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Content

CHARACTERIZATION OF OLIVE VARIETIES FROM ALBANIA: FATTY ACID PROFILE AND TOTAL PHENOLIC CONTENT

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ABSTRACT

The research study presented in this paper, the first of its kind in Albania, characterizes the fatty acid profile and total phenolic content of some olive varieties, namely, *Boçi*, *Ulli i Kuq, Ulli i bardhe Lezha*, *Sterbjak* and *Micka* all harvested during crop year 2010-2011.

Annual production capacity of the country is 56 000 tons of olive fruits and 7000 tons of olive oil. Fatty acid (FA) profiles to the studied olive cultivars exhibit a great variation in oleic acid, from 66.32 ± 0.14 % (Ulli i Kuq) to 75.13 ± 0.31 % (Sterbjak), values which are within the normal range for such FA. The content of linoleic acid varies from 7.23 ± 0.04 % (Sterbjak) to whereas the content of linolenic acid varies from 0.45 ± 0.01 % (Ulli i Kuq) to 0.96 ± 0.01 % (Sterbjak). All studied olive varieties revealed moderate levels of palmitic acid, which varied between 10.76 ± 0.05 % (Sterbjak) and 13.05 ± 0.02 % (Boçi). From a nutritional point of view, it is worth noticing that the Sterbjak variety has an n-6/n-3 ratio of 7.68, while the Ulli i bardhe Lezha goes to 10.20.

The Total Phenolic Content for the studied olive cultivars varied from 63.02 ± 5.63 GA mg/kg olive oil (Ulli i kuq) to 322.05 ± 3.97 GA mg/kg olive oil Ulli i bardhe Lezha such variation reflect differentness among studied olive varieties.

KEYWORDS: *Albanian Native Olive Cultivars; Sterbjak; Ulli i Bardhe Lezha, Ulli i Kuq, Fatty acid Profiles*

INTRODUCTION

The origin of olive trees in Albania is not different from the road of the distribution of this tree in Mediterranean region. Archaeological evidences on the agriculture activities such as wheat cobs, truss grapes, olive lop are stamped in stones and coins of the Illyrian tribes. Olive tree is planted mainly in the Western Lowland, by penetrating the mainland through the river valleys (Kafazi, Muço, 1984*;* Thomaj, Panajoti, 2003). The analyzed olive cultivars belong to the regions of Tirana and Lezha, in the Central and Northern part of Country. The actual annual producing capacity is 56 000 tons of olive fruits and 7000 tons of Olive oil (FAOSTAT, 2011). Recent genetic studies concluded that Albania owns 22 native olive cultivars (Bacu-Grazhdani et al, 2008). They are grouped according to their region of cultivation and classified as primary and secondary cultivars based on cultivar distribution (Thomaj, Panajoti, 2003).

Olive oil is a vegetable oil, extracted by the olive fruits, that can be consumed without prior refining treatment (Angerosa et al, 2006*;* Aparicio, Luna, 2002). Triglycerides are its major components and represent more than 98 % of the total weight. The remaining part are non-saponifiable chemical compounds such as sterols, polyphenols, alcohols, waxes, hydrocarbons, etc (Servilli, Montedoro, 2003; Mannina et al, 2003). Its composition depends on numerous factors such as the interaction between the cultivar and the environment, cultivation techniques, fruit ripeness and the oil extraction system (Aparicio, Luna, 2002). The characterisation of fatty acid profiles of olive oils from different olive cultivars is usually proposed as a methodology to differentiate these products according to their cultivar and geographical origin (Aparicio, Luna, 2002; Mannina et al, 2003).

In this study, are analysed the fatty acid composition and total polyphenol content of five olive cultivars: *Boçi, Ulli i bardhe Lezha (UbL)*, *Sterbjak*, *Ulli i Kuq* and *Micka* which belong to the Tirana and Lezha regions. These cultivars are mainly used in the oil production, although *Boçi* is used also in parallel as table olives. The *UbL* is the most planted by 2 % of the total of 6 million olive trees.

The scope of the study presented herein is linked with chemical evaluation of the Monovarietal Virgin Olive Oils. Assessment of quality parameters and nutritional value of olive oils from studied cultivars is presented in this paper. Nowadays, an attempt to modify the national fund of olive tree is an ongoing process. Such pioneering study will allow for the identification of native cultivars that produce good quality olive oil and that are very well adapted to the pedo-climatic conditions in Albania.

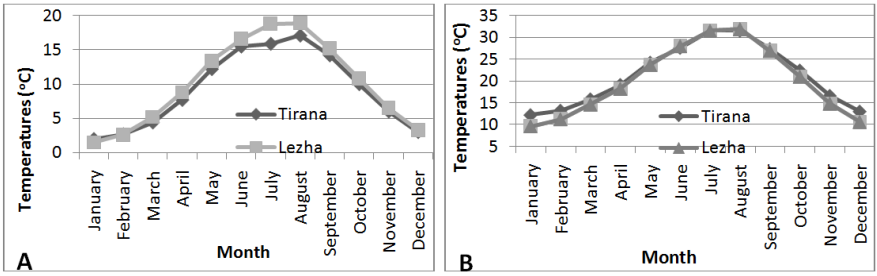

Figure 1: Monthly minimum (A) and maximum (B) average temperatures of Tirana and Lezha regions

MATERIALS AND METHODS

Sample collection and oil extraction

Native olive varieties, namely: *Boçi*, *Ulli i Bardhe Lezha*, *Sterbjak, Ulli i Kuq* and *Micka* were harvested in their main area of Tirana and Lezha (Table 1). The climatic characteristics of the production areas, in terms of temperature, are reported in Figure 1.

Oil extraction was performed with a SPREMOLIVA Press (an Italian TEM technology, Italy). The extraction condition was cold, mechanical pressing. SPREMOLIVA apply discontinuous process with 20-25 kg/cycle. After extraction the oil samples were stored in the dark at 4 oC until analysis.

Table 1: Olive varieties, harvesting and extraction day

Cultivar name	Harvesting day	Extraction day	Region
Boçi	25/10/2010	25/10	Tirana
Ulli kuq	13/11/2010	16/11	Tirana
Micka	11/11/2010	16/11	Tirana
Ulli i Bardhe Lezha	10/11/2010	13/11	Lezha
Sterbjaku	10/11/2010	13/11	Lezha

Chemicals

The chemical reagents were analytical grade, from Sigma-Aldrich Chemie (Steinheim, Germany). Internal standard C 15:0 was purchased from Sigma-Aldrich. Gallic acid and Folin-Ciocalteau reagent were supplied by Fluka Chemie GmbH (Buchs, Switzerland).

Determination of fatty acid profiles

Fatty acid methyl esters (FAME) were prepared through direct acidic transesterification, as originally proposed by Lepage and Roy (1984) and later modified by Carvalho and Malcata (2005), using Pentadecanoic acid (C 15:0) as internal standard. The assay of FAME was carried out with a HP-6890 Gas chromatograph,

equipped with a Flame Ionization Detector (GC-FID). Separation was achieved in a SP-2380 capillary column (60 m × 0.25 mm × 0.20 □m) from Supelco. Hydrogen was used as carrier gas at a flow rate of 1.0 mL/min. The temperatures in the injector and detector were 240 °C and 260 °C, respectively. The injection was performed in split mode (1:50). Oven temperature was set to 150 °C increased to 200 °C at a rate of 1 °C/min and held at 200 °C for 20 min. Calculations were performed according to AOCS Official Method Ce 1b-89 (AOCS, 1994).

Determination of Total Polyphenol Content

Fractionation of olive oils

The method used to perform the fractionation of oils was proposed by Kalantzakis *et al* (*2006*). Briefly, samples were dissolved in n-hexane (Sigma, Germany) and extracted with a methanol/water mixture (60:40, vol/vol). After dissolving 2.5 g of oil with 5 mL n-hexane, 5 mL of methanol/water 60:40 were added, and the mixture was shaken vigorously and centrifuged at 3500 rpm for 10 min. The polar fraction was used, as it was, for further analysis.

Colorimetric determination of total polyphenol content

The Colorimetric method was used to determine the total polyphenol content (TPC) of samples, according to method proposed by Kalantzakis *et al.* (2006). An UV-VIS Mini-1240 Spectrophotometer (Shimadzu) was used at 725 nm. Results were expressed as Gallic acid equivalent (mg/kg olive oil), calculated from the following calibration curve, determined by linear regression.

Statistical analysis

The complete data were evaluated by randomized block design, with three replicates from fatty acid analysis and duplicates for TPC values. Results were displayed as mean values and standard error (n=3). Significance of the differences among the values was determined by analysis of variance using One-way ANOVA test. The level of significance was determined at $P < 0.05$. The employed statistical program was SPSS 17.0 Statistics 2008 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Fatty acid profiles

Olive cultivars studied are described in Table 2. One-way ANOVA analysis showed that fatty acid profiles of the seven olive cultivars were statistically significantly different. Results revealed that, in what concerns Palmitic acid (PA), the cultivars can be grouped in two groups: (i) those with lower PA content, such as Sterbjak (10.76 ± 0.05 %) and Micka (10.79 ± 0.01 %) cultivars, and those with higher PA content, such as UbL (12.85 ± 0.01 %), Ulli i kuq (12.95 ± 0.03 %), and Boçi (13.05 ± 0.02 %).

The oleic acid (OA) content was: 67.65 ± 0.01 % (Boçi), 75.13 ± 0.31 % (Sterbjak), 70.84 ± 0.07 % (UbL); 66.26 ± 0.14 % (Ulli i kuq) and Micka 73.471 ± 0.14 %. Cluster analysis of the studied cultivars regarding the FA profiles indicate that olive cultivars are grouped in two groups; those with low content of OA such as: Boçi and Ulli i Kuq; and with high content of OA: UbL, Sterbjak and Micka.

Linoleic acid (LA) content showed high variation among the studied cultivars; olive cultivars such as Sterbjak (7.23 ± 0.04 %), Micka (9.10±0.02 %) and UbL (8.69 ± 0.00 %), presented low content of LA while the others Ulli i kuq (13.29 ± 0.02 %) and Boçi (12.47 ± 0.01 %), presented high contents – almost two-fold the amount in some cases. The □-Linolenic acid (ALA) is below 1 %, a condition for the quality of the Extra Virgin Olive Oils (EEC 2568/91). The ALA content varied according to the following ascending order: 0.45 ± 0.01 % (Ulli i kuq), 0.64±0.00 % (Boçi), 0.86 ± 0.00 % (UbL), 0.94 ± 0.01 % (Sterbjak) and 0.54±0.00 % (Micka).

A high content of ALA contributes to the n-6/n-3 ratio, a very important value for the nutritional evaluation of lipids of different origin. Regarding such ratio, the Sterbjak cultivar show a n-6/n-3 ratio of 7.68, followed

by 10.12 for UbL, while the remaining cultivars show higher values, 16.98 (Micka) and 19.5 for Boçi.

Table 2: Fatty acid profile (in % of total FA), Iodine Value, n-6/n-3 and 18:1/18:2 ratios of the olive cultivars

Formula	Boçi	UbL	Sterbjak	Ulli i kuq	Micka
14:0	ND	ND	ND	ND	N.D
16:0	13.05±0.009‡	12.85±0.003‡	10.76±0.028‡	12.95±0.02‡	10.799±0.010
16:1(n-9)	0.15±0.00‡	0.12±0.00‡	0.146±0.003‡	0.096±0.008‡	0.023±0.040
16:1(n-7)	0.63±0.00‡	0.97±0.00‡	0.646±0.003‡	0.826±0.003‡	0.606±0.003
17:0	0.12±0.00‡	0.00±0.00‡	0.00±0.00‡	0.15±0.00‡	0.084±0.001
17:1 (n-7)	0.223±0.003‡	0.00±0.00‡	0.00±0.00‡	0.25±0.00‡	0.175±0.003
18:0	2.113±0.003‡	2.536±0.003‡	2.206±0.006‡	2.643±0.008‡	2.306±0.005
18:1(n-9)cis	67.643±0.003‡	70.843±0.039‡	75.123±0.81‡	66.26±0.081‡	73.471±0.140
18:1(n-7)	2.24±0.00‡	2.49±0.00‡	2.10±0.01‡	2.28±0.025‡	2.271±0.031
18:2(n-6)trans	ND	ND	ND	ND	ND
18:2 (n-6)cis	12.467±0.008‡	8.686±0.003‡	7.233±0.021‡	13.286±0.008‡	9.098±0.022
20:0	0.346±0.006‡	0.423±0.006‡	0.363±0.012‡	0.41±0.005‡	0.347±0.008
18:3 (n-3)	0.64±0.00‡	0.86±0.00‡	0.943±0.006	0.443±0.003	0.538±0.003
20:1 (n-9)	0.24±0.01NS	0.176±0.048NS	0.236±0.008NS	0.246±0.033NS	0.208±0.025
22:0	0.116±0.008‡	0.056±0.003‡	0.063±0.003‡	0.153±0.012‡	0.050±0.087
Σ–SFA	15.75	15.87	13.39	16.27	13.587
Σ–MUFA	70.41	74.60	78.25	69.95	76.752
Σ–PUFA	13.09	9.72	8.17	13.73	9.663
n-6/n-3	19.52	10.12	7.68	29.8	16.976
18:1/18:2	5.38	8.15	10.39	4.99	8.075
MUFAs/SFAs	4.47	4.70	5.83	4.30	5.649
MUFAs/PUFAs	5.38	7.67	9.57	5.09	7.943
Iodine Value	85.807	82.765	83.881	85.71	84.05

End note 1: SFA= Saturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; MUFA= Monounsaturated Fatty Acids; ‡-Statistically significant among the different olive cultivars ($p<0.005$); and †-Statistically significant ($p<0.05$); NS-Statistically Non Significant among olive cultivars.

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Comparison of the FA profiles of the olive oils from Albanian native cultivars with those in neighbouring countries: Italy, Greece (Aparicio, Luna 2002; Pinelli et al. 2003; Paz Aguilera et al. 2005) and Northern Africa (Haddada et al. 2008) gives indication that they are comparable with Italian and Greek olive cultivars (Paz Aguilera, et al. 2005). For example, the level of palmitic acid in the studied native cultivars is comparable with Italian cultivars Leccino (14.3 %) and Moraiolo (10.5 %); Spanish cultivars Arbequina (14.3 %); Lechin (10.5 %) and Redondilla (12.5 %), and Greek cultivar Koreiniki (13.3 %). The level of oleic acid in Albanian cultivars is comparable with Frantoio (78.2 %), Arbequina (75.3 %) and Koreiniki (71.9 %) (Aparicio, Luna, 2002). Related to the linoleic acid trends is not as even since the Albanian olive cultivars present content differences. While the Boçi cultivar presents high content of linoleic acid comparable to the Spanish cultivars Redondilla and Lechin (Aparicio, Luna, 2002).

Oxidation stability

Analysis of the ratio 18:1/18:2 is an indication that refers to the oil oxidation stability. The lowest value proposed is 18:1/18:2 ≥ 7 (Kiritsakis et al. 1998). The results show that the Monovarietal olive oils from 8.08 (Micka) and 10.39 (Sterbjak) have acceptable oxidation stability, whereas the 4.99 (Ulli i kuq); and 5.38 (Boçi) cultivars have ratios under the minimum threshold meaning a minimal oxidation stability. The ratio between monounsaturated and saturated fatty acids of the studied cultivars had an average value of 4.86, whereas the ratio between monounsaturated and polyunsaturated fatty acids presented an average value of 7.09 (Table 2), which are relatively low; however, the high phenol content could indicate that oil quality was maintained without lipid deterioration.

The estimation of oil stability for the analyzed olive cultivars was analyzed based on the proposed equation for Iodine Value (Kiritsakis et al. 1998). The Iodine Values recorded for the seven cultivars are in the referred range of 80-90 for olive oils. The Iodine Value belongs vary from Ulli bardhe Lezha (82.76), to the max value Boçi (85.81) cultivars.

Phenolic compounds

Quantitative determination of phenolic compounds in olive oil was performed according to the colorimetric method (Kalantzakis et al. 2006). The amount of phenolic compounds in olive oil varies from 50 to 1000 mg/kg and depends on several factors such as: climate, and extraction technology, degree of maturation (Servilli, Montedoro, 2003) and cultivar (Montedoro et al. 1992).

The results for the five olive cultivars (Figure 2) reveal that the highest value belongs to the UbL cultivar 322.05 ± 3.97 mg/kg olive oil, and the lowest value belongs to Ulli i kuq by 63.02 ± 3.98 mg/kg olive oil. The results show that the polyphenol content of the studied olive oils had significant differences ($p<0.05$) among the cultivars. According to the classification for the TPC level of the olive oils proposed by Montedoro et al (1992) the studied cultivars can be classified as: "low" (50-200 mg/kg) Ulli i kuq, Sterbjak and Micka cultivars; "medium" (200-500 mg/kg) UbL and Boçi cultivars. The results obtained for the studied cultivars can be related mainly with the cultivar differences.

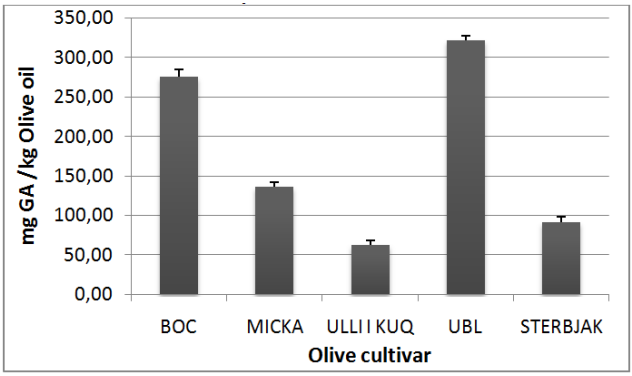

Figure 2: TPC values of the studied olive cultivars (as Gallic acid Equivalent mg/kg olive oil)

The actual stage of agriculture in Albania does not have premise for irrigation of the olive plantations. Furthermore, concerning the maximum and minimum temperature values in the different regions, respectively, give evidence that differences are not significant. Hence, differences reported in TPC content are not due to climatic conditions, but they might come from the cultivar caracteristics. The TPC values of UbL and Boçi are comparable with Koreiniki (Greece), Picual (Spain) and Frantoio (Italy) olive cultivars (Paz Aguilera et al. 2005).

CONCLUSIONS

The results presented show significant differences in the chemical composition of the studied cultivars. Those variations, observed in fatty acid composition and phenolic compounds, are probably due to both genetic factors and environmental conditions. By comparison with results from literature, it can be concluded that the levels of fatty acids in the oils of the studied cultivars are similar to those found in the group of olive cultivars typical from Northern Mediterranean regions. The nutritional profile of Sterbjak cultivar is highly interesting, as well as the UbL olive cultivar, due to its n-6/n-3 ratio and total phenolic content.

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