.19	0.11	15.6	72.3	12.0	6.3
OB2	12.87	0.74	0.05	0.07	2.39	71.62	10.26	0.72	0.55	0.39	0.02	0.20	0.12	16.2	72.8	11.0	7.0
OB3	12.37	0.75	0.05	0.08	2.44	71.78	10.68	0.66	0.54	0.37	n.d.	0.19	0.10	15.7	73.0	11.3	6.7
OB4	10.10	0.60	0.03	0.05	1.84	76.58	9.35	0.53	0.41	0.27	n.d.	0.14	0.08	12.6	77.5	9.9	8.2
OB5	12.00	0.69	0.05	0.07	3.07	70.51	11.83	0.61	0.53	0.38	n.d.	0.19	0.07	15.9	71.7	12.4	6.0
OB6	11.53	0.65	0.04	0.07	3.26	72.03	10.66	0.62	0.53	0.35	n.d.	0.17	0.09	15.6	73.1	11.3	6.8
OB7	12.00	0.74	0.05	0.07	2.44	71.49	11.30	0.69	0.54	0.39	n.d.	0.19	0.10	15.3	72.7	12.0	6.3
OB8	12.02	0.69	0.05	0.07	2.44	72.58	10.21	0.67	0.56	0.40	n.d.	0.20	0.11	15.4	73.7	10.9	7.1
OB9	11.48	0.55	0.03	0.07	1.78	71.55	12.71	0.67	0.54	0.37	n.d.	0.17	0.08	14.1	72.5	13.4	5.6
OB10	11.94	0.70	0.05	0.07	2.39	71.68	11.16	0.71	0.56	0.41	0.06	0.14	0.12	15.2	72.9	11.9	6.4
OB11	11.38	0.64	0.05	0.08	2.61	72.54	10.84	0.71	0.51	0.38	n.d.	0.18	0.10	14.8	73.6	11.5	6.7
OB12	10.47	0.67	0.07	0.11	2.58	76.24	7.99	0.65	0.55	0.40	n.d.	0.18	0.10	14.0	77.4	8.6	9.5
LA1	10.63	0.45	0.09	0.20	2.61	74.91	8.94	0.70	0.62	0.43	0.02	0.24	0.16	14.3	76.0	9.7	8.4
LA2	11.85	0.63	0.13	0.18	2.82	71.55	10.85	0.66	0.62	0.37	0.02	0.19	0.14	15.7	72.7	11.5	6.6
LA3	11.19	0.55	0.11	0.17	2.57	73.22	10.13	0.67	0.61	0.40	n.d.	0.21	0.15	14.9	74.3	10.8	7.2
LA4	12.28	0.68	0.12	0.17	2.65	71.03	11.19	0.64	0.57	0.36	n.d.	0.19	0.12	15.9	72.2	11.8	6.3
LA5	12.94	0.64	0.12	0.16	2.93	70.39	11.03	0.51	0.61	0.34	n.d.	0.19	0.13	16.9	71.5	11.5	6.4
LE1	11.60	0.72	0.05	0.07	2.90	73.95	8.89	0.63	0.57	0.34	n.d.	0.18	0.10	15.4	75.1	9.5	8.3
LE2	11.77	0.85	0.05	0.09	2.68	73.38	9.34	0.65	0.55	0.36	n.d.	0.19	0.10	15.3	74.7	10.0	7.9
LE3	11.54	0.75	0.05	0.08	3.65	74.53	7.48	0.66	0.61	0.36	n.d.	0.20	0.10	16.2	75.7	8.1	10.0
DR1	11.67	0.67	0.06	0.08	3.03	74.85	7.64	0.67	0.55	0.46	n.d.	0.22	0.12	15.6	76.0	8.3	9.8
MA1	11.16	0.76	0.04	0.07	2.12	70.23	13.93	0.64	0.45	0.37	n.d.	0.14	0.08	14.0	71.4	14.6	5.0

n.d. = not detected

due to a particularly high content of linoleic acid (13.9%). However, the last mentioned variety cannot be considerate as a representative sample because only sample of this cultivar was available. This aspect should be studied in future investigations. Further, in all oil samples a satisfactory content of palmitic acid (C16:0) has been detected, with media of 11.7%. The average for the amount of palmitoleic acid (C16:1) had the highest value in Levantinka oils (0.77%) and the lowest for Lastovka oils (0.59%). Very similar composition of some fatty acids was found in varieties Oblica and Lastovka, particularly, the amount of stearic acid (C18:0, 2.5 and 2.8% respectively), oleic acid (C18:1, 72.5 and 72.2% respectively), linoleic acid (C18:2, 10.7 and 10.4% respectively) and linolenic acid (C18:3, 0.7 and 0.6% respectively). All analyzed oil samples had content of oleic acid (C18:1) higher than 70%.

The highest level of oleic acid was in Oblica oil sample (76.6%), while the lowest value was found in Mastrinka oil. The amount of linoleic acid (C18:2) in Levantinka oils was a bit lower (8.6%) than in other samples. The level of C20:0 acid has been found to be comparable in three varieties (Oblica, Lastovka, Drobnica) with concentrations of 0.50%, 0.58% and 0.55% respectively. Polyunsaturated fatty acids had quite high content in olive oils from Oblica and Lastovka (11.4 and 11.1% respectively); while in Levantinka oils that value was lower (9.21%).

Phenolic fraction analyzed by ABTS and Electrochemical AOP

The antiradical power (ABTS) and electrochemical AOP were evaluated for the phenolic extracts of the olive oil samples (Table 4). The AOP values ranged in a strict interval (min=14.0, max=24.6 $QE_{0)}$. These can be ascribed to the fact that the oil samples were fresh and well preserved, in fact, the ABTS values were also concentrated in a strict interval (78% of samples showed an ABTS value in the range 0.75-1.5). By grouping the data in four groups, on the basis of the ABTS values (ABTS<0.75, 0.75<ABTS<1.0, 1.00<ABTS<1.5, ABTS>1.5) a correlation with the AOP value was observed (Figure 2).

An interesting correlation was observed when the samples where grouped according to the cultivar. The mean ABTS and AOP values of Oblica, Levantinka and Lastovka samples (the most represented) where calculated showing a strict linear correlation (r^2=0.995) showed in Figure 3.

These data confirm the possibility to evaluate the antioxidant activity of the phenolic extracts of olive oil with the simple electrochemical assay, reducing costs and time for analysis.

In conclusion the oils subject of this investigation showed interesting qualitative characteristics that underline their belonging to the extra-virgin category, even after six months from their production. All the oil

TABLE 4

ANTIOXIDANT ACTIVITY (EVALUATED BY ELECTROCHEMICAL AND ANTIRADICAL ASSAYS) OF MINOR POLAR COMPOUND OF MONOVARIETAL EXTRA VIRGIN OLIVE OIL SAMPLES.

Samples	AOP	$ABTS^{\bullet+}$
OB1	18.73	1.27±0.16
OB2	14.52	1.07±0.14
OB3	15.21	1.02±0.16
OB4	19.74	1.74±0.20
OB5	19.94	0.73±0.07
OB6	15.21	1.17±0.05
OB7	18.96	1.16±0.09
OB8	18.54	0.90±0.27
OB9	14.32	1.32±0.08
OB10	14.91	1.05±0.12
OB11	14.67	0.91±0.06
OB12	24.62	1.25±0.17
LA1	16.68	1.05±0.08
LA2	22.67	1.29±0.16
LA3	18.96	0.65±0.07
LA4	18.56	1.20±0.10
LA5	22.76	1.76±0.19
LE1	14.65	0.83±0.06
LE2	14.00	1.09±0.16
LE3	14.12	0.43±0.01
DR1	16.04	1.15±0.03
MA1	19.84	0.78±0.09

AOP, antioxidant power expressed as QE_0 quercetin equivalent with potential set to 0 mV (μg quercetin mL-1 phenolic extract); $ABTS^{\bullet+}$, mmol of Trolox kg-1 of oil.

samples showed a fatty acid composition that promotes oxidative stability, helped by the amount of phenolic compounds. The phenolic fraction had higher average value for oils after 6 months storage at room temperature, if compared with the amounts found in other works (21).

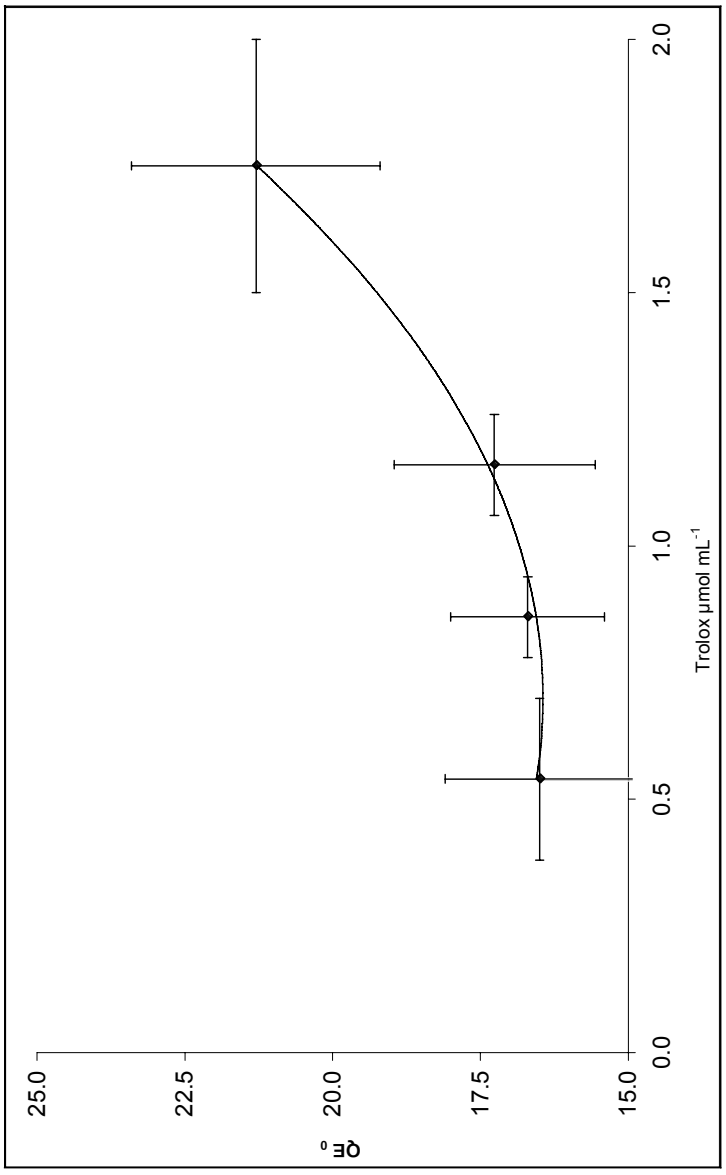

Fig. 2 - Correlation between ABTS values and QE0 values.

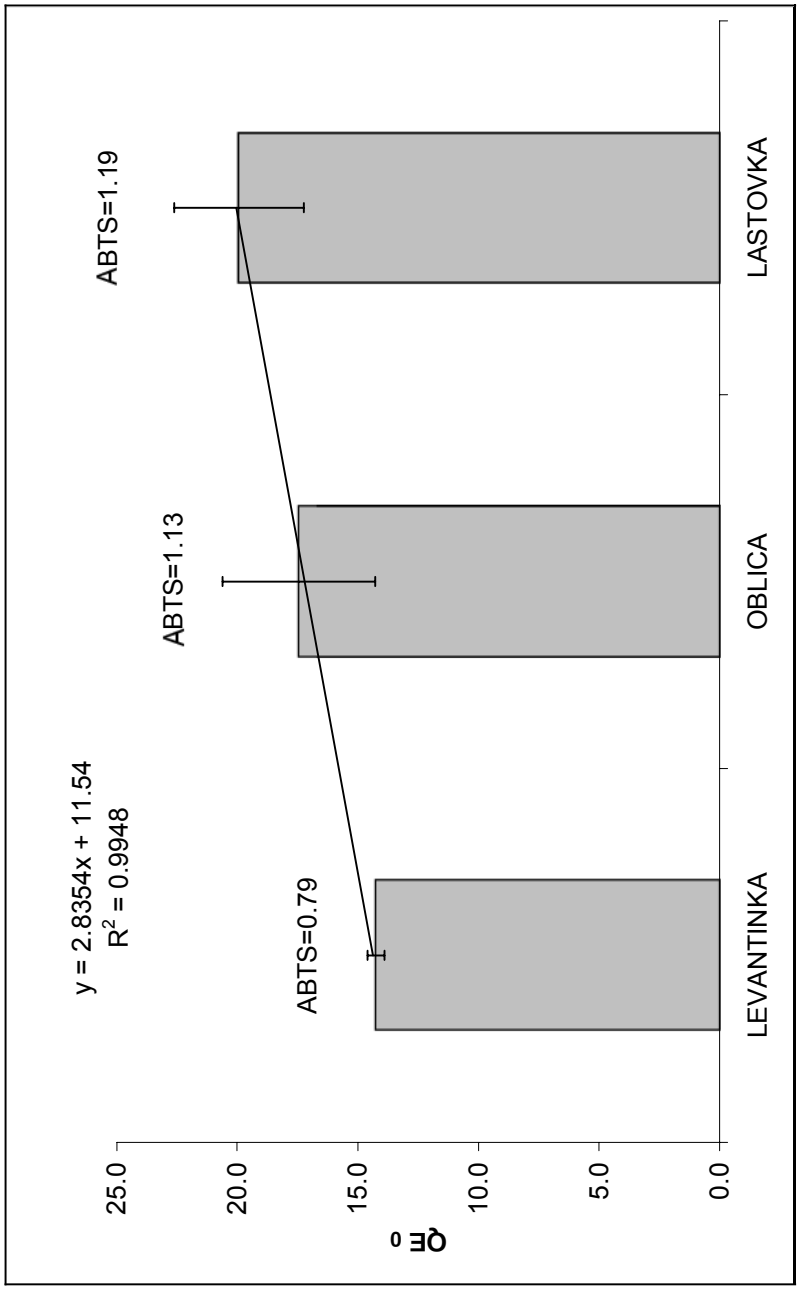

Fig. 3 - Cultivar dependence of the ABST and AOP values.

Regarding sensorial evaluation of virgin olive oils from varieties included in this investigation, from existing results it's possible to confirm that Oblica gives 'sweet' oil, with fruity attribute that reminds on mature olive, little pungent and almost without bitter attribute. Oils from Levantinka has stronger fruity sensation, that depends of ripening stage of olive, with stronger pungent attribute than Oblica oil and also existing bitterness. Lastovka gives oils with rather strong bitter attribute an also quiet pungent, with fruity flavor that reminds on olive leaf. Drobnica ad Mastrinka oils will be included with other oils in future sensorial evaluations.

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