Basic characteristics and quality of virgin olive oil produced in Corfu (cv. Lianolia) and South Albania (cv. Kalinioti)

Konstantoula Akrida-Demertzi[1], Christos Roukos[2] and Panagiotis Demertzis[1]

1 University of Ioannina, Department of Chemistry, Laboratory of Food Chemistry,
45500 Ioannina, Greece
2 Ministry of Rural Development and Food, Regional Department of Epirus & Western Greece,
Terma Panepistimiou Str, 45110 Ioannina, Greece

Abstract

In the present study the qualitative characteristics such as peroxide number, absorption coefficients (K_{232}, K_{270}), acidity, total phenols, a-tocopherols, chlorophylls and composition of fatty acids were determined in 19 virgin olive oil samples of "Lianolia" variety cultivated in Corfu and "Kalinioti" variety cultivated in South Albania, harvested in the crop year 2013/2014.

Obtained results indicate that all olive oil samples show chemical and physicochemical parameters included in the limits established for virgin olive oils and some of them in the limits for "extra" virgin olive oils.

Moreover it was found that the qualitative characteristics of olive oil samples are affected by variety, fruit treatment and the method of olive harvesting (in Corfu olives are collected by hands from plastic nets under the trees, whereas in Albania they are basically collected by hand after falling on the ground). The results of the present study are a first indication of the olive oil quality produced in Corfu and South Albania.

Keywords: Virgin olive oil; total phenols; a-tocopherols; chlorophylls; fatty acids

Introduction

Virgin olive oil (VOO) is the supernatant of the natural juice obtained exclusively by mechanical and physical processes from fresh and healthy olive fruits (*Olea europea* L.), such as washing, pressing, malaxation of olive paste, centrifugation, decantation, and filtration that do not modify its characteristics [1]. Virgin olive oil is a main agriculture product and plays an important role in the Greek agronomy and economy in Greece which is ranked third after Spain and Italy. The annual production is around 310.000 t olive oil produced from 531.000 oil farms, covering a total of ~730.000 ha, and the annual olive oil consumption is estimated around 220.000 t. [2].

Nowadays, it is well-known that regular dietary consumption of virgin olive oil manifests in health benefits associated with Mediterranean diet. The nutritional value of virgin olive oil arises from high level of oleic acid and from minor components such as phytosterols, carotenoids, tocopherols and total phenols. The major phenolic compounds are oleuropein derivatives, based on hydroxytyrosol, which are strong antioxidants and radical scavengers [3]. Considering that virgin olive oil is obtained from the olive fruit solely by mechanical and physical means, it retains more health beneficial phytochemicals as compared to other commercially available refined vegetable oils, which are generally solvent extracted [4].

Among minor components of olive oil, tocopherols and phenolic compounds represent antioxidants. In particular, phenolics make important contribution to the nutritional properties, the sensory characteristics, and the shelf life of olive oil. Indeed, it is known that the healthy properties of the phenolic compounds are correlated to their antioxidant activity [5].

The definition of the quality features of every edible product is of particular interest to both consumers and producers. Consequently it is justifiably necessary to define the quality level of olive oil which is an essential agricultural product of Greece and South Albania [6].

In the present study the quality characteristics such as peroxide number, absorption coefficients (K_{232}, K_{270}), acidity, total phenols, a-tocopherols, chlorophylls and composition of fatty acids were determined in 19 virgin olive oil samples of "Lianolia" variety cultivated in Corfu-Greece and "Kalinioti" variety cultivated in South Albania, harvested in the crop year 2013/2014.

The main objective of the present study was to investigate how olive oil quality is affected by variety and harvesting time and for this reason the two most common varieties in Corfu and South Albania ("Lianolia" and "Kalinioti") were comparatively studied.

Materials

Samples: 15 virgin olive oil samples of "Lianolia" variety cultivated in Corfu and "Kalinioti" variety cultivated in South Albania, collected in the crop year 2013/2014, were used for physicochemical analysis

Reagents and standards: The standards of methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate, methyl linolate and methyl linolenate were purchased from Sigma- Aldrich (Steinhaim, Germany). Methanol, n-hexane and 1-propanol were all of HPLC grade and were purchased from Lab-scan, Gliwice, Poland.

Apparatus:

-Spectrophotometer Milton Roy 1201

-A Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of a solvent delivery module (LC-10AD) with a double plunger reciprocating pump, UV-VIS detector (SPA-10A), column oven (CTO-10A) and 20 μl injection loop was used. The column used was an RP-Apex octadecyl C_{18} (15x0.4cm i.d.) with 5μm packing (Jones Chromatography limited, Colorado, USA).

-Gas Chromatograph Fisons GC-9000 series equipped with capillary column Carbowax 30m x 0.32mm i.d and FID detector.

Analytical Methodology

Free Acidity, Peroxide number, Specific UV Absorbances, Chlorophyll content, Polyphenols and α-tocopherols

Acidity (given in % of oleic acid) was measured by volumetric analysis [7].

Peroxide number (meq/Kg) and absorption coefficients (K_{232} and K_{270}) were determined according to 2.501 and 2.505 IUPAC methods [8].

Chlorophyll content was measured according to AOCS method [9].

Polyphenols (Total Phenolic Content-TPC) were determined by colorimetric Folin Ciocalteu method. Phenolic compounds were isolated by a triple extraction with a methanol/water mixture (60:40, v/v). The Folin–Ciocalteu reagent was added to a suitable aliquot of the combined extracts, and the absorption of the solution was measured spectrophotometrically at 725 nm. The total phenols were expressed as mg caffeic acid/kg oil using a standard curve (calibration curve with $r^2 = 0.99$) [6].

α-tocopherols were analyzed by HPLC at 294 nm using UV-VIS detector. Olive oil solutions (8% w/v) were prepared in a solvent mixture n-haxane/2-propanol 99:1 v/v and filtered through a 0.45 μm membrane filter. n-Hexane/2-propanol (99:1) v/v (A) and 2-propanol (B) were used as eluents. The gradient was 0% B for 10 min 0-5% B in 4 min, 5% B for 6 min, 5-0% B in 4 min and 0% b for 6 min. Separation was achieved on a 150 mm C18 column at a 1.2 ml/min flow rate. The injection volume was 20 μl. α-tocopherol standard solutions were prepared in solvent A. Tocopherols were detected at 294 nm by the UV-VIS detector and for quantification standard curves (concentration versus peak area) were calculated by linear regression analysis. Injections in duplicate were made at each concentration for both standards and samples [8–13].

Fatty acid analysis was performed by GC (8). A chromatographic analysis was performed in a Fisson 9000 gas chromatography, equipped with a FID detector and a Thermo Quest auto sampler using a capillary DB-wax column (length 30 m, internal diameter 0.32 mm and thickness 0.32 μm). The following GC parameters were kept constant: detector temperature: 240 ^{0}C, injector temperature: 220 ^{0}C, injector mode: split with split ratio 1:18, injection volume 1 μl. The applied temperature program was 180 ^{0}C (5 min), from 180 ^{0}C at a rate of 3 ^{0}C/min to 210 ^{0}C (20 min), cooling rate 50 ^{0}C/min to final 180 ^{0}C. Fatty acids were identified by comparing retention times with standard compounds.

Statistical analyses

Data were statistically analyzed using a factorial design where harvesting time (n=3) and sites (n=5) are the study factors, with interaction between harvesting time and sites [10]. The interaction harvesting time × sites was significant (P < 0.05), thus analyses of variance were conducted among harvesting times and among sites within harvesting times. Significant mean differences were detected using least square differences [11]. Pearson's correlations were calculated to examine interactions between quality parameters [11]. Differences were considered significant at the p<0.05 level.

Results and Discussion

In order to carry out the present investigation, the area of Albania was divided into 3 zones (Mountainous, Field and Coastal) and Corfu into 2 zones (North and South) i.e. into zones with different soil-climate characteristics. In the existing olive groves of every zone, an experimental field, planted with the above mentioned varieties and being of a suitable size, was chosen.

Olive fruit samples used for the production of olive oil to be analyzed were harvested from the above experimental fields. The samples were collected in three different periods: end of November, end of December and end of January. In Corfu olives are harvested by hands from plastic nets under the trees, whereas in Albania they are basically harvested by hand after falling on the ground.

In Table 1 data on origin, harvesting and storage of olives from "Lianolia" and "Kalinioti" varieties used for olive oil production as well as the type of olive mill are given.

Table 1. Data on origin, harvesting and storage method of olives from "Lianolia" and "Kalinioti" varieties and type of olive mill.

Sample number	Area	Variety	Site	Harvesting time	Harvesting method	Storage time	Storage method	Olive mill type
1	South Albania	Kalinioti	A	End of November				
6	South Albania	Kalinioti	A	End of December				
7	South Albania	Kalinioti	A	End of January				
2	South Albania	Kalinioti	B	End of November				
5	South Albania	Kalinioti	B	End of December				
8	South Albania	Kalinioti	B	End of January				
3	South Albania	Kalinioti	C	End of November				
4	South Albania	Kalinioti	C	End of December	By hand	5 days	In sacks	Centrifugal
9	South Albania	Kalinioti	C	End of January				
10	Corfu	Lianolia	D	End of November				
11	Corfu	Lianolia	D	End of December				
12	Corfu	Lianolia	D	End of January				
13	Corfu	Lianolia	E	End of November				
14	Corfu	Lianolia	E	End of December				
15	Corfu	Lianolia	E	End of January				

The quality characteristics of the investigated virgin olive oil samples are given in Table 2.

The chemical composition and quality of virgin olive oil are influenced by a variety of factors among them are geographical production area (altitude, soil composition, latitude) climatic conditions prevalent in the year of production, the cultivar and the extraction process [12].

In Table 3 the correlation coefficients between harvest time and quality characteristics of olive oil from five different sites are presented. During the study period, as ripening progresses, concentrations of α-tocopherols, and peroxide number increased significantly. Total polyphenols and chlorophyll contents showed significant (P<0.01) negative correlation with increasing level of maturation.

The quality characteristics of olive oil produced at the 5 different sites in relation to harvesting time are given in table 4.

Based on the data from Tables 2 and 4 and the national and European legislation [13, 14], it is noted that acidity, peroxide number and absorption coefficients (K_{232}, K_{270}) values for all samples show that they belong to the category of virgin olive oil. 3 samples (11, 12 and 15) belong to the category of extra virgin olive oil, 2 samples (9 and 14) to the category of lampante virgin olive oil [6, 13-18].

Acidity values show considerable variations among the studied samples of both varieties. These variations can be mainly attributed to the different harvesting techniques followed in Corfu and S. Albania.

Except for the first harvesting time (end of November), the acidity in all olive oil samples was significantly different among sites and among sites within harvest times,

Olive oil samples from Site C had the highest acidity values, while samples from Site D the lowest (Table 4). The most of the olive oil samples exhibited their peak values in acidity content at the second harvesting time (end of December).

Furthermore, peroxide number values vary among the studied varieties. Slightly variations are also observed in relation with both harvesting method and zone origin of olives.

Absorption coefficients values are within the limits established for edible virgin olive oils [6, 13-18]

The concentration of polyphenols and tocopherols is more or less influenced by the variety, harvesting time and harvesting method. The concentrations of total phenols for the studied samples were between 32.05 and 199.47 mg/kg. Similar concentrations for these ingredients in Greek olive oils were reported by other researchers [2].

Also the α-tocopherol (α-T) concentrations for the studied samples were found between 47.39 and 158.54 mg/kg. Past food chemistry reference books reported rather low levels of α-T for olive oils (<150 mg/kg). The concentration of α-T in a virgin olive oil depends on pedoclimatic, agronomic and technological factors. Genetic factors in some cases are becoming important. Current legislation on health claims gives an opportunity for advertisement of virgin olive oil as a good source of vitamin E and points to an urge for systematic knowledge of the potential of different olive cultivars and technological systems to yield extra virgin olive oil of high content in this bioactive compound. Moreover, its levels in commercial products should be also examined. Industrial and domestic handling conditions and practices are also critical factors for the maintenance of the prone to oxidation α-T. The recognition of the health benefits of polar phenolic compounds together with the fact that virgin olive oil is a good source of vitamin E are expected to influence consumption of virgin olive oil worldwide if their contents are in line with legislative requirements [2].

The polyphenols content in all olive oil samples was significantly different among sites and among sites within harvesting times (Table 4). During harvesting period (November through January) polyphenols contents of olive oils samples from Site A and B, showed a decrease with time of harvest. Total polyphenol level in the present study showed a clear negative correlation with increasing levels of maturation. Similar results were reported by other investigators [12].

Polyphenol content of oil strongly influences its oxidative stability, nutritional properties and flavor. The extent of quality deterioration of virgin olive oil depends on the storage conditions e.g. temperature, light, presence of oxygen in headspace, headspace volume, and on the package material. The changes expected are mainly caused by hydrolytic and oxidative phenomena. During storage, virgin olive oil antioxidants, as more oxidisable, contribute to extending the shelf life of the polyunsaturated fatty acids. The changes in the content and type of polyphenolic constituents affect the sensory characteristics of the oil. Less bitter and pungent oils are obtained even after six months of storage [2].

Chlorophyll content in the studied samples was ranged between 0.03 and 11.05 ppm and its content seems to be mainly depended on the variety and the harvesting method. As ripening progresses, photosynthetic activity decreases and the concentrations of chlorophylls decrease progressively [12]. Similar chlorophyll concentrations were found in virgin olive oil samples from other countries, in particular from Tunisia [19-21].

Variations in oleic acid content were observed among olive tree varieties and zone of origin.

Finally, variations in the ratios of unsaturated/ saturated and monounsaturated/ polyunsaturated fatty acids among the studied varieties were observed.

In conclusion, obtained results, although preliminary, could give a first indication of the quality of olive oil produced in Corfu and S. Albania.

Table 2: Results of two-way ANOVA showing degrees of freedom, F-statistic and the significance of the effects of harvesting time and sites interactions on quality characteristics of studied olive oil.

Source of variation	d.f.	Acidity F	Acidity P	Polyphenols (mg/Kg) F	Polyphenols (mg/Kg) P	α-Tocopherols (mg/Kg) F	α-Tocopherols (mg/Kg) P	Peroxide Number (meq/Kg) F	Peroxide Number (meq/Kg) P	Methyl Palmitoleate % F	Methyl Palmitoleate % P
Site (S)	4	192,796	0,000	338,020	0,000	5779,442	0,000	313,551	0,000	35,375	0,000
Harvest Time (H)	2	14,171	0,000	2529,660	0,000	240,103	0,000	454,399	0,000	3,549	0,041
S × H	8	148,294	0,000	1258,448	0,000	536,486	0,000	209,401	0,000	4,829	0,001

Source of variation	d.f.	UV K_{232} F	UV K_{232} P	UV K_{270} F	UV K_{270} P	Chlorophyll (ppm) F	Chlorophyll (ppm) P	Methyl Palmitate % F	Methyl Palmitate % P	Methyl Stearate % F	Methyl Stearate % P
Site (S)	4	4,691	0,005	5,598	0,002	6025,597	0,000	7717,999	0,000	364,316	0,000
Harvest Time (H)	2	12,089	0,000	11,413	0,000	4913,542	0,000	847,692	0,000	11,169	0,000
S × H	8	41,907	0,000	15,543	0,000	4845,205	0,000	1419,258	0,000	35,282	0,000

Source of variation	d.f.	Methyl Oleate % F	Methyl Oleate % P	Methyl Linoleate % F	Methyl Linoleate % P	Methyl Linolenate % F	Methyl Linolenate % P
Site (S)	4	1012,495	0,000	763,902	0,000	341,419	0,000
Harvest Time (H)	2	170,289	0,000	346,745	0,000	215,755	0,000
S × H	8	109,403	0,000	86,043	0,000	431,906	0,000

Table 3. Correlation coefficients between harvest time and quality characteristics of olive oil from five different sites.

	Harvesting time	Acidity	Polyphenols	α-Tocopherols	Peroxide_Number	Chlorophyll	Methyl_Palmitate_%	Methyl_Palmitoleate_%	Methyl_Stearate_%	Methyl_Oleate_%	Methyl_Linoleate_%	Methyl_Linolenate_%
Harvesting time	1											

Acidity	0,116	1									
Polyphenols	-0,268	-0,092	1								
α-Tocopherols	0,064	**0,171**[*]	-,059	1							
Peroxide_Number	**0,367**[*]	**-0,452**[**]	**-0,455**[**]	**-0,183**[**]	1						
Chlorophyll	**-0,298**[*]	-0,177	**0,708**[**]	**-0,196**[*]	**-0,356**[*]	1					
Methyl_Palmitate_%	-0,196	-0,248	0,129	**0,0313**[**]	0,272	**-0,314**[*]	1				
Methyl_Palmitoleate_%	-0,180	**-0,323**[*]	0,184	**0,352**[**]	0,257	-0,191	**0,867**[**]	1			
Methyl_Stearate_%	0,077	0,166	0,062	**-0,442**[**]	-0,201	**0,570**[**]	**-0,859**[**]	**-0,768**[**]	1		
Methyl_Oleate_%	0,246	**0,311**[*]	-0,104	**-0,247**[**]	**-0,317**[*]	**0,314**[*]	**-0,976**[**]	**-0,880**[**]	**0,862**[**]	1	
Methyl_Linoleate_%	**-0,335**[*]	**-0,521**[**]	-0,070	**-0,028**[**]	**0,320**[*]	-0,248	**0,527**[**]	**0,596**[**]	**-0,545**[**]	**-0,650**[**]	1
Methyl_Linolenate_%	0,060	0,043	-0,213	**-0,199**[*]	-0,008	**-0,507**[**]	0,158	0,136	-0,270	-0,199	**0,460**[**]	1

*: P<0.05, **:P<0,01

Table 4. Mean values of olive oil quality characteristics produced at five (5) different sites in relation to harvesting time.

	Site A	Site B	Site C	Site D	Site E	Mean	SEM	Sig.
	Acidity							
End of November	0,93	1,63	1,47	0,87	1,56	1,29	0,063	***
End of December	1,53	1,43	1,26	0,57	2,37	1,43	0,079	***
End of January	1,71	1,87	3,07	0,43	0,38	1,49	0,108	***
Mean	1,39	1,64	1,93	0,62	1,44	1,41		
SEM	0,112	0,082	0,128	0,047	0,107			
Sig.	***	**	***	***	***			
	Polyphenols (mg/Kg)							
End of November	171,3	77,6	48,1	85,9	52,3	87,0	1,30	***
End of December	45,3	73,9	177,4	173,6	136,6	121,4	1,95	***
End of January	50,6	54,4	79,2	53,1	36,6	54,8	2,76	***
Mean	89,1	68,6	101,6	104,2	75,2	87,7		
SEM	1,61	1,61	2,84	2,51	5,87			
Sig.	***	***	***	***	***			
	α-Tocopherols (mg/Kg)							
End of November	47,5	92,0	48,1	137,0	114,4	87,8	0,86	***
End of December	50,7	67,1	76,6	124,1	95,7	82,8	1,29	***
End of January	52,3	53,2	103,5	141,2	116,7	93,4	1,03	***
Mean	50,1	70,8	76,1	134,1	108,9	88,0		
SEM	0,72	0,94	1,00	1,34	1,24			
Sig.	***	***	***	***	***			
	Peroxide Number (meq/Kg)							
End of November	8,0	13,6	10,0	14,0	7,1	10,5	0,41	***
End of December	9,9	13,9	7,8	11,6	5,8	9,8	0,11	***
End of January	10,2	11,4	12,0	16,6	17,4	13,5	0,28	***
Mean	9,4	13,0	9,9	14,1	10,1	11,3		
SEM	0,19	0,12	0,10	0,13	0,58			
Sig.	***	***	***	***	***			
	UV K_{232}							
End of November	1,92	1,85	1,70	1,94	1,75	1,83	0,072	*
End of December	2,18	1,96	1,89	1,76	1,45	1,85	0,051	***
End of January	1,53	2,02	2,10	1,89	2,20	1,95	0,053	***
Mean	1,88	1,94	1,90	1,86	1,80	1,88		
SEM	0,056	0,047	0,041	0,057	0,044			
Sig.	***	*	***	*	***			
	UV K_{270}							
End of November	0,16	0,20	0,17	0,18	0,23	0,19	0,009	***
End of December	0,23	0,17	0,20	0,21	0,20	0,20	0,007	***
End of January	0,17	0,19	0,20	0,20	0,19	0,19	0,008	***
Mean	0,19	0,19	0,19	0,19	0,21	0,19		
SEM	0,007	0,008	0,008	0,005	0,006			
Sig.	***	*	*	***	**			
	Chlorophyll (ppm)							
End of November	9,73	2,98	3,27	2,09	0,85	3,79	0,059	***
End of December	2,78	2,35	11,08	3,46	0,60	4,05	0,070	***
End of January	1,51	1,87	2,26	1,37	1,04	1,61	0,051	***
Mean	4,67	2,40	5,54	2,31	0,83	3,15		
SEM	0,058	0,084	0,051	0,056	0,042			
Sig.	***	***	***	***	***			

	Site A	Site B	Site C	Site D	Site E	Mean	SEM	Sig.
	Methyl Palmitate %							
End of November	10,83	16,52	10,22	15,38	16,37	13,86	0,101	***
End of December	9,51	12,15	11,57	16,14	15,99	13,07	0,092	***

End of January	9,06	8,77	13,11	16,02	15,47	12,49	0,055	***
Mean	9,80	12,48	11,63	15,85	15,94	13,14		
SEM	0,065	0,067	0,052	0,119	0,062			
Sig.	***	***	***	***	***			
				Methyl Palmitoleate %				
End of November	0,14	0,77	0,14	0,90	0,79	0,55	0,031	***
End of December	0,06	0,23	0,50	0,91	0,62	0,47	0,096	*
End of January	0,08	0,15	0,38	0,69	0,72	0,40	0,076	***
Mean	0,09	0,38	0,34	0,83	0,71	0,47		
SEM	0,037	0,039	0,012	0,017	0,014			
Sig.	***	***	***	***	***			
				Methyl Stearate %				
End of November	3,11	2,48	2,80	2,15	1,83	2,47	0,055	***
End of December	2,75	2,50	2,89	1,94	2,13	2,44	0,037	***
End of January	2,92	2,82	2,50	2,18	2,30	2,54	0,088	***
Mean	2,93	2,60	2,73	2,09	2,09	2,49		
SEM	0,059	0,034	0,059	0,045	0,054			
Sig.	***	***	***	**	***			
				Methyl Oleate %				
End of November	80,37	73,78	80,83	73,23	73,80	76,40	0,378	***
End of December	82,40	78,21	79,51	74,37	74,93	77,88	0,185	***
End of January	82,62	81,80	78,62	73,59	75,68	78,46	0,148	***
Mean	81,80	77,93	79,66	73,73	74,80	77,58		
SEM	0,466	0,219	0,179	0,111	0,148			
Sig.	***	***	***	***	***			
				Methyl Linoleate %				
End of November	5,46	6,35	6,65	7,48	6,34	6,46	0,068	***
End of December	5,48	5,57	5,74	6,64	6,03	5,89	0,047	***
End of January	5,37	6,06	5,06	7,16	5,90	5,91	0,044	***
Mean	5,43	6,00	5,82	7,09	6,09	6,09		
SEM	0,039	0,042	0,039	0,083	0,050			
Sig.	***	***	***	***	***			
				Methyl Linolenate %				
End of November	0,13	0,11	0,21	0,22	0,14	0,16	0,004	***
End of December	0,12	0,09	0,08	0,13	0,27	0,14	0,003	***
End of January	0,15	0,20	0,13	0,18	0,18	0,17	0,004	***
Mean	0,13	0,13	0,14	0,18	0,20	0,16		
SEM	0,004	0,003	0,004	0,002	0,004			
Sig.	***	***	***	***	***			

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