Characterisation of virgin olive oils obtained from three different cultivars grown in the Albanian territory

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Chemical characterisation was carried out on samples of virgin olive oil obtained from three different cultivars, which were cultivated in three geographic areas of the Albanian territory. Two cultivars were typical of Albania (Kalinjoti and UBT Tiranë), while the third was originally from Spain (Arbequina). The olives, that came from three different geographical areas (in Albania) were obtained during the 2016-2017 harvesting season. Several evaluations have been carried out in order to determine qualitative parameters (free acidity, peroxide value and UV spectrophotometric indices), chemical composition of three classes of compounds (fatty acids, phenols and tocopherols) and genuineness (ΔECN42) of the analysed olive oils. The obtained results showed the Arbequina and UBT Tiranë cultivars characterised by significantly higher acidity values than those observed in Kalinjoti cultivar, which showed lower values also regarding the number of peroxides. No variations were instead evidenced in the UV spectrophotometric indices. Regarding the fatty acid profiles, the Arbequina cultivar grown in the Albanian territory resulted poorer of *w*9 isomer of oleic acid but, at the same time, showed greater concentrations of the *w*7 isomer. The UBT Tiranë was instead characterised by a lower content of linoleic acid compared to the other two cultivars. A further parameter worthy of attention concerned the total content of polyphenols, which are present at particularly higher concentrations in the Kalinjoti cultivar, whereas tochopherols seem to be more represented in the UBT Tiranë. This work, as a whole, has highlighted the undoubted strengths of Kalinjoti cultivar, which has been characterised by parameters that should guarantee superior health properties, and therefore should be of more interest for the consumer.

Keywords: Olive oil quality. Albanian olive cultivar. Fatty acids. Antioxidants

INTRODUCTION

Olive cultivation is widely spread in the Mediterranean region and is an important resource for the development of rural economy and the environment [1]. Olive oil is a typical lipid source of the Mediterranean diet and is accredited of beneficial effects that may be due to the presence of bioactive components, such as olive oil-derived polyphenols. Such compounds are reported to play biologically relevant activities which involve antioxidant activity, contributing to the prevention of human diseases [2, 3].

Despite the thorough characterisation performed on many bioactive compounds in the oil, less attention was given to the influence of cultivar and geographical origin on olive oil quality. The study of the qualitative characteristics of virgin olive oils of a pure cultivar or from a specific production area is of great scientific interest. It is also of interest to the local industrial sector, to the international olive oil business and to the consumer, who demands more information on the qualitative characteristics and properties of traditional local products [4]. The

olive production and the olive oil consumption in Albania has increased in the last decades. In the 1980s, Albania produced an average of 3,000 tons of olive oil per year. By the 1990s, the average annual production of olive oil rose to almost 4,000 tons per year. In the following years a decline in olive oil production was registered and stabilised at around 1,000 tons per year in 2016, based on a report released by Food and Agriculture Organisation (FAO) [5].
As reported by Chan-Halbrendt et al. (2010) [6], olive oil has a relevant role in the diet of Albanians and the olive oil industry has been targeted as a top strategic sector for growth and development by the Albanian government. For these reasons, different studies have been carried out in order to characterise the olive oils coming from distinct albanian cultivars, both from an organoleptic point of view, and as regards the aroma profiles [7, 8].
Taking into consideration the increased interest in this production sector, three olive oils obtained from as many olive cultivars grown in the Albanian territory, have been characterised and compared.

MATERIALS AND METHODS

PLANT MATERIAL

In this study, the olive oils obtained from three different cultivars have been involved: two of which were typical of Albania (Kalinjoti and UBT Tiranë), while the third was originally from Spain (Arbequina).
Kalinjoti is a cultivar population with multiple forms distinguished by the fruit and other agronomic characters. It represents the more widespread autochthonous cultivar at national level and is especially distributed on the coastal areas of Vlorë, Mallakastër, and Ionian. In these areas it occupies 85% of the olive grove and is particularly used to produce good quality oil (with a high yield, 28%), as well as aromatic and table olives [8].
The UBT Tiranë cultivar is mostly diffused in the Middle Albania, mainly in Tirana, Durrës, and Krujë. It is hardy to biotic and abiotic stress, in particular it is resistant against the prolongated summer drought and the coldness. As a result, it has a good tolerance to olive-knot disease (*Pseudomonas syringae* subsps. avastanoi). Such cultivar is characterised by late ripening and is reported to ensure high yield in good quality oil [8].
Arbequina is a Spanish cultivar particularly widespread in Catalogna. It is resistant to cold and is a salt tolerant variety, it is very tolerant to peacock and turmeric diseases and is highly appreciated for constant productivity. It enters production prematurely, fruits are moderately resistant to breakage, and their small size makes it difficult to harvest with mechanical tools. The oil quality is excellent, mainly for the high and well recognised organoleptic properties [9].

EXPERIMENTAL DESIGN AND SAMPLING

The olives were harvested by hand are immediately underwent a continuous extraction process, under the Protocol and the Rules of IOC [10]. Sampling of olive oils was carried out directly in an industrial mill during the harvesting season 2016-2017: samples were stored at room temperature in the dark until analysis.

FREE ACIDITY, PEROXIDE VALUE (PV) AND UV ABSORPTION

Free acidity (% oleic acid), peroxide value (PV) and UV spectrophotometric indices (K_{232} and K_{270}) were carried out according to the EEC Regulation No 2568 [11]. Each determination was performed in triplicate. PV and was expressed as milli-equivalents of active oxygen per kilogram of oil (meq O2/kg). Oil solutions (0.5%, wt/vol) were prepared in iso-octane, and the UV spectrum was recorded between 190 and 350 nm. K_{232}, and K_{270} and values were calculated from absorption at 232 and 270 nm, respectively. Repeatability of the K_{232} and K_{270} determinations was checked for five replicate solutions and was satisfactory (1.7 and 8.8%, respectively). Consequently, for each oil sample, one solution was prepared, and absorption was then measured in duplicate.

FATTY ACID PROFILE OF OLIVE OIL FROM SELECTED CULTIVARS

The GC analysis of FAMEs was carried out in a HRGC 8000 Series (Fison Instruments, Milan, Italy), equipped with a SPB2380 column (30 m, 0.32 mm, 0.2 lm i.d.) and a flame ionisation detector (FID). The GC oven temperature program was set at 165°C for 10 min, and then increased to 220°C at 10 C/min. Helium was supplied as the carrier gas at an initial pressure of 43 kPa. The injection and detector temperatures were set at 220°C and 230°C, respectively. The injection mode was split (1:40) and injection volume was 1 µL. Relative quantification was carried out according to the procedure reported in the IOC document [10].

DIFFERENCE BETWEEN THE THEORETICAL AND ACTUAL AMOUNTS OF TRIACYLGLYCEROLS

The analysis of intact TAGs was carried out using an Infinity 1260 HPLC (Agilent Technologies, Milan, Italy), which comprised a quaternary pump system, a column oven (Infinity 1260 TCC), an autosampler 1260 ALS, and a RID. The analytical procedure was carried out following the IOC methods for ΔECN42 [10].

DETERMINATION OF TOTAL PHENOLS

The total polyphenols content in olive oil samples was estimated by the Folin-Ciocalteu colorimetric method, according to the Singleton and Rossi procedure (1965) [12]. 10 µL of each sample were mixed with 90 µL of distilled water and 500 µL of freshly prepared 0.2 N Folin-Ciocalteu's reagent (1:10 v/v with water). After

10 min, 400 µL of satured sodium carbonate (75 g/L) were added. After incubation at 23°C (room temperature) for 1.5 h, the absorbance of the resulting blue coloured solution was measured at 765 nm with JENWAY 6305 UV/vis spectrophotometer. Quantitative evaluations were performed by using a standard calibration curve of six points (R^2 = 0.9944) ranging from 0 to 50 µg/mL of gallic acid in 80% methanol. The total phenolic content was expressed as gallic acid equivalents (GAE), in mg per kg of sample.

TOCOPHEROLS DETERMINATION

The tocopherols determination was performed following the procedure previously reported by Procida et al. (2009) [13]. A solution was prepared containing 1 g of oil in acetone (10 mL) and analysis by HPLC was performed by using a RP-C18 column (particle size 4 µm, l 250 mm, 4 mm i.d.). The injected volume was equal to 20 µL and sample was eluted with 0.5% H_3PO_4 water solution and acetonitrile/methanol 1:1 (v/v) with a flow rate of 1.3 mL min–1. A UV detector with a selected wavelenght of 295 nm was used. An integrator was used for the determination of the standard calibration curves and for the calculation of the tocopherols amounts in the oil samples. Results associated to each sample are the mean values of two replicates.

STATYSTICAL ANALYSIS

Statistical analysis of data was carried out by the Student's t-test. All numerical data are expressed as mean ± SD of at least three different experiments. Statistical significance was set at $P<0.01$ and $P<0.05$.

RESULTS AND DISCUSSION

In this study, several analytical approaches have been carried out in order to characterize the olive oils coming from three distinct cultivars of the Albanian territory. Specifically, evaluations on UV absorbance indices, free acidity, number of peroxides, fatty acid profiles, total polyphenols and tocopherols were performed.

As reported in Table I, the spectrophotometric absorption in the UV region did not show variations among the selected cultivars, performing the analysis both at 232 nm (K_{232}) and 270 nm (K_{270}). In Table I, interesting and significant differences were instead reported with regard to free acidity and number of peroxides in olive oil samples.

The free acidity observed in all samples was much lower than the upper limit of 0.8% established for the best commercial quality olive oil, indicated as 'extra' virgin (Regulations EEC 2568/91) [11]. About this, the Kalinjoti cultivar showed a significant lower value ($P<0.01$) with respect to the other samples, and this is a very interesting qualitative aspect, since the increase in free acidity is frequently correlated to an increase of lipolysis due to enzymatic mechanisms, and greater sensitivity to pathogenic infections and mechanical demage. As reported by Salvador et al. (2001) [14] the extent of these processes is amplified during the oil ripening, but, since no variables were introduced regarding the sampling period, the storage and the analysis of the samples, it is presumable that the observed difference is to be totally attributed to the variety of harvested olives.

Also, in the evaluation of the number of peroxides (Tab. I), the Kalinjoyi cultivar distinguished itself with respect to the other oil varieties, showing significantly lower values ($P<0.05$). Since the peroxide value indicates the presence of products deriving from primary lipid oxidation (hydroperoxides), this index is often used as indicator of oil stability and has been reported to be particularly influenced by the activity of lipoxygenases, whose action therefore contributes to the deterioration of the matrix and the production of volatile compounds that could negatively affect flavour and odour scores. Considering what has been previously reported by Frankel (1980) [15] for soy oil, flavor reversion could occur at peroxide values up to 10 mekO_2/kg, while oxidative rancidity occurs at values greater than 10 mekO_2/kg. In this work all the values found are less than 10, but the data concerning the kalinjoti cultivar must certainly be framed in a higher quality perspective.

Regarding the fatty acid analyses, it could be useful to consider the study of Stefanoudaki et al. (1999) [16] who demonstrated that the fatty acid composition of olive oils could be characterised by significant variations even within close geographical areas. In this study (Tab. II), significant differences were found between Spanish and Albanian cultivars at the level of

Table I - Basic analysis (acidity, number of peroxides, UV absorption characteristics) of olive oils from selected cultivars (harvesting 2016/17)

	Arbequina	Kalinjoti	UBT Tiranë
Acidity (% oleic acid)	$0.75^a \pm 0.08$	$0.27^b \pm 0.04$	$0.74^b \pm 0.08$
Number of peroxides (mekO_2/kg)	$8.50^a \pm 0.79$	$6.32^b \pm 0.61$	$8.38^a \pm 0.81$
Absorbance UV			
K_{232}	1.90 ± 0.16	1.81 ± 0.15	1.98 ± 0.17
K_{270}	1.37 ± 0.12	1.54 ± 0.14	1.46 ± 0.12

Data are reported as mean values ± standard deviation. [a,b] Different letters in the same row indicate significant differences ($P<0.05$).

palmitic acid (C16:0), palmitoleic acid (C16:1), *w*9 and *w*7 isomers of oleic acid (C18:1*w*9 and C18:1*w*7), and linoleic acid (C18:2) (no difference between Albanian cultivars).

Palmitic and palmitoleic acids were more represented in olive oil samples obtained from Arbequina cultivar ($P<0.05$ and $P<0.01$, respectively), and it is interesting to underline as the same samples are also differentiated with regard to the concentrations of oleic acid isomers ($P<0.01$). In fact, C18:1*w*9 is less represented in Arbequina in comparison to samples coming from Kalinjoti and UBT Tiranë cultivars, whereas for C18:1*w*7 the Spanish cultivar has the highest content. Overall, the oil which has higher concentrations of unsaturated compounds was detected in the Kalinjoti cultivar (85.84%) against 83.05% in UBT Tiranë and 80.41% in Arbequina. This finding is particularly interesting if we consider that a high dietary proportion of saturated fatty acid (SFA), is commonly associated to several diseases, which mainly affect the cardiovascular system [17, 18]. The variation in fatty acid composition due to an increase of unsaturated fatty acids is generally associated to an increase of oxidative susceptibility as reported by Cert et al. (1996) [19]. At the same time, it must also be considered that oxidative stability is greatly influenced by the presence of natural bioactive compounds with potential antioxidant activity. About this, the evaluation of total polyphenols in olive oil samples (Tab. III), has contributed in a decisive way in understanding the framework highlighted by the analyses carried out.

Phenols, reported as gallic acid equivalent, resulted as more represented in Kalinjoti samples ($P<0.01$); The detected concentrations were about 2.5 times higher with respect to Arbequina and 3.5 times higher in comparison with UBT Tiranë. This finding could, at least in part, justify the higher oxidative stability of olive oil obtained from Kalinjoti cultivars, in the face of a greater concentration of unsaturated fatty acids. It is in fact well known, that polyphenols are characterised by strong antioxidant properties that allow to protect a wide range of biological macromolecules from oxidative damage. In addition to this, it should also be considered that polyphenols have been credited over time with several nutraceutical properties; for instance numerous studies highlighted the role of these compounds in reducing the extent of inflammatory processes and curbing the onset and progression of cancer diseases [20]. According to what has just been reported, the finding concerning the higher amount of polyphenols in olive oil samples obtained from the Kalinjoti cultivar is rather interesting, although the exact characterisation of these compounds requires further and more specific analysis.

The analysis and characterisation of tocopherols (Tab. III) in the olive oil samples showed a fairly complex picture with UBT Tiranë which showed a higher content of these compounds both with respect to Kalinjoti ($P<0.01$) and Arbequina, although the difference was not significant ($P>0.05$) in this last case. Since these compounds are given undoubted antioxidant properties [21], the UBT Tiranë samples should have shown better oxidative stability than previously observed. This finding therefore testifies that multiple compounds are responsible for protecting the matrix from the oxidative damage and their mechanisms of action, as well as the mutual interactions, undoubtedly deserve more specific and in-depth evaluations.

Table II - Fatty acid composition of olive oils

	Arbequina	Kalinjoti	UBT Tiranë
C16:0	$17.27^{a} \pm 1.92$	$11.53^{b} \pm 1.06$	$13.34^{b} \pm 1.28$
C16:1	$2.57^{a} \pm 0.23$	$0.56^{b} \pm 0.07$	$0.61^{b} \pm 0.06$
C17:0	0.09 ± 0.01	0.09 ± 0.01	0.13 ± 0.02
C17:1	0.22 ± 0.03	0.16 ± 0.02	0.19 ± 0.02
C18:0	1.64 ± 0.15	2.34 ± 0.21	2.68 ± 0.24
C18:1 *w*9	$60.22^{a} \pm 3.02$	$71.36^{b} \pm 4.16$	$71.62^{b} \pm 3.88$
C18:1 *w*7	$4.39^{a} \pm 0.36$	$1.72^{b} \pm 0.16$	$1.53^{b} \pm 0.15$
C18:2	$12.09^{a} \pm 1.22$	$10.67^{a} \pm 0.97$	$8.14^{b} \pm 0.79$
C18:3	0.64 ± 0.07	0.62 ± 0.07	0.63 ± 0.06
C20:0	0.36 ± 0.04	0.40 ± 0.05	0.51 ± 0.06
C20:1	0.28 ± 0.04	0.33 ± 0.04	0.33 ± 0.03
C22:0	0.11 ± 0.01	0.10 ± 0.01	0.15 ± 0.02
C24:0	0.06 ± 0.01	0.06 ± 0.01	0.09 ± 0.01
UFA (%)	80.47	85.42	83.05
SFA (%)	19:47	14.52	16.90

Data are expressed as mean percentage of total fatty acids ± standard deviation.

[a,b] Different letters in the same row indicate significant differences ($P<0.05$)

Table III - Total polyphenols and tocopherols content in selected olive oils.

	Arbequina	Kalinjoti	UBT Tiranë
Total Phenols (mg GAE/kg)	$251.02^{a} \pm 14.88$	$636.13^{b} \pm 48.21$	$181.33^{c} \pm 17.59$
Total Tocopherols (mg/kg)	$192.32^{a} \pm 11.91$	$151.25^{b} \pm 8.54$	$204.73^{a} \pm 16.13$
α-Tocopherol (mg/kg)	$187.46^{a} \pm 12.82$	$145.42^{b} \pm 9.27$	$203.02^{a} \pm 15.56$
β-Tocopherol (mg/kg)	2.93 ± 0.25	3.57 ± 0.26	nd
γ-Tocopherol (mg/kg)	1.93 ± 0.17	2.26 ± 0.21	1.71 ± 0.19
δ-Tocopherol (mg/kg)	nd	nd	nd

Data are reported as mean values ± standard deviation. GAE = gallic acid equivalent. nd = not detectable. [a,b,c] Different letters in the same row indicate significant differences ($P<0.05$).

CONCLUSIONS

In this study the olive oils obtained from three different cultivars (Arbequina, Kalinjoti and UBT Tiranë) grown in the Albanian territory (harvesting 2016/17) were compared from a qualitative point of view. From the analysis performed, high qualitative properties emerged in all the selected cultivars, although the oil samples obtained from Kalinjoti showed overall greater oxidative stability, greater amount of unsaturated fatty acids and a higher concentration of bioactive compounds credited with antioxidant properties.
All this is particularly interesting if we consider the growing attention that the consumers have been showing on this matter for some time, the choice of food products associated to a greater health value.

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