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Research Note

Composition and qualitative characteristics of virgin olive oils produced in northern Adriatic region, Republic of Croatia

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RESUMEN

Composición y parámetros de calidad de aceites de oliva vírgenes producidos en el norte de la región Adriática, República de Croacia.

Istria y la isla Krk están localizadas en el norte de la región Adriática, República de Croacia. La mayoría de los aceites producidos en las islas de esta región corresponden a la clasificación extra virgen de las variedades (Debela, Naska, Rosulja, Slatka, Buza, Carbonera, Bianchera, Leccino). La caracterización de estos aceites es poco conocida.

El objetivo de este trabajo fue la caracterización de los aceites de oliva vírgenes durante las campañas 1997/98, 1998/99 y seis meses de 1999/2000. Para todas las muestras se determinó la acidez, el índice de peróxido y las constantes de absorción en el UV. Para los aceites de oliva vírgenes durante la campaña 1997/98 se determinaron la composición en ácidos grasos, los contenidos en esteroles y alcoholes alifáticos, los ácidos grasos saturados en posición 2 de los triglicéridos y el contenido en trilinoleína. Los análisis químicos se completaron con la determinación del contenido en polifenoles expresado como ácido cafeico, y la determinación de escualeno y α-tocoferol.

PALABRAS-CLAVE: Aceite de oliva virgen– Composición química – República de Croacia.

SUMMARY

Composition and qualitative characteristics of virgin olive oils produced in northern Adriatic region, Republic of Croatia.

Istria and Island Krk are located in the Northern Adriatic region, Republic of Croatia. The majority of oils produced on the islands of this Region correspond to extra virgin classification as a consequence of olive cultivars (Debela, Naska, Rosulja, Slatka, Buza, Carbonera, Bianchera, Leccino). The characterisation of these oils is little known.

The objective of this work was the characterisation of virgin olive oils during the 1997/98, 1998/99 and six months of 1999/2000 harvest. Acidity, peroxide value and UV absorption constants were determined for all samples. Fatty acid composition, sterol and aliphatic alcohol contents, saturated fatty acids in the 2-position of the triglyceride and trilinolein content were determined for the virgin olive oils during 1997/98 harvest. The chemical analyses were supported by the determination of polyphenol content expressed as caffeic acid, squalene and α-tocopherol content.

KEY-WORDS: Chemical composition – Republic of Croatia – Virgin olive oil.

1. INTRODUCTION

Croatia is a country, which consists of diverse climatic and cultural geographic region. The production and consumption of olive oil are traditional in the Mediterranean region. Mediterranean characteristics are noticeable on the islands, peninsula Istria and on the Adriatic coast.

Olive oil is an important commodity in the daily diet of the Mediterranean people and a main ingredient of the famous Mediterranean diet.

Olive oil characteristics depend on generic, agronomic and environmental factors (Cimato, 1990; Mousa *et al*., 1996) as well as on the quality and the physiological condition of the olives from which it is extracted (Garcia *et al*., 1996). Some cultivars have been developed in various ways, including the correlation between chemical and physical parameters in order to identify the optimum period for harvesting (Finotti et al, 2001). In the Republic of Croatia, the major cultivars used for oil production include Buga, Bianchera, Lastovka, Drobnica, Rosulja, Zutica, Uljarica, Slivnjaca, Puljka, Plominka, Ostrika, Oblica, Levantica, Kosmaca, Grozdaca, Duzica and Crnica. Some of these cultivars are grown in the Northern Adriatic region of the Republic of Croatia (Buga, Bianchera, Drobnica, Rosulja, Plominka, Oblica and Crnica). The same name may be given to similar cultivars and different names may be used for identical ones (i.e. Buza is a synonym for Buga, Belica is a synonym for Bianchera, Naska is a synonym for Drobnica, Slatka is a synonym for Plominka, Debela is a synonym for Oblica, etc.). Since 1970s, some cultivars such as Leccino were introduced, especially in Istria.

The existing olive fruit processing capacity satisfies the current need (9000 t olive fruit per day). Total production processing olive fruit period range is 20-40 days.

Olive oil production varied during last five years (Table I) as the result of insufficient olive cultivation (no irrigation, insufficient fertilization, etc.).

Table I
Production, import, export and consumption of olive oil in the Republic of Croatia for the years 1995 to 1999

Year	Olive oil (hl)			
	Production	Import	Export	Consumption
1995	54470	3389	378	57481
1996	22927	189	1200	21916
1997	15717	10711	1722	24706
1998	31721	12078	2800	40999
1999	52849	7822	2400	58271

Source: Statistical yearbook 2000, Central Burean of Statistics Republic of Croatia.

The major olive oil production is covered by private family farms, which produce olive oil for their own consumption and the surplus is placed on the market.

Lately, the consumption of olive oil has increased in traditional and in other regions of the Republic of Croatia, which resulted from a the higher quality of olive oil and the education of population.

The European Community (EC) has promulgated a regulation to set the characteristics of olive oil and necessary analytical methods (Regulation 2568/91, July, 1991). In recent years, this regulation has been updated and widened to take into account new knowledge on olive oils and to vary the concentration limits for many components to be analyzed. Two years ago, the Republic of Croatia promulgated National Regulation for determining characteristics of olive oils and pomace oils, which is identical to the European Community (EC) Regulations 2568/91 and 656/95, but majority of laboratories in Croatia are not trained to perform the chemical analyses in accordance with the above-mentioned Regulation (sterol and aliphatic alcohol contents, determination of saturated fatty acids in the 2-position of the triglyceride, determination of composition of trilinolein content, etc.). Some analytical procedures were modified.

Since the above-mentioned regulation is volunary, a small number of olive oil samples was characterized in laboratories in the Republic of Croatia.

The purpose of this work is to acquaint readers of the journal with the new results of chemical analysis of the olive oils from the Northern Adriatic region of the Republic of Croatia which were poorly known.

Previous studies (Procida *et al.*, 1994; Procida *et al.*, 1995) have reported on the chemical characterization of the typical olive oils from the Island Krk and Istria.

2. EXPERIMENTAL PART

The samples of virgin olive oils produced in the Croatian Northern Adriatic region in different years were analyzed according to the official methods (Regulation EEC No. 2568/91).

Olive oil samples were obtained from olive oil produced in Kvarner islands from a mixture of various olive cultivars (Debela, Naska, Rosulja, Slatka, Plominka) and processed at an industrial level in an oil mill located in Punat, Island of Krk, Croatia, by using a centrifugation system in the three-phase mode.

Olive oil samples were obtained from olive oil produced in Istria from a mixture of various olive cultivars (Buga, Carbonera, Bianchera, Leccino) and processed at an industrial level in oil mills located in Vodnjan and Tar, Istria, Croatia, by using a centrifugation system in the three-phase mode. Before processing the olive fruit was not classified in terms of cultivar, method of harvesting and condition. All olive oil samples were collected from private family farms and analyzed as part of the Primorsko-Goranska County Program for Revitalisation of Olive Cultivation and Olive Oil Production.

The month of production (september and december), cultivars, type of milling plant and storage time and condition are known for each sample.

Quality parameters (acidity, peroxide value and UV absorption) were determined in all samples, 41 samples during the 1997/98, 45 samples during 1998/99 and in 59 samples during six months 1999/2000 harvest were determined. The composition of major samples (fatty acids, sterol and aliphatic alcohol contents, determination of saturated fatty acids in the 2-position of the triglyceride, determination of composition of trilinolein content) and polyphenol content expressed as caffeic acid, squalene and α-tocopherol content during the 1997/98 harvest were also determined.

All chemicals were obtained from Fluka (Buchs, Switzerland) in the adequate purity and standards from Sigma (St.Louis, MO, USA).

Aminopropyl solid-phase extraction column (SPE) were obtain from Varian (Harbor City, CA, USA).

2.1. Apparatus

UV-spectrophotometer Cary 1 (Varian, CA, USA) was used for the determination of UV absorbance constants and total polyphenols.

Gas chromatographic (GC) analyses were carried out using an Gas Chromatograph Autosystem XL (Perkin-Elmer, Norwalk, CT, USA) with a flame-ionization detector (FID). Chromatography software from Perkin-Elmer Nelson (Turbochrom 4, rev.4.1.) was used for data acquisition from the FID. Hydrogen was obtained using a Claind hydrogen generator.

The composition of triglyceride in olive oils in terms of their equivalent carbon number (ECN) was

determined using an High-performance liquid chromatography (HPLC) TSP Spectra System with P2000 gradient binary pump, SCM1000 vakuum membrane degasser, Rheodyne injector 7725i, loop 20 μl, refractive index detector Spectra System RI-150. Chromatography software from TSP (PC 1000) was used for data aquisition from RI detector.

2.2. Analytical methods

2.2.1. *Analyses of oil quality characteristics*

Titrable acidity, peroxide value (PV) and UV absorbance at 232 (K_{232}) and 270 nm (K_{270}) were determined according to EC Regulation 2568/91.

2.2.2. *Phenol analysis*

The total phenol content was determined according to the colorimetric method proposed by Swain and Hillis (Swain & Hillis, 1959) with the internal modification. 50 g of oil dissolved in hexane (50 ml) was extracted with methanol:water (60:40, v/v, 3 x 30 ml). Each extract was treated once with hexane (50 ml). Total extract, which contained polar fraction, was collected and its volume noted. From each sample there were transferred 2, 4 and 10 ml polar fraction solution into 25-ml volumetric flasks and Folin-Ciocalteau reagent (0.5 ml) was added; after 3 min, 1 ml of a saturated sodium-carbonate solution was added and the flasks were made up to volume with water and stored in the dark for 1 hour. The absorbance of the solutions were measured at 725 nm. The total polyphenol content was expressed as ppm of caffeic acid.

2.2.3. *Fatty acids determination*

The fatty acid composition was determined according to modified EC Regulation 2568/91 by GC analysis within 5% coefficient of variance. Test portions, in form of the fatty acid methyl esters were performed in duplicate and 1 μl of each sample solute in hexane was injected. An capillary column SP-2330 (Supelco, Bellafonte, USA), 30 m x 0.32 mm id, 0.2 μmfilm trickness, was used.

Helium was used as the carrier gas with split injection (100:1). The analyses were carried out in isothermal condition at 185°C, detector and injector temperature were 220°C. The results were elaborated by the normalisation method.

2.2.4. *Sterols and aliphatic alcohols determination*

The sterols and aliphatic alcohols composition were determined according to the modified EC Regulations 2568/91 by GC analysis within 5% coefficient of variance (Giacometti, 2001). The test portions, in the form of TMS derivatives, were examined in duplicate and 1 μl of each test portion in chloroform was injected into the GC system. An SPB-5 capillary column (Supelco, Bellafonte, PA, USA), 30 m x 0.53 mm x 0.5 μm film trickness, was used. Helium was used as the carrier gas with split injection (100:1). The analyses were carried out in the programmed temperature mode from 180 to 270°C, with rate 8 °C min^{-1} and then isothermal for 65 min. The detector temperature was 300 °C and the injector temperature was 290°C. The external standard for sterols was dihydrocholesterol and the internal standard for aliphatic alcohols was arachidyl alcohol. The results were expressed as both percentage and ppm of total sterols and aliphatic alcohols in the unsaponifiable fraction. α-Tocopherol and squalene were determined in the same fraction under the identical analytical conditions.

2.2.5. *Determination of saturated fatty acids in the 2-position of the triglycerides in olive oils.*

The samples of olive oil in duplicate were lipolysed by a modification of the EC Regulations 2568/91. The solution which contained 20 mg of crude lipase from Porcine pancreas (activity 220 U mg^{-1} of protein), 2 ml of the TRIS-buffer solution (pH 8.0), 0.5 ml of the sodium cholate solution and 0.2 ml of the calcium chloride solution was added into 0.1 g olive oil sample dissolved in hexane. The test tube was closed and shaken continuously in the thermostat maintained at 40 ± 0.5 °C during three minutes.

The reaction mixture was cooled immediately in running water and 1 ml of hydrochloric acid and 1 ml of diethyl ether were added. After centrifugation, the organic layer was taken by Pasteur pipette.

Monoglycerides were separated from the lipolysis products by elution from the SPE cartridge aminopropylsilica column (Pinkart et al., 1998).

After transmethylation, fatty acids were determined in accordance with the above-mentioned method (*2.2.3.*).

2.2.6. *Determination of triglyceride composition in olive oils in terms of their equivalent carbon number (ECN)*

The chromatographic separation was achieved on a Kromasil 100 C18, 7 μm column (250 mm x 4 mm i.d.), obtained from MZ Analysentechnik (Mainz, Germany) at 40°C. Isocratic elution was carried out at flow rate of 0.7 ml min^{-1} with a mixture of acetone: acetonitrile (60:40, v/v) as mobile phase. The test portions of olive oils were examined in duplicate and 20 μl of each test portions in acetone (5%, w/v) injected into the HPLC system. The results were expressed as a relative percentage of each triglyceride.

3. RESULTS AND DISCUSSION

The appropriate climatic condition, geographic area and olive cultivars in North Adriatic Region of

Croatia are important factors for the production of the highest quality virgin olive oil (Favretto, 1996). After the well known problems affecting the area in recent past, now, some parts of the coastal area participate in the revitalisation of olive fruit and olive oil production. Since 1997, Primorsko-Goranska County has established the collaboration with the University of Rijeka. The olive oil samples were collected from individual producers as accidental samples and included in the Program.

Table II shows the quality parameters in olive oil samples during the 1997/98, 1998/99 and 1999/2000 harvest. During the 1997/98 harvest, 83% of the collected and analyzed samples had the acidity lower than 1.0, 93% showed the peroxide value lower than 20 meq/kg and 80.5 % showed UV spectrophotometric constants according to Regulation CEE 2568/91 and 656/95 for the extra virgin olive oil.

As shown in Table II, in all the collected and analyzed samples of virgin olive oil produced during the 1998/99 harvest, acidity was lower than 1.0%, 95.5% showed the peroxide value lower than 20 meq/kg and 98% showed UV spectrophotometric constants according to Regulation CEE 2568/91 and 656/95 for the extra virgin olive oil.

The quality parameters in virgin olive oil samples during six months of 1999/2000, as shown in Table II, resulted in 96.6% of the collected and analyzed samples acidity lower than 1.0%, 98.3% the peroxide value lower than 20 meq/kg and 91.5% showed UV spectrophotometric constants according to Regulation CEE 2568/91 and 656/95 for the extra virgin olive oil. On the basis of the results of quality parameters obtained during the above-mentioned period the virgin olive oil from this geographic area indicated the highest quality.

Table II

The quality parameters of the virgin olive oil samples from Northern Adriatic region of Croatia during the 1997/98, 1997/98 and six months of the 1999/2000 harvest

	Acidity (%)	Peroxide value (meq O_2/kg)	K_{232}	K_{270}	ΔK
1997/98 harvesting					
Minimal value	0.20	2.49	1.19	0.07	-0.01
Maximal value	2.78	39.53	3.27	0.46	0.03
Average value	0.67	12.91	2.01	0.16	0.00
Variance	0.30	53.50	0.17	0.0044	0.0001
1998/99 harvesting					
Minimal value	0.13	3.18	0.71	0.04	-0.09
Maximal value	0.74	22.99	2.72	0.23	0.01
Average value	0.27	9.03	1.88	0.14	-0.01
Variance	0.02	17.55	0.11	0.0013	0.0005
1999/2000 harvesting					
Minimal value	0.15	4.76	1.47	0.07	-0.02
Maximal value	2.10	22.46	2.71	0.27	0.01
Average value	0.40	9.25	1.95	0.15	0.00
Variance	0.0963	11.29	0.0455	0.0012	0.0000
	M 1.0*	M 20*	M 2.50**	M 0.20*	M 0.01*

(*) Regulation CEE 2568/91; (**) Regulation CEE 656/95.

According to Bruni *et al*. (Bruni *et al*, 1994) various factors such as harvest period, cultivar and origin, affect the formation of the main fatty acids of olive oil in the different ways. The composition of fatty acids in virgin olive oil produced during the 1997/98 harvest showed a high concentration of oleic acid, which ranged from 74.17 to 77.32% and oleic/linoleic acids ratio from 7.08 to 13.96, with small a variance. The highest variance was found in linoleic acid, which content ranged from 5.54 to 10.47%. The concentration of oleic acid was decreased as the concentration of NaCl was increased (Marzouk *et al*., 1986). Dipping in sea water is a traditional and still very frequent method of preserving olives in Croatia (especially in South Adriatic region). This preserving method is not frequent in Northern Adriatic region. One of the collected olive oils, was obtained from olive fruit previously stored in aqueous media and did not change significantly basic physicochemical quality indicators but the oil quality was reduced primarily due to undesired changes of sensory characteristics and contained very low total polyphenol content.

As shown in Table III, in all the analyzed olive oil produced during the 1997/98 harvest, the variance of fatty acids was in accordance with the results of Italian oils (Bruni *et al*., 1994; Esti *et al*., 1996).

Table III

The fatty acids composition in the virgin olive oil from Northern Adriatic region of Croatia during the 1997/98 harvest

	Minimal value (%)	Maximal value (%)	Average value (%)	Variance
C14:0	0.010	0.030	0.016	4.50E-05
C16:0	10.560	13.710	12.124	0.970
C16:1	0.660	1.510	1.082	0.069
C17:0	0.050	0.100	0.067	2.00E-04
C18:0	1.310	2.250	1.855	0.064
C18:1	74.160	77.320	75.566	1.055
C18:2	5.540	10.470	8.233	1.757
C18:3	0.450	1.090	0.592	0.031
C20:0	0.120	0.610	0.248	0.015
C20:1	0.100	0.340	0.195	4.00E-03
C22:0	0.000	0.170	0.023	2.30E-03
C24:0	0.000	0.000	0.000	0.000
C18:1/ C18:2	7.083	13.957	9.439	3.293

Table IV shows the sterol compositions in virgin olive oils during the 1997/98 harvest. The concentrations of β-sitosterol ranged from 93.88 to 98.44%, stigmasterol concentrations from 0.06 to 2.81% and campesterol concentrations from 1.26 to 3.32%. El-Agaimi et al. (1994) reported on the effect of irrigation water salinity on the sterol and tocopherol composition of olive oil. The majority of olive trees in this Region grow near the sea and that can be the reason, with cultivar, origin and environment, for the different concentrations of campesterols, stigmasterol and β-sitosterol.

The concentration of aliphatic alcohols, as shown in Table V, indicated the highest content of docosanol, tetracosanol and hexacosanol.

The concentrations of α-tocopherols were 1.49 to 33.66 mg/100 g and squalene were 99.69 to 1245.26 mg/100 g. Minor compounds, such as α-tocopherol, squalene and β-sitosterol, influence on the kinetic of lipoperoxidation reactions and thus confirm their protective affect on fatty acids. Finally, the optimal ratio in the concentrations of these compounds in a mixture, such as olive oil, can be a relevant factor for the determination of antioxidity power of virgin olive oil (Quaglia, 1996). (Table VI).

The concentrations of total polyphenols expressed as caffeic acid were 52.52 to 356.15 ppm. The content of total polyphenols was 52.52 ppm in the sample where olive fruits were stored in sea water. (Table VI).

The concentration of saturated fatty acids in the 2-position of the triglyceride was low, where the average value was 0.45%. SPE separation method was chosen in preference to thin-layer chromatography (TLC). SPE column with bonded aminopropyl groups proved of great value for the separation of different lipid classes (polar lipids, sterols, steryl esters, poly-β-hydroxyalkanoate, triacylglycerols, diacylglycerols, monoacylglycerols) in a reasonable degree of purity by sequentional elution with solvents of increasing polarity. In comparison with TLC, the samples were purer and the amount of fatty acid methyl esters was higher. (Table VII).

In all analyzed samples, the value of trilinolein (LLL) did not exceed the maximum limit of 0.5% determined by the EC Regulation for different olive grades. In our preliminary results we compared UV detection (210 nm) and RI detection. ECN 46 and ECN 48 showed inverse results in comparison with these both detections. UV detection does not allow a quantitative estimation of these compounds, due to the absence of the specific absorption peak.

Table IV

The sterol compositions of the virgin olive oil from Northern Adriatic region of Croatia during the 1997/98 harvest

	Minimal value (%)	Maximal value (%)	Average value (%)	Variance
Cholesterol	0.002	0.056	0.021	0.0004
Brassicasterol	0.000	0.048	0.013	0.0002
Campesterol	1.260	3.320	2.069	0.4807
Stigmasterol	0.06	2.810	0.610	0.7747
β-sitosterol	93.880	98.470	97.073	2.2657
Δ-7-stigmastenol	0.003	0.440	0.214	0.0252
Total sterol content (mg/kg)	***1211.60***	***1850.42***	***1462.07***	

Table V

The aliphatic alcohol compositions of the virgin olive oil from Northern Adriatic region of Croatia during the 1997/98 harvest

	Minimal value (%)	Maximal value (%)	Average value (%)	Variance
C22	10.630	23.790	14.625	14.106
C23	1.044	6.860	4.243	4.508
C24	20.840	38.940	27.499	29.118
C25	0.130	2.630	0.918	0.695
C26	30.700	46.990	40.288	32.797
C27	0.180	3.800	1.471	1.441
C28	4.750	17.040	10.958	18.935
Total alcohol content (mg/kg)	***121.310***	206.720	163.761	

Table VI

Squalene, α-tocopherol and total polyphenols (as caffeic acid) concentrations of the virgin olive oil from Northern Adriatic region of Croatia during the 1997/98 harvest

	Minimal value mg/100g	Maximal value mg/100g	Average value mg/100g	Variance
Squalene	99.69	1245.26	668.32	1.36E+05
α-tocopherol	1.49	33.66	10.25	8.10E+03
	mg/Kg	mg/Kg	mg/Kg	
Total polyphenols	52.52	356.15	214.67	1.00E+02

Table VII

Fatty acid concentrations in the 2-position and trilinolein concentrations (ECN 42) of the virgin olive oil from Northern Adriatic region of Croatia during the 1997/98 harvest

	Minimal value %	Maximal value %	Average value %	Variance
2-position	0.28	0.60	0.45	0.008
Trilinolein	0.04	0.50	0.19	0.020

CONCLUSION

With the promulgation of the National Regulation for differentiation between various types of oil, physical and chemical characteristics of each of them and organoleptic characteristics of virgin oil, in order to guarantee the purity and quality of the products, which is identical to the European Community (EC) Regulations 2568/91 and 656/91, the Republic of Croatia comes up to the EC.

The olive oil characterisation helps to understand the olive oil situation in the Northern Adriatic region.

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