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Effect of region of cultivation, tree age, and harvest time on the quality of Lebanese virgin olive oil

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PAPER

Abstract

In Lebanon, olive oil plays an essential role at both economic and social levels. However, factors influencing its quality are rarely addressed. This preliminary study is the first analysis to show a comparison between ancient and adult virgin olive oil trees in Lebanon. Analysis of pedo-climatic conditions and physicochemical parameters of virgin oil samples taken from ancient and adult trees from three Lebanese regions (Bechmizine, Kfaraaka, and Kawkaba) and at two different harvest periods showed that these parameters differed significantly among cultivation regions, while the tree age and harvest time had a lower effect. We observed that the oil obtained from adult trees of Kawkaba region during the first harvest period (green to reddish stage of fruit maturity) had the best quality, compared to all other samples. Oil produced from these trees showed the highest polyphenol content, a relatively higher composition of tocopherols, oleic acid, monounsaturated fatty acids, and trans fatty acids C18: 1–C18: 2, with relatively low acidity and peroxide values. It was concluded that the quality of virgin olive oil was associated with its chemical composition, and was the result of a complex interaction between several environmental factors associated with the area of cultivation, tree age, and harvest time.

Keywords: adult trees, ancient trees, harvest date, Lebanon, region of cultivation, virgin olive oil

Introduction

Olive oil is an integral part of cultural and culinary heritage of Mediterranean countries, as it is the oldest crop in their history and their main edible oil, while other countries, such as the United States, Chile, and New Zealand, have made it a valuable commodity (Aparicio and Harwood, 2013). Olive oil is mainly appreciated for its characteristic taste and therapeutic, dietary, and nutritional values (Sotiroudis *et al.*, 2003).

Composition of olive oil is closely correlated with its production. The quality and quantity of olive oil is influenced by different interrelated factors, leading to a dynamic multivariate structure (Inglese *et al.*, 2011). Although the genotype is a key factor (Ripa *et al.*, 2008), olive trees are subjected to environmental factors (geographic locations, soil type, and soil water content), agronomic practices (crop load, irrigation, and pruning), and climatic conditions (temperature, rainfall, and humidity), all playing crucial roles in determining oil content and composition. In addition, age of tree (Chtourou Bouchaala *et al.*, 2014, 2017; Rouas *et al.*, 2016), and especially oil extraction method (Bendini *et al.*, 2012; García and Jesús, 2012) and storage conditions (Ouedrhiri *et al.*, 2017), plays an important role in determining the quantity of some olive oil components.

Lebanon is historically known for olive cultivation that dates back to the Phoenician period. Olive trees are on average 150 years old, and centennial trees are distributed in many areas from the north to the south under

different pedo-climatic conditions (Chalak *et al.*, 2011). Olive fruits are harvested for oil production from adult and ancient olive trees distributed all over the country in mid-October in coastal areas, and from mid-October to mid-November, at higher altitudes, after the first rains (Chehade *et al.*, 2012). Several agricultural practices applied by olive farmers, such as late harvesting period, managing the harvest by beating tree shoots using wood sticks, collecting of damaged olive fruits from the ground and mixing them with healthy fruits, transporting of olive fruits into plastic bags, and the long time spent prior to processing, decreased the quality of olive oil produced in Lebanon (Consultation and Research Institute and ACTED Lebanon, 2018).

Although few studies have investigated the quality of olive oil produced from Lebanese olive cultivars (El Riachy, 2019) as well as the effect of geographical origin (agro-climatic conditions) and harvest time (ripening stage) (Dib *et al*., 2020; El Riachy, 2018; Merchak, *et al*., 2017), no study has classified and characterized the influence of tree age on the quality of olive oil. Hence, in the present study, effects of age of olive tree (ancient and adult), harvest period (two harvest periods were chosen), and region of cultivation were investigated to find the best growth area and conditions for obtaining high virgin olive oil quality in Lebanon.

Materials and Methods

Plant material

The study was conducted during the growing season 2018–2019. Two groups of tree age from "Baladi" cultivar were chosen: ancient "An" (trees aged more than 150 years old) and adult "Ad" (trees aged between 80 and 100 years) from three different Lebanese regions, namely Bechmizzine "B," Kfaraaka "L" (north of Lebanon), and Kawkaba "K" (south of Lebanon). The choice of these regions was based on their characterization by different microclimates and the presence of two different tree ages in each region. All trees were rain-fed and fertilized twice a year: 20 kg of poultry manure in autumn (mainly after the harvest season) and 5 kg/tree of ammonium sulfate (21-0-0) in spring (mid-February to first week of March). A total of 5 kg of olives from each tree age in each region were picked at two harvesting periods: the first was at veraison (ripening) stage (ripening index 2 to 3), as it was proved to obtain the best quality of olive oil (Bellincontro *et al.*, 2012; Chtourou Bouchaala *et al.*, 2014; Gutiérrez *et al.*, 1999; Inglese *et al.*, 2011), and the second was on harvest date chosen by olive grower of each orchard (Table 1). Only healthy fruits without any kind of infection or physical damage were harvested manually, placed in a plastic box, and stored in a portable igloo isulated box for transportation to laboratory for processing.

The weather data for Bechmizzine "B" and Kfaraaka "L" regions were obtained online from the website (weatherlink.com, private weather stations) and for Kawkaba region from the Lebanese Agricultural Research Institute (LARI) through their weather station located in Hasbaya.

Soil from each of the selected orchards was analyzed. Using a sheathed auger, soil sample was taken randomly at five points under trees from a depth of 30–40 cm. All soil samples were oven-dried at 40°C for 48 h, ground, and sieved through a 2-mm aperture to remove pebbles–roots–leaves etc. The analyses were performed according to the official methods indicated by the D.M. No. 79 of 11/05/1992 and D.M. No. 185 of 13/09/1999 and subsequent amendments approved by Italy's Ministry for Agricultural and Forestry Policies (1999).

Oil extraction

For oil extraction, 5 kg of healthy olives were chosen from each tree age group alone. The oil was extracted within 24 h of olive harvesting using a small-size Abencor mill (MC2; Ingenieria y Sistemas, Seville, Spain), simulating commercial oil extraction systems. The olives were first washed with water and then crushed in a hammer mill (Abencor MM-100, Ingenieria y Sistemas, Seville, Spain) equipped with a 4-mm sieve. The obtained paste was then processed using a malaxer (Abencor TB-100, Ingenieria y Sistemas, Seville, Spain) (30 min at 25°C) and centrifuged (Abencor, CF-100, Ingenieria y Sistemas, Seville, Spain) at 3500 rpm for 2 min. The oil was poured

Table 1. Olive harvest dates for two harvest periods.

Sample name	Crop season	Date of 1st harvest	Ripening index (RI)	Date of 2nd harvest	Ripening index (RI)
BAn	2018–2019	26 Sep	2.5	24 Oct	4
BAd	2018–2019	26 Sep	2.4	24 Oct	3.8
LAn	2018–2019	02 Oct	2.2	29 Oct	3.5
LAd	2018–2019	02 Oct	2.3	29 Oct	3.6
KAn	2018–2019	25 Sep	2.8	13 Nov	4.5
KAd	2018–2019	25 Sep	2.3	17 Oct	3.5

into amber 225-mL glass bottles without head space and stored at 4°C in dark until analysis.

Determination of oil content

The oil content of the olive paste was determined by gravimetric analysis using a solvent automatic extractor (Series 158 series; Velp Scientifica, Usmate Velate MB, Italy). Olive paste, 2 g, was stored at 4°C until extraction, and dried for 24 h at 105°C to determine oil content in dry matter. Each sample was placed in previously weighed flasks. Petroleum ether was used as a solvent. The total length of the program was 2 h and 30 min. It consisted of the following five cycles: immersion for 1 h, removing for 10 min, washing for 50 min, recovery for 30 min, and cooling for 10 min. Once the process was complete, the flasks were removed, placed in an oven at 105°C for 1 h, and then placed in desiccators for 12 h before being weighed. Oil content was expressed as percentage of dry matter. Every sample was subjected to three repetitions.

Analytical determinations

Routine parameters, such as free acidity (% oleic acid) and peroxide value (PV), were determined according to the methods described by International Olive Council (IOC, 2017a, 2017b). Other analysis was carried out in duplicate to determine the following:

Chloropylls

Chlorophylls were measured according to the spectrophotometric method reported by Pokorny *et al.* (2007) using a spectrophotometer (Model. 8453; Hewlett-Packard, Palo Alto, CA, USA). The chlorophyll content (C) is expressed in terms of milligram of pheophytin A per kilogram of oil.

Tocopherols

Tocopherols were determined and quantified by the method proposed by Conte *et al*. (2019) using an HPLC Agilent 1100 Series (Agilent Technologies, Palo Alto, CA, USA) equipped with a fluorescence detector (FLD; Agilent Technologies, Palo Alto, CA, USA) set at 290 nm for excitation and at 330 nm for emission, and a Gemini C18 column (100-mm length × 4.6-mm internal diameter; 3-mm particle size; Phenomenex, Torrence, CA, USA). The mobile phase was methanol–water (98:2 v/v) and the flow rate was 2 mL/min (Gimeno *et al.*, 2002).

Total polyphenols

Total polyphenols were extracted according to the method proposed by Pirisi *et al.* (2000). In particular, 4 mL of hexane and 4 mL of methanol–water (ratio 70:30 v/v) were added to 10 g of olive oil and mixed on vortex for 3 s. Subsequently, the mixed solution was agitated for 10 min using a magnetic stirrer. After agitation, the solution was centrifuged for 10 min at 4°C and 6000 rpm. After that, the residue obtained was removed and centrifuged for 5 min at 4°C and 9000 rpm. The obtained hydroalcoholic phase was filtered and collected using a 0.45-mm syringe filter.

The extract was subjected to spectrophotometric determination of total polyphenols following Singleton and Rossi (1965) using the above-mentioned spectrophotometer. Results were expressed as milligram of gallic acid per kilogram of oil.

Antioxidant activity

A changed free radical 2,2-diphenyl, 1-picrylhydrazyl (DPPH) approach, defined by Atoui *et al.* (2005), was used to assess the antioxidant activity of olive oil. A DPPH solution 0.25 g L^{-1} (0.0634 μmol mL^{-1}) was prepared. The antioxidant activity was determined on the polyphenol extract with the DPPH stable radical. Under continuous stirring, 50 mL of extract was added to 2.8 mL of DDPH solution. Decrease in absorbance at 515 nm was recorded every 60 s until the plateau was reached (30 min) against a blank of methanol using the above-mentioned spectrophotometer. Results were expressed as percentage decrease in absorbance per milligram of oil when 0.17 μmol of DPPH was available for reaction.

Fatty acids

The content of fatty acids for olive oil samples was analyzed according to Gerber method for milk and the Gerber-Van Gulik method (International Organization for Standardization [ISO], 1975). In brief, 1 g of olive oil was added to 0.4-mL ammonia (NH_3) 25%, 2-mL ethyl alcohol (C_2H_6O) 95%, and 4-mL hexane (C_6H_{14}). Samples were vortexed for 1 min and centrifuged at 3000 rpm, and the upper layer was collected. Another extraction was executed, this time using ethyl alcohol 95% (1 mL) and hexane (5 mL); samples were also vortexed for 1 min and centrifuged at 3000 rpm and the upper layer was collected. A final extraction was done by adding 5 mL of hexane and the samples were vortexed for 1 min and centrifuged at 3000 rpm before obtaining the upper layer. The fatty acid methyl esters (FAME) were prepared in conjunction with the FIL-IDF standard protocol (Cantellops *et al.*, 1999) with base-catalyzed transesterification.

Statistical analysis

A principal component analysis (PCA) was performed on all physicochemical parameters of oil to investigate the factor–among cultivation region, harvest time, and tree age—that mostly affected the oil parameters analyzed. Therefore, to do a more in-depth evaluation of the

data, one-way ANOVA analysis was conducted for each region to evaluate whether the other two factors (tree age and harvest time) could have influenced the chemical parameters at least within the individual region. The statistical analysis was performed using GraphPad Prism (version 9). Fisher's least significant differences (LSD) test was used to evaluate differences when analyzing factors for different regions of culture.

Results and Discussion

Results of climatic conditions for the three studied Lebanese regions

Climatic data for the three studied regions are represented in Table 2. Bechmizzine region "B" has the lowest altitude above the sea level (265 m), with an annual average rainfall of 643 mm/year and an annual average temperature of 21°C. Kfaraaka "L" region, which is also located at 334 m above the sea level, had an annual average rainfall and average temperature of 108 mm/year and 19.5°C, respectively. Kawkaba "K" region has the highest altitude above the sea level (672 m), with an annual average rainfall of 777 mm/year and an annual average temperature of 18°C.

Temperatures above 30°C, registered in Bechmizzine region for 7 months of the year, are as follows: March 30.4°C, May 34.6°C, June 33.4°C, July 35.7°C, August 34.2°C, September 34.1°C, and October 32.2°C. No temperature below 0°C was recorded in this region. Rainfall took place for 10 months per year, with the rainiest months being December (308.32 mm) and February (146.30 mm). At Daher el Ain station, the total amount of precipitation recorded was 643.09 mm (Figure 1).

In Kfaraaka, temperature above 30°C was recorded only for the following 2 months: July 30.2°C and August 30.4°C. Temperature below 0°C on any day was not recorded in this region. Rainfall took place for 12 months per year, with the rainiest months being January (16 mm) and December (40 mm). The total amount of precipitation recorded was 108.2 mm for Amioun weather station (Figure 2).

In Kawkaba, temperatures above 30°C were registered for the following 5 months successively: May 30.1°C, June 30.9°C, July 32.6°C, August 32.4°C, and September 32.2°C. Temperature below 0°C on any day was not recorded in this region. Rainfall took place for 9 months per year, with the rainiest months being January (379 mm) and February (142 mm). The total amount of precipitation recorded at Hasbaya weather station was 777 mm (Figure 3).

Differences in temperature were observed, with the highest temperature recorded in Bechmizine, followed by Kfaraaka and Kawkaba. In addition, different amount of rainfall was also recorded in these three regions, with Kawkaba having the highest rainfall and Kfaraaka experiencing the lowest. These environmental changes had an impact on the physiological behavior of olive trees and consequently on the quality of olive oil (Angerosa *et al.*, 1996; Aparicio *et al.*, 1994; Ponti *et al.*, 2014). In our study, no clear relationship was observed between climatic conditions and the analyzed olive oil parameters. This could be because our study studied climatic conditions for only 1 year, which was not a sufficient period to confirm a relationship between climatic changes and the quality properties of the analyzed olive oil. Therefore, the future work should study climatic conditions for a longer period to confirm the effect of environmental conditions of Lebanon on the quality of olive oil.

Results of pedological characterization of the three studied Lebanese regions

Results of physicochemical soil analysis for the three studied Lebanese regions are presented in Table 3.

KAd soil had the highest pH (H_2O) value, followed by KAn, LAd, LAn, and soil samples of Bechmizzine "B." The pH (KCl) was measured only for Bechmizzine "B" soil samples after obtaining a pH (H_2O) value lower than 6.5.

Table 2. Regions, locations, annual rainfall, temperatures, and altitude of the studied Lebanese regions.

Region	Location	Year	Rainfall (mm/year)	Temperature (°C)*†	Altitude (m)
Bechmizzine "B"	North	2018	643	21 (12.2–29.5)	265
Kfaraaka "L"	North	2018	108	19.5 (15.5–24.3)	334
Kawkaba "K"	South	2018	777	18 (12.8–25.3)	672
Kawkaba "K"	South	2019	424	18.8 (12.1–24.7)	672

*Average values of minimum and maximum temperatures of the year.
†Minimum and maximum temperatures are reported in parentheses.

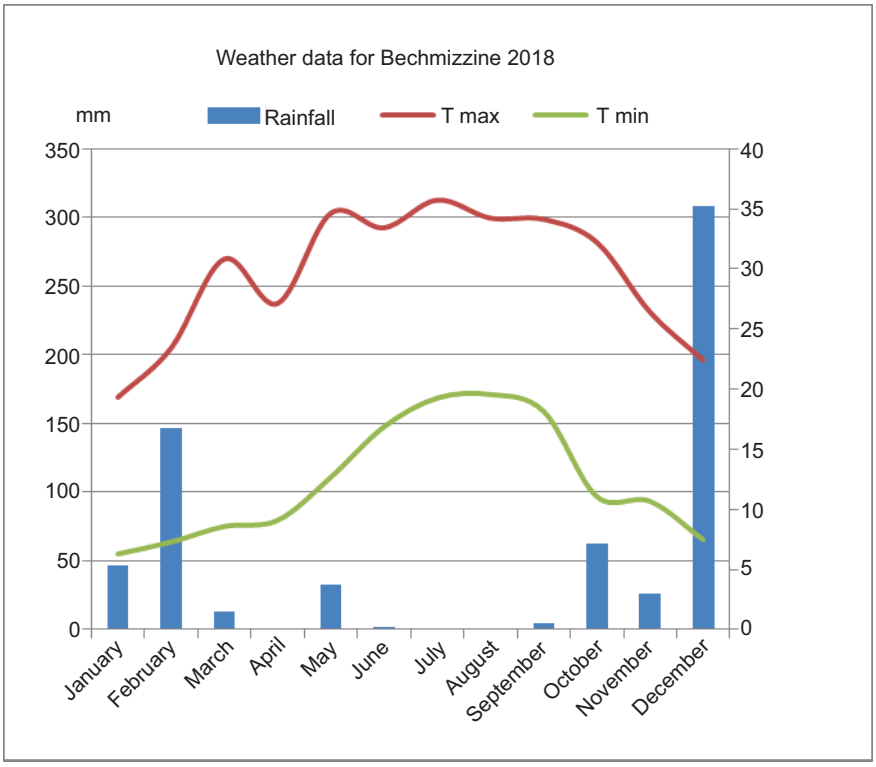

Figure 1. Minimum and maximum temperature (°C) and rainfall (mm) recorded at Daher el Ain station near Bechmizzine orchards in 2018.

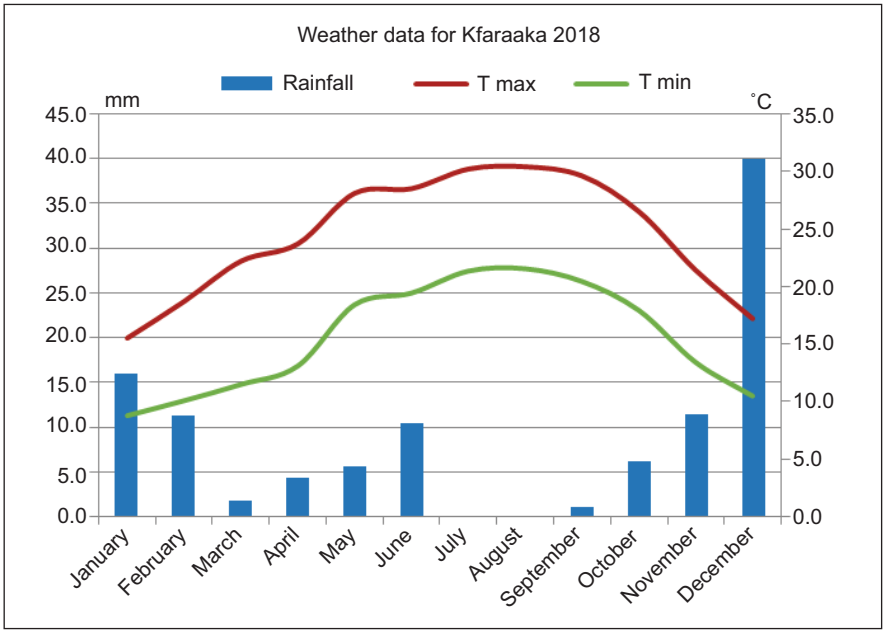

Figure 2. Minimum and maximum temperature (°C) and rainfall (mm) recorded at Amioun station near Kfaraaka orchards in 2018.

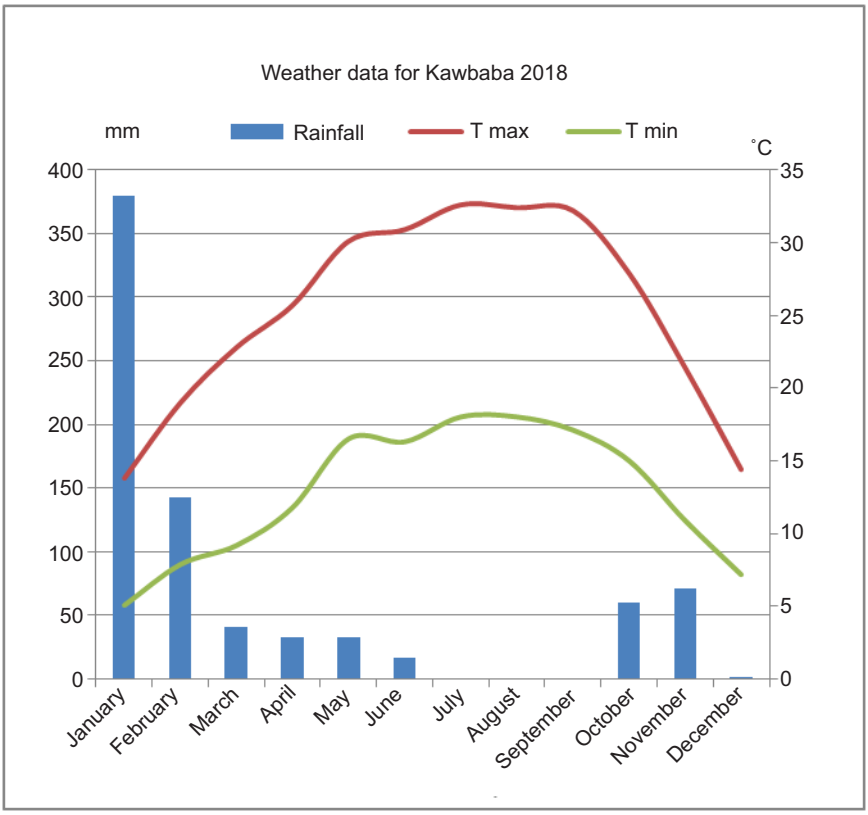

Figure 3. Minimum and maximum temperature (°C) and rainfall (mm) recorded at Hasbaya station near Kawkaba orchards in 2018.

Table 3. Results of chemical analysis of soil for the three studied Lebanese regions.

Chemical analysis of soil							
Chemical parameters of soil	**Unit**	**Bechmizzine "B"**		**Kfaraaka "L"**		**Kawkaba "K"**	
		BAn	**BAd**	**LAn**	**LAd**	**KAn**	**KAd**
pH (H_2O)		5.5	5.5	7.4	7.5	7.8	8.1
pH (KCl)		4.8	4.8				
EC	(dS/m)	0.794	0.794	1.421	0.308	0.287	0.189
Total limestone	(g/kg)	NDA	NDA	252	31	71	504
Active limestone	(g/kg)	NDA	NDA	153	NDA	NDA	210
Total nitrogen	(g/kg)	1.2	1.2	3.5	1.9	5.9	1.1
Carbon		11	11	27	17	43	9
Organic matter		19	19	47	29	74	16
C/N		9	9	8	9	7	8
Assimilable phosphate (P_2O_5)		46	46	128	18	174	9
Exchangeable potassium (K_2O)		328	328	1455	436	2083	1455

NDA: not detectable analytically; EC: electrical conductivity in deciSiemens per meter (dS/m); BAn: Bechmizzine ancient trees; BAd: Bechmizzine adult trees; LAn: Kfaraaka ancient trees; LAd: Kfaraaka adult trees; KAn: Kawkaba ancient trees; KAd: Kawkaba adult trees.

The highest electrical conductivity was discovered for LAn soil, followed by LAd, KAn, KAd, and soil samples of Bechmizzine "B." As for total limestone, it was not detectable analytically in the soil samples of Bechmizzine "B" region, while KAd soil had the highest value followed by LAn, KAn, and LAd. Regarding active limestone, it was not detectable analytically neither in soils of region Bechmizzine "B," nor in LAd and KAn, while KAd had

higher value of active limestone than LAn. With respect to total nitrogen, the highest value was detected in KAn, followed by LAn, LAd, Bechmizzine "B" soil samples, and finally in KAd soil. Carbon content was maximum in KAn, followed by LAn, LAd, Bechmizzine "B," and KAd soil samples. As for organic matter, KAn soil had the maximum value, followed by LAn, LAd, Bechmizzine "B," and KAd soil samples. The carbon:nitrogen (C:N) ratio was the same for Bechmizzine "B" and LAd soil samples but lower for both LAn and KAd and the lowest for KAn soil sample. The assimilable phosphate (P_2O_5) had the highest value in KAn soil, followed by LAn, Bechmizzine "B," and KAd. As for exchangeable potassium (K_2O), the maximum value was detected in KAn, followed by KAd, LAn, LAd, and Bechmizzine "B" soil samples. In Bechmizzine, soil typology (clay soil) was the same for both ancient and adult trees, since they were planted together in the same olive orchard (Table 3). Regarding Kfaraaka, similar soil type was also registered for adult and ancient trees (loamy clay soil). On the other hand, in Kawkaba, soil types were different: the adult trees were grown in a silty clay soil, while the ancient trees were planted in loamy clay soil. In the literature, few studies have established that soil type influences the quality of olive oil, but the relation between soil components and olive oil physicochemical parameters is scarcely debated (Çetinkaya and Kulak, 2016; Rached *et al.*, 2017a, 2017b; Rouas *et al.*, 2016).

PCA Results for physicochemical parameters of oil

Table 4 shows that the first two components account for the majority (76.11%) of the total variance, and therefore these two components can be used in further analyses.

Scores of first principal component (PC1) and second principal component (PC2) were plotted and color-coded according to the cultivation region, the harvest time, and the tree age (Figure 4).

In Figure 4, a clear clustering at PC1 based on the region of cultivation is observed, where the data from "B" region are clustered on the left-hand side of the *x*-axis (PC1), the data of region "L" are clustered toward the right-hand side of the same axis, and the data of region "K" are less regrouped but distributed below the *x*-axis on the right and left-hand sides. These observations were supported by the weight of each variable of oil physicochemical and fatty acid parameters of extra virgin olive oil (EVOO) on the two major principal components (Table 5).

Bold values shown in Table 5 explain the strong positive or negative loadings of PC1 and PC2 on olive oil parameters. The results showed that, except acidity, all variables such as total polyphenol, antioxidant activity, palmitic acid, palmitoleic acid, linoleic acid, region of cultivation,

Table 4. Physicochemical oil data showing the percentage values of individual variance and cumulative variance for each principal component.

Principal component	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Individual variance (%)	54.37	21.74	12.63	4.12	3.19	2.05	0.82	0.58	0.35	0.13	0.03
Cumulative variance (%)	54.37	76.11	88.74	92.86	96.04	98.09	98.91	99.49	99.83	99.97	100

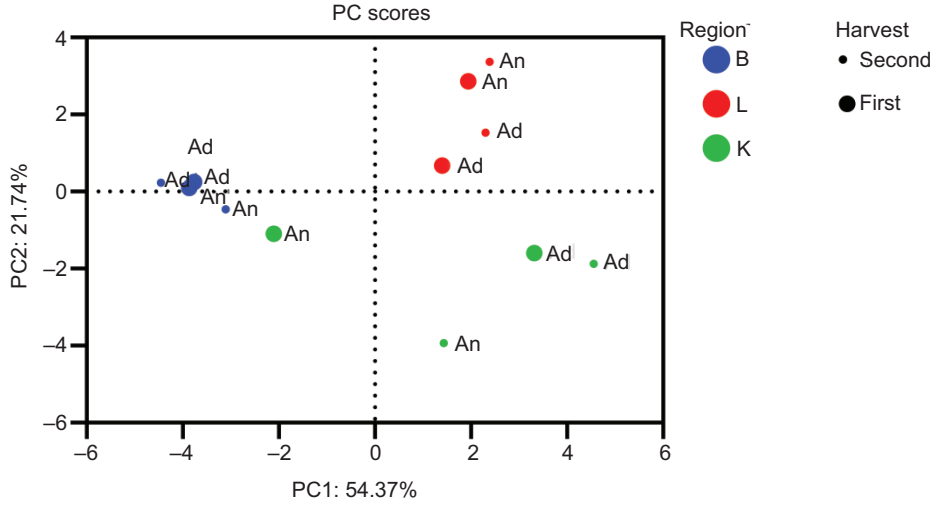

Figure 4. PCA score plot of first principal component (PC1) and second principal component (PC2) illustrating the distribution of all oil physicochemical parameters color-coded according to the region, sized according to the harvest time, and labeled with "An" for ancient trees and "Ad" for adult trees.

tree age, and harvest time were negatively associated with PC1, and heptadecanoic acid, heptadecenoic acid, arachidic acid, tocopherols, and the region of cultivation had the maximum impact on PC1 (Table 5). On the other hand, the antioxidant activity, palmitic acid, palmitoleic acid, and lignoceric acid had the maximum influence on PC2 (Table 5). The PCA indicated that the observed diversity was primarily due to the "cultivation region" factor, with the "age" and the "harvest" factors playing a minor role.

Influence of the studied Lebanese regions on the physicochemical parameters of EVOOs

One-way ANOVA analysis showed that region Kfaraaka "L" had the maximum oil yield (% of dm) among the three tested regions (Table 6).

This may be attributed to the higher weight of fresh olive fruits in this region (Trentacoste *et al.*, 2010), which reported a positive relation between oil yield and fresh fruit weight. Altitude could have probably affected oil yield, as indicated by other authors (Lombardo *et al.*, 2008; Mousa *et al.*, 1996), who found significantly higher fruit weight and lower oil yield at lower altitudes than at higher altitudes. These findings could explain the results of oil yield obtained in the present study for olive oil obtained in Bechmizzine "B," which has the lowest altitude between the three tested regions.

Table 5. Loading weights of each variable on principal components.

Variable	PC1	PC2
Acidity	0.75	–0.44
Peroxide value	–0.68	0.21
Chlorophyll content	–0.59	–0.01
Total polyphenol	0.65	–0.53
Tocopherols	**–0.88**	–0.07
Antioxidant activity	0.62	**–0.70**
Myristic acid (C14: 0)	–0.33	–0.56
Palmitic acid (C16: 0)	0.65	**0.75**
Palmitoleic acid (C16: 1)	0.50	**0.84**
Heptadecanoic acid (C17: 0)	**–0.96**	–0.19
Heptadecenoic acid (C17: 1)	**–0.98**	0.02
Stearic acid (C18: 0)	–0.79	–0.59
Oleic acid (C18: 1 cis9)	–0.66	–0.15
Linoleic acid (C18: 2)	0.56	–0.43
Arachidic acid (C20: 0)	**–0.93**	–0.26
Eicosenoic acid (C20: 1)	–0.88	–0.39
Linolenic acid (C18: 3)	–0.67	0.47
Behenic acid (C22: 0)	–0.86	–0.20
Lignoceric acid (C24: 0)	–0.68	**0.69**
Area	**0.78**	–0.43
Age	0.19	–0.04
Harvest	0.18	–0.11

The higher free acidity value in oils from Kfaraaka "L" and Kawkaba "K," compared to Bechmizzine "B," was most likely due to the lipase enzyme activity; either by an active lipase existing in olive seeds (Peres *et al.*, 2017) or by fruit microbiota (lactic and enteric bacteria, fungi, and *Pseudomonas*), which had the main influence in the extent of hydrolytic process (Vichi *et al.*, 2011). These significant differences in free acidity detected among the three tested regions (Table 6) could result in differences in the quality of olive oil and its stability during storage (Ayton *et al.*, 2012). It is well known that acidity is an important parameter for assessing the quality of olive oil (IOC, 2019). Owing to the presence of lipase enzymes in the oil, hydrolysis continues even after extraction and may lead to an increase in oil acidity during storage, thus reducing its quality (Ayton *et al.*, 2012).

The higher peroxide value obtained in oils from Bechmizzine "B," compared to the other two regions (Table 6), could be related to lower content of polyphenols and tocopherols in oils from this region, as indicated in the dedicated section of the paper. It has been shown that polyphenols and tocopherols are natural antioxidants which act as chain-breaking components (Shahidi and De Camargo, 2016), leading to a removal of free radicals and suppressing peroxidation (Roginsky, 2003).

The highest chlorophyll content obtained in oils from Bechmizzine "B," compared to the other two regions (Table 6), was probably due to the very low—or almost non-detectable—limestone content in this region (Table 3). This finding was in line with the results of Rouas *et al.* (2016), who found that chlorophyll content decreased with an increased percentage of limestone in the soil. Another factor that could have affected the chlorophyll content was the altitude, as reported previously by other authors (Dabbou *et al.*, 2010), who found higher chlorophyll content at lower-altitude cultivation area as in Bechmizzine "B."

Total polyphenol content was higher in oils from Kawkaba "K," which has the highest altitude of 672 m above the sea level (Table 6). These results were in accordance with those reported previously by Dabbou *et al.* (2010), Issaoui *et al.* (2010), Ripa *et al.* (2008), and Rouas *et al.* (2016), who observed a significant higher polyphenols content in higher-altitude cultivated regions. These authors ascribed this difference to the tree cultivar and to the effect of pedo-climatic conditions that differed between regions.

Table 6. Influence of the studied Lebanese regions on EVOO's analytical indices during 2018–2019 crop season.

Oil parameters	Regions		
	B	L	K
Oil content (% of dm)	35 ± 2[c]	48 ± 6[a]	46 ± 6[b]
Fruit fresh weight (g)	1.50 ± 0.06[c]	2.74 ± 0.07[a]	2.58 ± 0.04[b]
Free acidity (% oleic acid)	0.22 ± 0.04[c]	0.34 ± 0.02[b]	0.43 ± 0.17[a]
Peroxide value ($meqO_2\ kg^{-1}$)	6.50 ± 0.59[a]	5.81 ± 0.44[b]	5.66 ± 0.64[b]
Chlorophyll content (mg pheophytin A kg^{-1})	47 ± 11[a]	27 ± 15[b]	32 ± 22[b]
Total polyphenols (mg gallic acid kg^{-1})	274 ± 98[b]	293 ± 110[b]	550 ± 233[a]
Tocopherols (mg kg^{-1})	325 ± 59[a]	191 ± 45[b]	192 ± 47[b]
Antioxidant activity (%/mg)	0.67 ± 0.35[b]	0.73 ± 0.42[b]	2.16 ± 0.99[a]

Mean values ± standard deviation: within rows, values with the same superscript letters do not differ significantly from each other for each oil parameter according to LSD test ($p < 0.05$). B: Bechmizzine region; L: Kfaraaka region; K: Kawkaba region; EVOO: extra virgin olive oil.

The higher antioxidant activity observed in region "K," compared to regions "B" and "L" (Table 6), is explained by high polyphenols content discovered in this region. A two-tailed Pearson's correlation test was performed, and high correlation was found between antioxidant activity and total polyphenols content in regions "B" (r = 0.9704, $p < 0.001$), "L" (r = 0.9974, $p < 0.001$), and "K" (r = 0.8409, $p = 0.0089$).

The tocopherol content (Table 6) was in accordance with those obtained by other authors in oils obtained in low altitude regions (Aguilera *et al.*, 2005; Jukić Špika *et al.*, 2016; Mousa *et al.*, 1996). In fact, it was shown that formation of tocopherols was augmented with increase in temperature during fruit maturation (Kalogeropoulos and Tsimidou, 2014; Mailer *et al.*, 2010). The discussion on fatty acids was focused only on major fatty acids (Table 7), as minor fatty acids had a less important role in the determination of olive oil quality (Douzane, 2012).

Oleic acid (C18: 1) and linoleic acid (C18: 2) showed an opposite trend in the three regions. The higher content of oleic acid and the lowest of linoleic acid observed in the oil samples from the hottest region "B" were not in agreement with the results of other studies, which discovered lower oleic acid (C18: 1) and higher linoleic acid (C18: 2) levels in oils obtained from areas of hot temperature (Kalogeropoulos and Tsimidou, 2014; Mailer *et al.*, 2010; Ripa *et al.*, 2008), and from lower altitude regions (Issaoui *et al.*, 2010; Piravi-Vanak *et al.*, 2012; Mansour *et al.*, 2015). These results could be related to the mean daily thermal amplitude as reported by García-Inza *et al.* (2018), who found a significant but nonlinear relationship between oleic acid and mean daily thermal amplitude. In this study, the mean daily thermal amplitude was not calculated due to the absence of daily temperature in meteorological data. Therefore, the future daily thermal amplitude data need to be collected to confirm the results of the literature. Palmitic acid (C16: 0) and ΣSFA are known to be affected by temperature and altitude of the geographical place; both components increase with increase in temperature (Gargouri *et al.*, 2013; Ripa *et al.*, 2008). In this study, palmitic acid showed a contradictory behavior with regions' temperature. There is no clear explanation for the content of palmitic acid, but it could be related to the dilution effect by oleic acid. No significant results were observed for ΣMUFA and ΣPUFA, and MUFAs–PUFA and C18:1–C18:2 ratios and need to be further investigated.

Influence of tree age and two different harvest periods on the physicochemical parameters and fatty acid composition of EVOOs in the regions of cultivation

Analysis of physicochemical parameters and fatty acid composition of olive oil showed a pronounced clustering according to the region of cultivation. Moreover, within each region, significant differences were observed in oils from trees of different ages as well as at different harvest periods (Tables 8–10).

As reported by other authors (Bengana *et al.*, 2013; Fregapane *et al.*, 2017), lower amounts of polyphenols and tocopherols are detected with the advancement of ripening stage. The results of the present study confirmed this statement by revealing low polyphenol and tocopherol content in olive oil produced from all trees during the second harvest time when the maturity index was high irrespective of the tree age and the region of cultivation. On the other hand, free acidity and peroxide values did not change between ancient and adult trees between the two harvest periods in Bechmizzine and Kfaraaka. These values were significantly different in Kawkaba, where free acidity increased during the second harvest in olive oil produced by both ancient and adult trees, while

Table 7. Influence of the studied Lebanese regions on the composition of fatty acids of EVOOs during 2018–2019 crop season.

Fatty acids	Samples		
	B	L	K
Myristic acid (C14: 0)	0.027 ± 0.008^{b}	0.020 ± 0.005^{a,b}	0.031 ± 0.01^{a}
Palmitic acid (C16: 0)	11 ± 0^{c}	14 ± 0^{a}	11 ± 0^{b}
Palmitoleic acid (C16: 1)	0.37 ± 0.02^{c}	0.64 ± 0.09^{a}	0.38 ± 0.07^{b}
Heptadecanoic acid (C17: 0)	0.31 ± 0.02^{a}	0.18 ± 0.01^{c}	0.20 ± 0.04^{b}
Heptadecenoic acid (C17: 1)	0.34 ± 0.02^{a}	0.23 ± 0.008^{b}	0.22 ± 0.04^{c}
Stearic acid (C18: 0)	4.84 ± 0.09^{a}	3.63 ± 0.30^{c}	4.26 ± 0.43^{b}
Oleic acid (C18: 1)	72 ± 1^{a}	69 ± 1^{c}	70 ± 1^{b}
Linoleic acid (C18: 2)	8.95 ± 1.18^{c}	9.93 ± 0.94^{b}	11 ± 1^{a}
Arachidic acid (C20: 0)	0.69 ± 0.007^{a}	0.57 ± 0.01^{c}	0.59 ± 0.05^{b}
Eicosenoic acid (C20: 1)	0.39 ± 0.01^{a}	0.31 ± 0.01^{c}	0.34 ± 0.03^{b}
Linolenic acid (C18: 3)	0.70 ± 0.04^{a}	0.64 ± 0.07^{b}	0.53 ± 0.07^{c}
Behenic acid (C22: 0)	0.19 ± 0.009^{a}	0.16 ± 0.003^{c}	0.16 ± 0.02^{b}
Lignoceric acid (C24: 0)	0.09 ± 0.002^{a}	0.09 ± 0.006^{b}	0.07 ± 0.005^{c}
ΣSFA	17 ± 0^{b}	18 ± 0^{a}	17 ± 1^{b}
ΣMUFA	73 ± 1^{a}	70 ± 1^{a}	71 ± 1^{a}
ΣPUFA	9.65 ± 1.22^{a}	11 ± 1^{a}	11 ± 2^{a}
MUFAs–PUFAs	7.56 ± 1.08^{a}	6.04 ± 1.37^{a}	6.09 ± 1.06^{a}
C18: 1–C18: 2	8.02 ± 1.07^{a}	6.98 ± 1.35^{a}	6.29 ± 1.02^{a}

Mean values ± standard deviation: within rows, values with the same superscript letter do not differ significantly from each other according to LSD test ($p < 0.05$).
B: Bechmizzine region; L: Kfaraaka region; K: Kawkaba region. ΣSFA: sum of saturated fatty acids; ΣMUFA: sum of monounsaturated fatty acids; ΣPUFA: sum of polyunsaturated fatty acids; MUFAs–PUFAs: monounsaturated–polyunsaturated fatty acids ratio; C18: 1–C18: 2: oleic acid–linoleic acid ratio.

Table 8. Influence of tree age and harvest time factors on EVOO's physicochemical parameters in region "B" during 2018–2019 crop season.

Sample name	Free acidity (%)	Peroxide value (meqO_2 kg^{-1})	Chlorophyll content (mg pheophytin A kg^{-1})	Total polyphenols (mg gallic acid kg^{-1})	Tocopherols (mg kg^{-1})	Antioxidant activity (%/mg)	Oil yield (dry matter) (%)	Fruit fresh weight (g)
BAn1	0.22 ± 0.001^{a}	6.26 ± 0.18^{b}	55 ± 1^{b}	332 ± 0^{b}	282 ± 21^{c}	0.86 ± 0.01^{b}	38 ± 2	1.54 ± 0.08
BAd1	0.22 ± 0.001^{a}	6.40 ± 0.07^{b}	59 ± 1^{a}	212 ± 5^{c}	299 ± 8^{b,c}	0.35 ± 0.01^{c}	34 ± 2	1.45 ± 0.05
BAn2	0.22 ± 0.0002^{a}	6.42 ± 0.23^{b}	30 ± 0^{d}	345 ± 7^{a}	392 ± 4^{a}	1.01 ± 0.004^{a}	34 ± 0	1.49 ± 0.04
BAd2	0.22 ± 0.01^{a}	7.19 ± 0.30^{a}	45 ± 0^{c}	140 ± 0^{d}	347 ± 28^{a,b}	0.27 ± 0.0003^{d}	35 ± 2	1.50 ± 0.08

Mean values ± standard deviation: within columns, values with the same superscript letter do not differ significantly from each other for each oil parameter according to LSD test ($p < 0.05$).
BAn1: Bechmizzine ancient trees, first harvest; BAd1: Bechmizzine adult trees, first harvest; BAn2: Bechmizzine ancient trees, second harvest; BAd2: Bechmizzine adult trees, second harvest.

peroxide values also significantly changed, but there was no clear relation with the tree age (Table 10).

Results concerning fatty acids were statistically significant for the three regions; however, no clear relation was observed with respect to the tree age (Tables 11–13).

Oleic acid, MUFA, MUFAs–PUFAs, and C18: 1–C18: 2 were found to be always higher in the oil produced during the first harvest, regardless of the tree age. Linoleic acid and PUFAs always showed an inverse relation to oleic acid. Palmitic acid did not show a clear pattern neither with the tree age nor with the harvest time (Tables 11–13).

Conclusion

Summarily, it is important to mention that all virgin olive oil samples analyzed in this study were within the limit of EVOO according to the standards of IOC (Peres *et al.*,

Table 9. Influence of tree age and harvest time factors on EVOO's physicochemical parameters in region "L" during 2018–2019 crop season.

Sample name	Free acidity (% oleic acid)	Peroxide value (meqO_2 kg^{-1})	Chlorophyll content (mg pheophytin A kg^{-1})	Total polyphenols (mg gallic acid kg^{-1})	Tocopherols (mg kg^{-1})	Antioxidant activity (%/mg)	Oil yield (dry matter) (%)	Fruit fresh weight (g)
LAn1	0.34 ± 0.00^{a}	5.55 ± 0.17^{a}	8 ± 0^{d}	265 ± 11^{b}	227 ± 35^{a}	0.58 ± 0.0^{c}	43 ± 2^{c}	2.55 ± 0.08^{c}
LAd1	0.36 ± 0.04^{a}	5.47 ± 0.30^{a}	47 ± 1^{a}	393 ± 8^{a}	222 ± 9^{a}	1.08 ± 0.00^{b}	50 ± 2^{b}	2.81 ± 0.01^{b}
LAn2	0.33 ± 0.01^{a}	6.02 ± 0.14^{a}	24 ± 0^{c}	138 ± 5^{c}	128 ± 11^{b}	0.15 ± 0.00^{d}	42 ± 1^{c}	2.50 ± 0.02^{c}
LAd2	0.33 ± 0.00^{a}	6.21 ± 0.68^{a}	31 ± 0^{b}	377 ± 1^{a}	186 ± 23^{a,b}	1.10 ± 0.01^{a}	56 ± 3^{a}	3.10 ± 0.02^{a}

Mean values ± standard deviation: within columns, values with the same superscript letters do not differ significantly from each other for each oil parameter according to LSD test ($p < 0.05$).
LAn1: Kfaraaka ancient trees, first harvest; LAd1: Kfaraaka adult trees, first harvest; LAn2: Kfaraaka ancient trees, second harvest; LAd2: Kfaraaka adult trees, second harvest.

Table 10. Influence of tree age and harvest time factors on EVOO's physicochemical parameters in region "K" during 2018–2019 crop season.

Sample name	Free acidity (%)	Peroxide value (meqO_2 kg^{-1})	Chlorophyll content (mg pheophytin A kg^{-1})	Total polyphenols (mg gallic acid kg^{-1})	Tocopherols (mg kg^{-1})	Antioxidant activity (%/mg)	Oil yield (dry matter) (%)	Fruit fresh weight (g)
KAn1	0.22 ± 0.00^{d}	6.41 ± 0.10^{a}	58 ± 0^{a}	227 ± 4^{c}	259 ± 19^{a}	0.55 ± 0.001^{c}	38 ± 2^{d}	1.77 ± 0.21^{d}
KAd1	0.34 ± 0.00^{c}	5.19 ± 0.03^{c}	41 ± 0^{b}	765 ± 13^{a}	190 ± 4^{b}	2.64 ± 0.02^{b}	49 ± 1^{b}	3.13 ± 0.09^{b}
KAn2	0.62 ± 0.00^{a}	4.98 ± 0.004^{c}	4 ± 0^{d}	475 ± 2^{b}	179 ± 13^{b}	2.73 ± 0.01^{a}	42 ± 1^{c}	2.14 ± 0.21^{c}
KAd2	0.56 ± 0.00^{b}	6.06 ± 0.21^{b}	24 ± 0^{c}	734 ± 1^{a}	139 ± 12^{c}	2.70 ± 0.02^{a}	53 ± 2^{a}	3.30 ± 0.30^{a}

Mean values ± standard deviation: within columns, values with the same superscript letters do not differ significantly from each other for each oil parameter according to LSD test ($p < 0.05$).
KAn1: Kawkaba ancient trees, first harvest; KAd1: Kawkaba adult trees, first harvest; KAn2: Kawkaba ancient trees, second harvest; KAd2: Kawkaba adult trees, second harvest.

Table 11. Influence of tree age and harvest time factors on fatty acids composition of EVOOs in region "B" during 2018–2019 crop season.

Fatty acids	Samples			
	BAn1	BAd1	BAn2	BAd2
Myristic acid (C14: 0)	0.023 ± 0.008^{a}	0.027 ± 0.008^{a}	0.034 ± 0.01^{a}	0.35 ± 0.005^{a}
Palmitic acid (C16: 0)	11 ± 0^{b}	11 ± 0^{a}	11 ± 0^{a}	11 ± 0^{b}
Palmitoleic acid (C16: 1)	0.37 ± 0.002^{b}	0.35 ± 0.001^{c}	0.40 ± 0.0002^{a}	0.35 ± 0.002^{c}
Heptadecanoic acid (C17: 0)	0.32 ± 0.001^{b}	0.31 ± 0.001^{b}	0.28 ± 0.003^{c}	0.33 ± 0.0008^{a}
Heptadecenoic acid (C17: 1)	0.35 ± 0.001^{a}	0.34 ± 0.003^{b}	0.31 ± 0.0001^{c}	0.35 ± 0.005^{a}
Stearic acid (C18: 0)	4.86 ± 0.03^{b}	4.76 ± 0.004^{c}	4.78 ± 0.002^{c}	4.96 ± 0.02^{a}
Oleic acid (C18: 1)	73 ± 0^{b}	73 ± 0^{a}	70 ± 0^{c}	70 ± 0^{c}
Linoleic acid (C18: 2)	8.02 ± 0.005^{c}	7.69 ± 0.01^{b}	10 ± 0^{b}	10 ± 0^{a}
Arachidic acid (C20: 0)	0.68 ± 0.002^{b}	0.68 ± 0.002^{b}	0.69 ± 0.001^{b}	0.70 ± 0.005^{a}
Eicosenoic acid (C20: 1)	0.40 ± 0.006^{a}	0.37 ± 0.001^{c}	0.40 ± 0.002^{a,b}	0.39 ± 0.0001^{b}
Linolenic acid (C18: 3)	0.72 ± 0.02^{a}	0.63 ± 0.005^{b}	0.71 ± 0.0004^{a}	0.71 ± 0.006^{a}
Behenic acid (C22: 0)	0.18 ± 0.001^{b}	0.19 ± 0.002^{b}	0.18 ± 0.003^{b}	0.20 ± 0.0004^{a}
Lignoceric acid (C24: 0)	0.09 ± 0.001^{a}	0.09 ± 0.001^{a}	0.09 ± 0.001^{a}	0.009 ± 0.002^{a}

(*continues*)

Table 11. Continued.

Fatty acids	Samples			
	BAn1	BAd1	BAn2	BAd2
ΣSFA	17 ± 0[a]	17 ± 0[a]	17 ± 0[a]	17 ± 0[a]
ΣMUFA	74 ± 0[b]	74 ± 0[a]	71 ± 0[c]	71 ± 0[c]
ΣPUFA	8.74 ± 0.02[c]	8.33 ± 0.01[d]	11 ± 0[b]	11 ± 0[a]
MUFAs–PUFAs	8.46 ± 0.01[b]	8.91 ± 0.01[a]	6.69 ± 0.001[c]	6.64 ± 0.03[d]
C18: 1–C18: 2	9.08 ± 0.005[b]	9.52 ± 0.01[a]	7.06 ± 0.002[c]	7.00 ± 0.02[d]

Mean values ± standard deviation: within rows, values with the same superscript letters do not differ significantly from each other for each fatty acid according to LSD test ($p < 0.05$).
BAn1: Bechmizzine ancient trees, first harvest; BAd1: Bechmizzine adult trees, first harvest; BAn2: Bechmizzine ancient trees, second harvest; BAd2: Bechmizzine adult trees, second harvest.
ΣSFA: sum of saturated fatty acids; ΣMUFA: sum of monounsaturated fatty acids; ΣPUFA: sum of polyunsaturated fatty acids; MUFAs–PUFAs: monounsaturated–polyunsaturated fatty acids ratio; C18: 1–C18: 2: oleic acid–linoleic acid ratio.

Table 12. Influence of tree age and harvest time factors on fatty acids composition of EVOOs in region "L" during 2018–2019 crop season.

Fatty acids	Samples			
	LAn1	LAd1	LAn2	LAd2
Myristic acid (C14: 0)	0.02 ± 0.0002[a]	0.01 ± 0.001[a]	0.02 ± 0.01[a]	0.01 ± 0.0002[a]
Palmitic acid (C16: 0)	15 ± 0.02[b]	13.39 ± 0.01[d]	14.91 ± 0.05[a]	14.21 ± 0.02[c]
Palmitoleic acid (C16: 1)	0.73 ± 0.002[a]	0.50 ± 0.003[d]	0.71 ± 0.001[b]	0.59 ± 0.0003[c]
Heptadecanoic acid (C17: 0)	0.17 ± 0.001[b]	0.19 ± 0.001[a]	0.16 ± 0.001[c]	0.19 ± 0.0009[a]
Heptadecenoic acid (C17: 1)	0.24 ± 0.006[a]	0.22 ± 0.001[c]	0.23 ± 0.004[b,c]	0.23 ± 0.0009[a,b]
Stearic acid (C18: 0)	3.41 ± 0.02[c]	4.02 ± 0.008[a]	3.32 ± 0.01[d]	3.75 ± 0.006[b]
Oleic acid (C18: 1 cis9)	70 ± 0[b]	71 ± 0[a]	68 ± 0[d]	67 ± 0[c]
Linoleic acid (C18: 2)	9.19 ± 0.04[c]	8.93 ± 0.009[d]	11 ± 0[a]	11 ± 0[b]
Arachidic acid (C20: 0)	0.56 ± 0.009[b]	0.59 ± 0.004[a]	0.56 ± 0.003[b]	0.57 ± 0.002[b]
Eicosenoic acid (C20: 1)	0.31 ± 0.0006[a]	0.32 ± 0.003[b]	0.30 ± 0.007[b]	0.29 ± 0.002[b]
Linolenic acid (C18: 3)	0.70 ± 0.01[a]	0.55 ± 0.006[c]	0.70 ± 0.01[a]	0.59 ± 0.001[b]
Behenic acid (C22: 0)	0.16 ± 0.002[a]	0.16 ± 0.002[a]	0.16 ± 0.001[a]	0.15 ± 0.0008[a]
Lignoceric acid (C24: 0)	0.09 ± 0.001[a]	0.09 ± 0.008[a]	0.09 ± 0.002[a]	0.08 ± 0.002[a]
ΣSFA	19 ± 0[b]	18 ± 0[c]	19 ± 0[a]	19 ± 0[b]
ΣMUFA	71 ± 0[b]	72 ± 0[a]	69 ± 0[d]	70 ± 0[c]
ΣPUFA	9.90 ± 0.06[c]	9.49 ± 0.01[d]	11 ± 0[a]	11 ± 0[b]
MUFAs–PUFAs	7.17 ± 0.05[b]	7.59 ± 0.01[a]	5.90 ± 0.01[d]	6.24 ± 0.0004[c]
C18: 1–C18: 2	7.58 ± 0.04[b]	7.94 ± 0.01[a]	6.17 ± 0.01[d]	6.48 ± 0.001[c]

Mean values ± standard deviation: within rows, values with the same superscript letters do not differ significantly from each other according to LSD test ($p < 0.05$).
LAn1: Kfaraaka ancient trees, first harvest; LAd1: Kfaraaka adult trees, first harvest; LAn2: Kfaraaka ancient trees, second harvest; LAd2: Kfaraaka adult trees, second harvest.
ΣSFA: sum of saturated fatty acids; ΣMUFA: sum of monounsaturated fatty acids; ΣPUFA: sum of polyunsaturated fatty acids; MUFAs–PUFAs: monounsaturated–polyunsaturated fatty acids ratio; C18: 1–C18: 2: oleic acid–linoleic acid ratio.

2017). The present study is the first one conducted in Lebanon to show differences between physicochemical and fatty acid composition of virgin oils obtained from ancient and adult trees in the three selected regions during two harvest periods. Based on the analysis of virgin olive oil characteristics and in combination with meteorological and soil analysis data, we conclude that the quality of virgin olive oil is associated with its chemical composition. This further is the result of complex interactions between several environmental factors associated with the area of cultivation, tree age, and harvest time, with the maximum influence of the cultivation region, and less influence of both harvest time and tree age.

Table 13. Influence of tree age and harvest time factors on fatty acids composition of EVOOs in region "K" during 2018–2019 crop season.

Fatty acids	Samples			
	KAn1	KAd1	KAn2	KAd2
Myristic acid (C14: 0)	0.04 ± 0.01^{a}	0.02 ± 0.005^{a}	0.03 ± 0.01^{a}	0.02 ± 0.004^{a}
Palmitic acid (C16: 0)	11 ± 0.04^{c}	12.54 ± 0.01^{b}	10.90 ± 0.04^{d}	12.99 ± 0.006^{a}
Palmitoleic acid (C16: 1)	0.31 ± 0.002^{c}	0.42 ± 0.004^{b}	0.31 ± 0.006^{c}	0.45 ± 0.008^{a}
Heptadecanoic acid (C17: 0)	0.24 ± 0.004^{a}	0.16 ± 0.0001^{c}	0.23 ± 0.003^{b}	0.15 ± 0.002^{d}
Heptadecenoic acid (C17: 1)	0.27 ± 0.003^{a}	0.19 ± 0.003^{c}	0.24 ± 0.002^{b}	0.18 ± 0.003^{d}
Stearic acid (C18: 0)	4.55 ± 0.02^{b}	3.90 ± 0.01^{c}	4.76 ± 0.01^{a}	3.81 ± 0.01^{d}
Oleic acid (C18: 1)	72 ± 0^{a}	71 ± 0^{b}	68 ± 0^{d}	69 ± 0^{c}
Linoleic acid (C18: 2)	9.87 ± 0.01^{c}	9.81 ± 0.001^{d}	13 ± 0^{a}	11 ± 0^{b}
Arachidic acid (C20: 0)	0.63 ± 0.001^{b}	0.54 ± 0.002^{c}	0.64 ± 0.002^{a}	0.54 ± 0.006^{c}
Eicosenoic acid (C20: 1)	0.36 ± 0.002^{a}	0.31 ± 0.002^{b}	0.36 ± 0.001^{a}	0.31 ± 0.002^{b}
Linolenic acid (C18: 3)	0.58 ± 0.001^{b}	0.45 ± 0.001^{d}	0.60 ± 0.001^{a}	0.46 ± 0.004^{c}
Behenic acid (C22: 0)	0.18 ± 0.002^{a}	0.14 ± 0.0001^{b}	0.18 ± 0.002^{a}	0.14 ± 0.003^{b}
Lignoceric acid (C24: 0)	0.08 ± 0.0001^{a}	0.07 ± 0.002^{b}	0.07 ± 0.002^{b}	0.07 ± 0.0001ns
ΣSFA	17 ± 0^{c}	17 ± 0^{b}	18 ± 0^{c}	18 ± 0^{a}
ΣMUFA	72 ± 0^{a}	72 ± 0^{b}	69 ± 0^{d}	70 ± 0^{c}
ΣPUFA	11 ± 0^{c}	10 ± 0^{d}	14 ± 0^{a}	12 ± 0^{b}
MUFAs–PUFAs	6.95 ± 0.0003^{b}	7.05 ± 0.0002^{a}	5.01 ± 0.002^{d}	5.77 ± 0.01^{c}
C18: 1–C18: 2	7.27 ± 0.001^{a}	7.28 ± 0.001^{a}	5.17 ± 0.002^{c}	5.92 ± 0.02^{b}

Mean values ± standard deviation: within rows, values with the same superscript letters do not differ significantly from each other according to LSD test ($p < 0.05$).
KAn1: Kawkaba adult trees, first harvest; KAn2: Kawkaba ancient trees, second harvest; KAd2: Kawkaba adult trees, second harvest.
ΣSFA: sum of saturated fatty acids; ΣMUFA: sum of monounsaturated fatty acids; ΣPUFA: sum of polyunsaturated fatty acids; MUFAs–PUFAs: monounsaturated–polyunsaturated fatty acids ratio; C18: 1–C18: 2: oleic acid–linoleic acid ratio.

Considering the analysis of physicochemical parameters and fatty acids discussed above, we observed that oil obtained from the adult trees of Kawkaba region during the first harvest period (greenish to reddish stage of fruit maturity; KAd1 sample) had the best quality of olive oil, compared to all other samples. Oil produced from these trees showed the highest polyphenol content, a relatively high composition of tocopherols, oleic acid, MUFAs, and C18: 1–C18: 2, with relatively low acidity and peroxide values. All these parameters combined together reflect a high quality of the olive oil produced from these trees.

Conflict of interest

There was no conflict of interest to declare.

Author Contributions

Conceptualization, A.P (Antonio Piga), P.C. A.E., G.H.; methodology, P.C. (Paola Conte), A.P., A.E.; validation, P A.P (Antonio Piga), P.C. A.E., G.H.; formal analysis, A.E. (Antonio El Chami), P.C.; investigation, A.E. (Antoino El Chami), P.C.; data curation, A.E. (Antonio El Chami), P.C., AP.; writing—original draft preparation, A.E. (Antonio El Chami); writing—review and editing, A.E. (Antonio El Chami), P.C., A.P.; funding acquisition, A.P. (Antonio Piga). All authors have read and agreed to the published version of the manuscript

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