



Characterization of Kalinjoti and Nisioti Monocultivar Virgin Olive Oils produced in Albania

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A number of native olive cultivars are grown in the Western and Southern regions of Albania, along the Mediterranean sea. Genetic studies have found 22 native olive cultivars. Present study presented the chemical characteristics of Kalinjoti and Nisioti cultivars, which are grown mainly in Vlora region, Southern Albania. Their chemical characteristics are compared with other olive varieties important: Ullibardhë Tirana (Bianco di Tirana), Mixan, Ullibardhë Kruja and Frëngu. The olive oil samples analyzed belong to the harvesting year, 2015-2016. Results on fatty acid profiles exhibit a great variation in oleic acid, from 71.91 ± 0.06 % (Mixan) to 80.07 ± 0.04 % (Nisiot), linoleic acid varied from 4.10 ± 0.00 % (Nisiot) to 10.11 ± 0.01 % (Kalinjot). Moderate levels of palmitic acid, between 9.57 ± 0.01 % (Kalinjot) to 12.38 ± 0.02 % (Mixan) resulted. The total phenolic content for two cultivars Kalinjoti (216.63 ± 10.76 mg GAE/kg olive oil) and Nisioti (275.45 ± 11.63 mg GAE/kg olive oil) was found lower than Olive cultivars that belong to the Western central region of country like Ullibardhë Tirana (445.03 ± 16.83 mg GAE/kg olive oil).

Keywords: Olive oil, Kalinjoti, Nisioti, Fatty acid, polyphenols, Albania.

INTRODUCTION

The olive tree (*Olea europaea* L.) is present in Albania, like in other Northern Mediterranean neighboring countries. According to the geography of the country, its cultivation is mainly present in the Western regions alongside to the Adriatic and Ionian seas, by penetrating the mainland through the river valleys [1,2]. Genetic studies have concluded that Albania owns 22 native olive cultivars [3], strictly distributed into six regions: Kruja, Tirana, Vlora, Elbasani, Berati and Lezha regions [4]. Olive cultivars are mainly used for oil extraction, while a limited number of cultivars is used as table olive. Kalinjoti, classified as the 'principal cultivar', belongs to the Vlora region, Southern Albania. This cultivar is more distributed and covers 50 % of the total plantation area in country. The second important native olive cultivar to Vlora region is Nisioti, but due to relatively lower plantation areas it is considered as 'secondary olive cultivar' to this region. Other native olive cultivars in Albania belonging to other regions are: Ullibardhë Tirana (UBT), Frëngu, Ullibardhë Kruja and Mixan [2].

The analysis of several principal and minor compounds to olive oils, are thought to depend on agronomic practices, variety, climatic, ripening stage [5-9] and extraction technologies [10].

The aim of this study was to characterize the fatty acid profile and total polyphenols of monovarietal virgin olive oils

for native olive cultivars: Kalinjot, Nisiot belonging to Vlora regions and comparing them with other principal varieties of the country: Ullibardhë Tirana (Bianco di Tirana), Mixan, Ullibardhë Kruja and Frëngu. The chemical characterization of monovarietal virgin olive oils may be of relevance both for the geographical indication, as well as the policies undertaken for the selection of native cultivars with higher productivity and quality.

EXPERIMENTAL

Olive samples and extraction: Virgin olive oils (VOO) samples from six native olive cultivars, namely, Kalinjot, Nisiot, Ullibardhë Tirana (Bianco di Tirana), Mixan, Ullibardhë Kruja and Frëngu, were collected during the harvesting season November-December 2015. The olive fruits were harvested from different olive trees of the same plantation in the period where the maturity index, resulted about 5. Their extraction was carried out in the laboratory using a SPREM Oliva Press. The oil was transferred into glass bottles and stored in dark at 4 °C.

Analytical methods: Fatty acid methyl esters (FAME) were prepared through direct acidic transesterification, as originally proposed by Lepage and Roy [11] and later modified by Carvalho and Malcata [12] by using pentadecanoic acid as

internal standard. The assay of FAME was carried out with a HP-6890 Gas chromatograph, equipped with a Flame Ionization Detector (GC-FID). Calculations were performed according to AOCS Official Method Ce 1b-89 [13]. Identification of fatty acids was undertaken with pure standards (Sigma-Aldrich, Supelco), based on the comparison of retention times. Fatty acids were calculated as the percentage of the total fatty acids. Each sample was analyzed in triplicate.

Total polyphenolic content (TPC) was measured by a colorimetric method proposed by Kalantzakis *et al.* [14]. Briefly, samples were dissolved in *n*-hexane (Sigma, Germany) and extracted with a methanol/water mixture (60:40, vol/vol). The insoluble fraction (non-polar) in methanol-water fraction was removed, whereas the polar fraction was used, as it was, for further analysis. The absorbance of mixture was measured in UV-VIS Mini-1240 spectrophotometer (Shimadzu) at 725 nm. Results were expressed as gallic acid equivalent (mg/kg olive oil), calculated from the following calibration curve, determined by linear regression, where (GA) is the concentration of Gallic acid, expressed as mg/kg olive oil: $A_{725} = 3.049 (GA) + 0.004$ ($r^2 = 0.999$). Each sample was measured in triplicate.

Statistical analysis: The complete data was evaluated by randomized block design, with three replicates from fatty acid analysis and duplicates for total polyphenolic content 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, with a level of significance at $P < 0.05$ and using SPSS 17.0 Statistics 2008 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Characterization of the Virgin olive oil from two cultivars Kalinjoti and Nisioti shows an interest for the area that this two cultivars cover in the total of the country, over 60 %. To distinguish, this two cultivars, they are compared with four other cultivars that belong to three other regions of the country Mixan Cultivar (Elbasani region), UBT (Tiran region) and UBK and Frengu cultivars (Kruja region).

Fatty acid composition: The major fatty acids (FA) present in the six olive cultivars were: oleic acid (C18:1), palmitic acid (16:0), linoleic acid (C18:2) and stearic acid (C18:0), according to the decreasing order. The fatty acid content falls in the average percentage intervals described by FAO and IOOC [10]. A major variation presented in these olive varieties belonged to the oleic acid (OA): Kalinjoti, Nisiot, UBT, Mixan, Frëngu, UBK (from 70.87 to 76.58 %).

All six olive varieties revealed moderate levels of palmitic acid, found at concentrations five to eight-fold lower than oleic acid, *i.e.* between 9.57 % (Kalinjoti) to 12.38 % (Mixan), with no considerable differences among them. The content of linoleic acid (LA) varied from 4.10 % (Nisiot) to 10.11 % (Kalinjoti), whereas the content of α -linolenic acid showed a small variation from 0.48 % (UBK) to 0.58 % (UBT).

There was a similarity among Albanian studied cultivars and other cultivars from neighbor countries, Italy and Greece [5-8]. Comparison of olive cultivars in present study with data of cultivars from Southern coast of Mediterranean sea, showed a profile with a high content of polyunsaturated fatty acids

[15,16]. Statistical analyses reveal differences among the cultivars for individual fatty acids ($p < 0.05$) (Table-1): only the main fatty acids, C16:0, C18:1 (n-9) and C18:2 (n-6), presented statistically differences among them. This could be as a tool to distinguish mono-varietal olive oils produced by the studied olive cultivars.

Oxidative stability: Different authors suggest the ratio C18:1/C18:2 on evaluation of the oxidative stability of virgin olive oils, with a proposed minimum accepted value of 7.0 [17]. In this study, two olive cultivars, Frengu and Nisioti exhibited higher values, 13.37 and 19.54, respectively, while the remaining four cultivars produced monovarietal olive oil of good oxidative stability (Table-1).

Iodine value: Another indicator proposed to evaluate the oxidative stability of vegetative oils is the iodine value (I.V.). The values are calculated according to the proposed equation: $I.V. = (\% \text{ Palmitoleic acid} \times 1.001) + (\% \text{ Oleic acid} \times 0.899) + (\% \text{ Linoleic acid} \times 1.814) + (\% \text{ Linolenic acid} \times 2.737)$

The majority of the studied cultivars reached values in the range 80-90 (Table-1), as recommended, with the Kalinjoti I.V. value 86.82, while the I.V. value to Nisioti cultivar was 81.22. There is considerable difference with two cultivars from Central Albania UBK (95.6) and Frengu (90.3).

Analysis of saturated and unsaturated fatty acids families: According to families, as saturated (SFA), mono-unsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) can be used to distinguish cultivars [17]. Regarding to SFA, all the six olive cultivars did not differ, with the lower and higher value Nisiot (12.95 %) and Mixan (16.03 %), respectively. Based on the MUFA and PUFA, Kalinjot presented the higher contents of PUFA, 10.86 %.

The ratio between MUFA and SFA was an average value of 5.54, whereas the ratio between MUFA and PUFA presented an average value of 10.34 (Table-2), the high phenol content could indicate that oil quality was maintained without lipid deterioration.

The n-6/n-3 ratio used to indicate interest on nutritional aspect. It shows that the two cultivars Nisioti (8.11) and Kalinjoti (13.57) present high interest as lipid sources. Compared to other cultivars in this study, two cultivars Mixan and UBK show low interest, since they present high values.

Polyphenol content: Quantitative determination of phenolic compounds in olive oil was performed according to the colorimetric method Folin-Ciocalteau [14]. Phenolic compounds in olive oil vary from 50 to 1000 mg/kg and depends on several factors such as: climate and extraction technology [18], degree of maturation [19] and cultivar [20].

Results on total polyphenol content (TPC) show that two cultivars Kalinjoti and Nisioti show similar values, which may indicate that the main factor is region, while other pedo-climatic factors are same. Meanwhile the TPC frUBT cultivar presented the highest values, 445.03 ± 16.83 mg kg⁻¹ gallic acid in olive oil, whereas Frëngu cultivar shows the lowest levels, 42.78 ± 7.04 mg GAE/kg olive oil. Results also show major differences among cultivars, which may be correlated to the cultivars and region of cultivation, rather than to agriculture practices or other factors. The comparison of TPC content in the six studied cultivars with other cultivars from

TABLE-1
FATTY ACID COMPOSITION OF VOO IN SIX NATIVE OLIVE CULTIVARS,
AVERAGE \pm STANDARD ERROR IN % OF TOTAL FAME (n = 3)

Fatty acid	Kalinjoti	Nisjoti	UbT	Mixan	UbK	Frëngu
14:0	ND	ND	ND	ND	ND	ND
16:0	9.57 \pm 0.01	9.94 \pm 0.01	10.88 \pm 0.01	12.38 \pm 0.02	11.16 \pm 0.01	9.62 \pm 0.01
16:1(n-9)	0.14 \pm 0.00	0.12 \pm 0.00	0.07 \pm 0.00	0.08 \pm 0.01	0.09 \pm 0.00	0.12 \pm 0.00
16:1(n-7)	0.26 \pm 0.00	0.41 \pm 0.00	0.35 \pm 0.01	0.51 \pm 0.01	0.55 \pm 0.00	0.60 \pm 0.00
17:0	0.10 \pm 0.00	0.00 \pm 0.00	0.13 \pm 0.00	0.12 \pm 0.01	0.13 \pm 0.00	ND
17:1 (n-7)	0.15 \pm 0.00	0.00 \pm 0.00	0.19 \pm 0.00	0.17 \pm 0.01	0.22 \pm 0.00	ND
18:0	3.02 \pm 0.00	2.56 \pm 0.01	2.83 \pm 0.01	2.88 \pm 0.01	2.35 \pm 0.00	3.87 \pm 0.00
18:1(n-9)t	ND	ND	ND	ND	ND	ND
18:1(n-9)cis	73.61 \pm 0.02	80.07 \pm 0.04	74.61 \pm 0.06	71.91 \pm 0.06	72.59 \pm 0.01	76.58 \pm 0.02
18:1(n-7)	1.48 \pm 0.00	1.55 \pm 0.00	1.53 \pm 0.00	1.76 \pm 0.01	1.97 \pm 0.00	2.14 \pm 0.00
18:2(n-6)t	ND	0.00 \pm 0.00	ND	0.11 \pm 0.01	ND	ND
18:2 (n-6)cis	10.11 \pm 0.01	4.10 \pm 0.00	8.00 \pm 0.07	8.66 \pm 0.032	9.90 \pm 0.01	5.73 \pm 0.00
20:0	0.46 \pm 0.01	0.45 \pm 0.02	0.43 \pm 0.01	0.46 \pm 0.01	0.35 \pm 0.00	0.54 \pm 0.02
18:3 (n-3)	0.75 \pm 0.01	0.51 \pm 0.00	0.58 \pm 0.01	0.51 \pm 0.01	0.43 \pm 0.01	0.51 \pm 0.00
20:1 (n-9)	0.34 \pm 0.01	0.31 \pm 0.01	0.28 \pm 0.01	0.25 \pm 0.03	0.25 \pm 0.01	0.28 \pm 0.01
21:0	ND	ND	ND	ND	ND	ND
22:0	ND	ND	0.07 \pm 0.00	0.18 \pm 0.01	ND	ND
n-6/n-3	13.57	8.11	13.75	17.34	23.18	11.13
SFA	13.15	12.95	14.34	16.03	14.00	14.04
MUFA	75.99	82.46	77.05	74.69	75.66	79.71
PUFA	10.86	4.60	8.59	9.29	10.33	6.24
18:1/18:2	7.28	19.54	9.33	8.30	7.33	13.37
MUFAs/SFAs	5.78	6.37	5.37	4.66	5.40	5.68
MUFAs/PUFAs	7.00	17.92	8.97	8.04	7.33	12.77
Iodine value	86.82	81.22	83.53	82.26	95.6	90.3

SFA = total content of saturated fatty acids; PUFA = total content of polyunsaturated fatty acids; MUFA = total content of monounsaturated fatty acids.

Statistically significant ($p < 0.05$); NS-Statistically non-significant among olive cultivar. UbT-Ullibardhe Tirana, UbK-Ullibardhë, Kruja.

TABLE-2
TOTAL POLYPHENOL CONTENT (mg gallic acid kg⁻¹ olive oil)

Olive cultivar	Mean	SD
UBT	445.03	16.83
Kalinjot	216.63	10.76
Mixan	139.24	6.56
Nisiot	275.45	11.63
Frëngu	42.78	7.04
UBK	322.05	5.61

neighbouring countries reveal that these cultivars are similar with cultivars from the Toscana region (Italy) and Dalmatian coast (Croatia) [15].

Montedoro *et al.* [20] have grouped the monovarietal olive oils according to the TPC in three groups. The olive cultivars analyzed can be classified as: “low” (50-200 mg GAE/kg olive oil) Frëngu and Mixan cultivars; “medium” (200-500 mg/kg) UbT, UbK and Kalinjoti cultivars. The results obtained for the studied cultivars can be related mainly with the cultivar differences. The results show that the polyphenol content of the studied virgin olive oil had significant differences ($p < 0.05$) among the cultivars. The olive plantations in Albania are mainly in arid and semi-arid areas with non-irrigation practices. Concerning the maximum and minimum temperature values for the different regions, differences are not significant. Differences reported on the TPC content do not come due to climatic conditions. The TPC values of UbT are comparable with Koreiniki (Greece), Picual (Spain) and Frantoio (Italy) cultivars [5,15].

Conclusion

Results found a similarity among Albanian studied olive cultivars and other cultivars from neighbor countries, Italy and Greece. Regarding to the oxidative stability we can say that Nisioti present highest interest. The TPC for two cultivars Kalinjoti (216.63 \pm 10.76 mg GAE/kg olive oil) and Nisioti (275.45 \pm 11.63 mg GAE/kg olive oil) was found lower than Olive cultivars that belong to the Western central region of country like Ullibardhë Tirana (445.03 \pm 16.83 mg GAE/kg olive oil). According to the TPC Kalinjoti and Nisioti cultivars belong to “medium” (200-500 mg/kg) group. Further scientific elaboration will need to focus on the blending of this two cultivars from the Vlora region, which may contribute on production of PDO olive oils.

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