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Characterization of Turkish Virgin Olive Oils Produced from Early Harvest Olives

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Abstract Olives were collected from various districts of Turkey (North and South Aegean sub-region, Bursa-Akhisar, South East Anatolia region) harvested over seven (2001–2007) seasons. The aim of this study was to characterize the chemical profiles of the oils derived from single variety Turkish olives including Ayvalik, Memecik, Gemlik, Erkence, Nizip Yaglik and Uslu. The olive oils were extracted by super press and three phase centrifugation from early harvest olives. Chosen quality indices included free fatty acid content (FFA), peroxide value (PV) and spectrophotometric characteristics in the ultraviolet (UV) region. According to the FFA results, 46% (11 out of 24 samples) were classified as extra virgin olive oils; whereas using the results of PV and UV, over 83% (over 19 of the 24 samples) had the extra virgin olive oil classification. Other measured parameters included oil stability (oxidative stability, chlorophyll pigment, pheophytin-α), *cis–trans* fatty acid composition and color index. Oxidative stability among oils differed whereas the *cis–trans* fatty acid values were within the national and international averages. Through the application of two multivariate statistical methods, Principal component and hierarchical analyses, early harvest virgin olive oil samples were classified according to the geographical locations categorized in terms of fatty acid profiles. Such statistical clustering gave rise to defined groups. These data provide evidence of the variation in virgin olive oil quality, especially early harvest and *cis–trans* isomers of fatty acid profiles from the diverse agronomic conditions in the olive growing regions of Turkey.

Keywords Turkey · Virgin olive oil · Quality · *cis–trans* Fatty acid composition · Oxidative stability · Characterization · Chemometry

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Introduction

Virgin olive oil is the main oil used in the Mediterranean diet. Virgin olive oil is valued for its organoleptic and nutritional characteristics and is resistant to oxidation due to its high monounsaturated fatty acid content (MUFAs), and low polyunsaturates (PUFAs) and the presence of natural antioxidants such as phenols, tocopherols and carotenoids. The fatty acid composition, especially the MUFA content, and the natural antioxidants provide advantages for health [1, 2].

Olives (*Olea europaea* L.) are one of the most important crops in Mediterranean countries, especially Spain, Italy, Greece and Turkey. The economically important Turkish olive cultivars include Ayvalik, Memecik, Gemlik, Erkence, Nizip Yaglik, and Uslu. A total of 88 Turkish olive varieties and 26 foreign olive cultivars were registered by the Ministry of Agriculture and Rural Affairs of Turkey in 1990 based on their pomological and morphological parameters [3]. The main olive growing regions in Turkey are the North and South Aegean where Ayvalik and Memecik, respectively, are the main cultivars of economic importance. Uslu is a domestic cultivar in the Akhisar district of the Middle Aegean zone. The major olive variety of the Marmara region, notably in the

Gemlik Gulf is the Gemlik cultivar. Gemlik has also been cultivated widely in other olive growing regions in Turkey for over 20 years. The Erkence variety is commonly grown in Izmir Peninsula and some coastal districts of the Aegean and Mediterranean regions. Nizip Yaglik is the most common domestic variety of the South Anatolia region [4].

Early harvest olives, especially green to pink, give higher quality oils than the late harvest olives. However, many olives in Turkey continue to be harvested late, traditionally, and are more economical to harvest after they are fully ripened because all of the olives are collected at once. Today, early or fall harvest is practiced in different parts of Turkey because the early harvest olives have low free fatty acid (FFA) and peroxide values (PV) which are important in high quality olive oil. Early harvest olive oils are more stabile to oxidation than mature oils because they are rich in antioxidants and aromatic compounds, including chlorophyll, phenolics, and aromatic components. Thus, early harvest oils are of high quality and have an increased market value compared to late harvest oils. Because of the higher polyphenols and antioxidants, early harvest oils have longer shelf lives and are often blended with late harvest oils to increase the shelf life of the resulting product [5]. Olive oil in Turkey is extracted by pressure and three phase centrifugation systems. The extraction systems vary in the physical force employed and in the water requirements. The dry and wet pressure systems have currently fallen into disuse in Turkey. A three phase continuous centrifugation system is most commonly used today because the system allows the processing of large amounts of olives in a short period of time.

The aim of the present study was to characterize the chemical profiles of the oil of economically important Turkish olive cultivars, including Ayvalik, Memecik, Gemlik, Erkence, Nizip Yaglik and Uslu. Oils were extracted from early harvest olives in different locations of Turkey. Quality index parameters, including free fatty acid content (FFA), peroxide value (PV), and UV spectrophotometric characteristics and other physico-chemical data [oxidative stability, chlorophyll, fatty acid (FA) composition and color index] were evaluated to determine the chemical profiles of early harvest virgin olive oil using official methods. The FA profile of early harvest virgin oil samples from different locations of Turkey were subjected to the Principal component (PC) and Hierarchical cluster (HC) analyses, aimed at establishing differences in FA profiles according to the olive varieties or growing region. Although some studies report on the chemical characterization and chemometric classification of Turkish virgin olive oils [6–13] and domestic olive varieties [14–17], there is limited or no information available on early harvest olives.

Experimental Procedures

Material

Twenty-four early harvest olives from domestic olive varieties Ayvalik, Memecik, Gemlik, Uslu, Nizip Yaglik and Erkence, were analyzed over 2001–2007 harvest seasons. These samples were divided into four groups based on the important olive cultivation districts of Turkey: (1) North Aegean (NA, $n = 7$), (2) South Aegean (SA, $n = 6$), (3) Manisa and Bursa sub-region (MB, $n = 6$), and (4) South Eastern Anatolia (SEA, $n = 5$). All except the two dry super press and two Abencor extracted oil samples were extracted using the three phase centrifugal systems from early harvested olives in November and the first week of December. Three hundred milliliter oil were placed in each of two dark glass bottles and stored in a refrigerator at 4 °C until they were analyzed.

The Oil Extraction Systems

Early harvest virgin olive oil samples were produced by the super press, three phase decanter and Abencor processes. The processing steps of these systems are given in Schemes 1, 2, and 3, respectively.

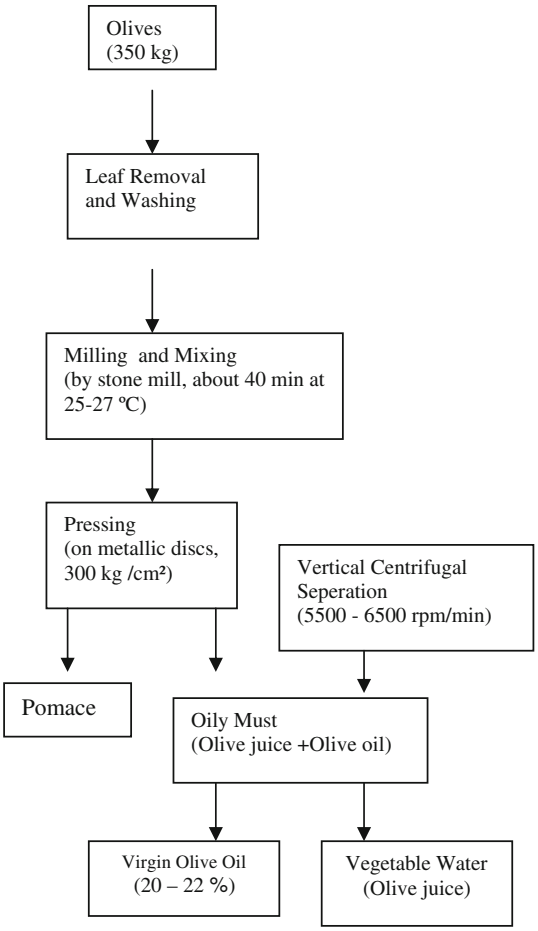
The Abencor extraction (MC2 Ingenierias y Sistemas, Sevilla, Spain) process, a laboratory model of the three phase continuous centrifugation system, predicts the industrial yield of oil. The apparatus used in the Abencor process consisted of a hammer mill, a mixer and a pulp centrifuge.

The varieties, locations and crop years of early harvest olives used for extracting oil are shown in Table 1.

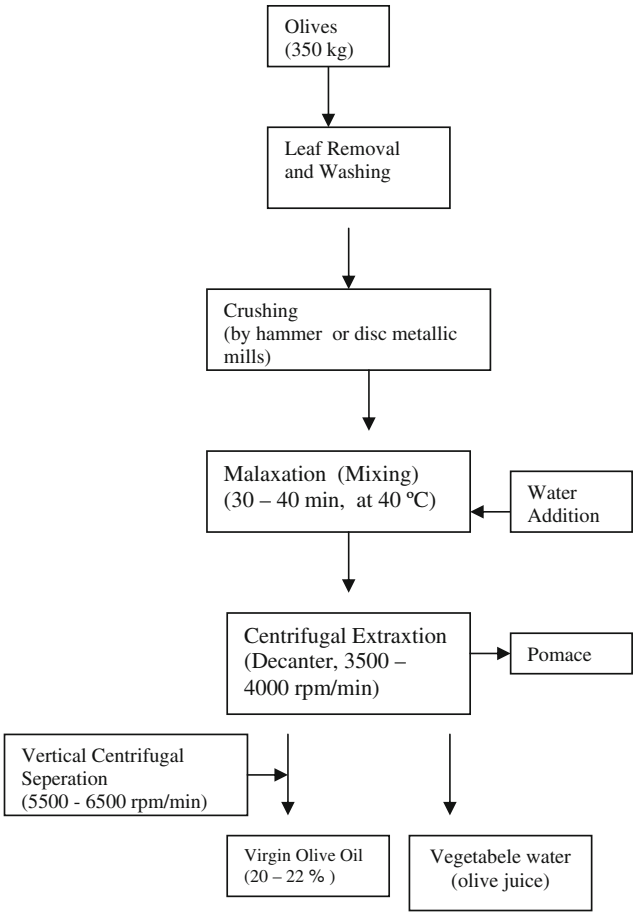
Methods

Determination of free fatty acids (FFA), peroxide values (PV) and UV absorption characteristics were carried out following the analytical methods described in Regulation EEC/2568/91 and EE/1429/92 of the European Union Commission (EU) [19].

Free fatty acidity (FFA), given as a percentage of oleic acid, was determined by titration of a solution of oil dissolved in 1:1 ethanol:ether with ethanolic potash. The peroxide value (PV), expressed in milliequivalents of active oxygen per kilogram of oil (mequiv O_2/kg oil), was determined by reacting oil and 3:2 chloroform:acetic acid with potassium iodide in darkness. The free iodine was then titrated with a thiosulfate solution. K_{232} and K_{270} extinction coefficients were calculated from UV absorption at 232 and 270 nm, respectively, collected on a UV spectrophotometer (Carry 50 UV–Vis, Varian Inc, Australia), using 1% oil in cyclohexane and path length of 1 cm. The



Scheme 1 The processing steps of the dry super-press system



Scheme 2 The processing step of the centrifugation system (three phases)

oxidative stability of virgin olive oil was estimated by the Schaal oven method, by heating a sample for 24 h at 98 °C (±2 °C), described in Škevin et al. [18]. Briefly, 10 g of oil was placed in each of 2 open petri dishes and placed in an oven at 98 °C (±2 °C) for 24 h. Stability was expressed as the change in peroxide value (CPV%) according to the formula:

$$\mathrm{CPV}\% = \mathrm{PV}_2 - \mathrm{PV}_1/\mathrm{PV}_1 \times 100$$

PV_1 peroxide value of sample at the beginning
PV_2 peroxide value at the end

The total chlorophyll content was calculated at 630, 670 and 710 nm, using carbon tetrachloride and a spectrophotometer (Carry 50 UV–Vis, Varian Inc, Australia). The calculation of total chlorophyll content [2] is as follows:

$$\mathrm{Ch}\ (\mathrm{mg/kg}) = [A_{670} - (A_{630} + A_{710})/2]/0.901L$$

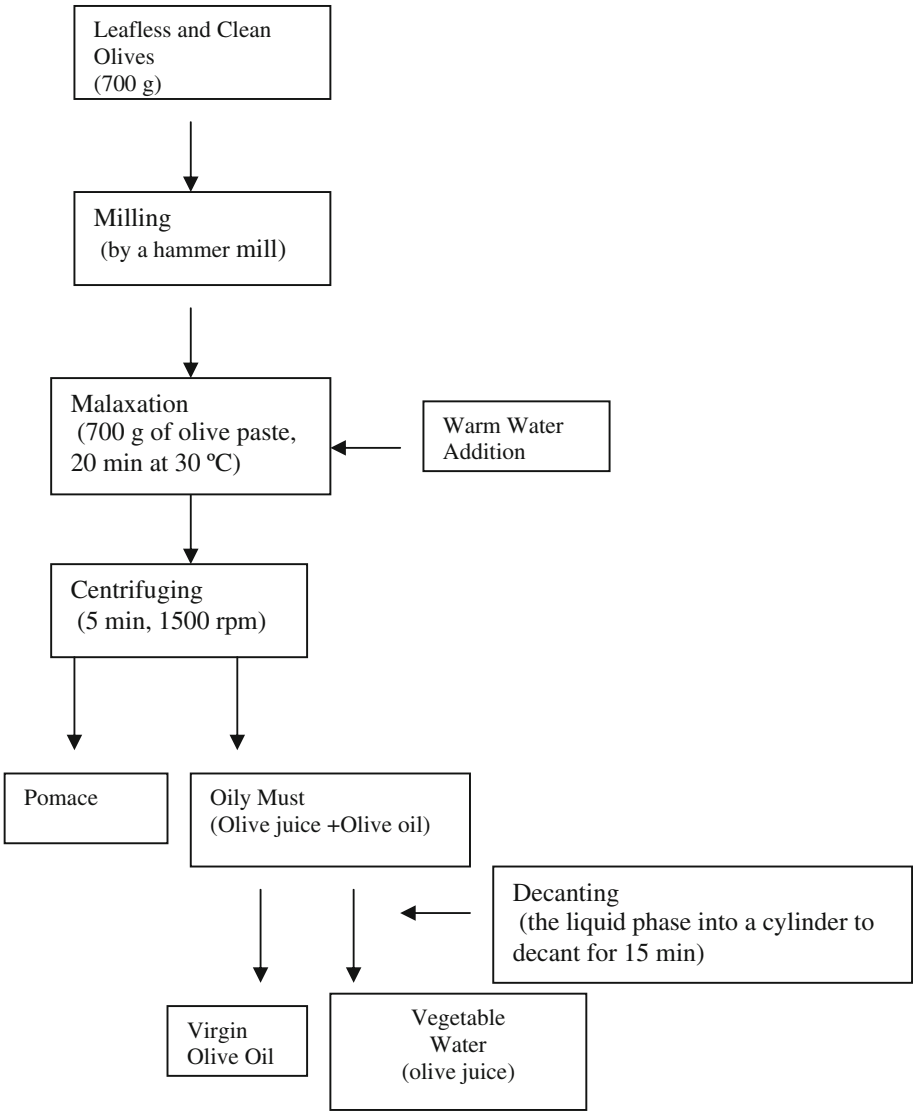
where A is the absorbance of the oil at the respective wavelength and L is the cell thickness (cm).

Pheophytin-α, an important compound from chlorophyll pigments, was calculated according to the equation, C (Pheophytin-α in mg/kg) = 345.3 $[A_{670} - (A_{630} + A_{710})/2]/L$ where A is the absorbance of the oil at the respective wavelength and L is the cell thickness (mm) as described by Psomiadou and Tsimidou [20].

Virgin olive oil color was determined according to the Quick Method for the definition and classification of the color of virgin olive oils [21]. The 60 standard solutions for the color determination were prepared with increasing volumes of 0.04% Brom Thymol Blue (BTB) in 1/15 M KH_2PO_4/Na_2PO_4 solution, according to the established procedure [21]. The BTB standard was stored in the dark at 20 °C.

The *cis–trans* fatty acid contents were determined using a capillary gas chromatographic method described in the European Union Commission [19]. Fatty acid methyl esters (FAMEs) were prepared by saponification/methylation with sodium methylate according to a cold methylation method [8]. Fatty acid analyses were carried out by gas chromatography (HP 6890, Hewlet Packard Inc., USA) using a DB-23 capillary column (30 m × 0.25 mm ID and 0.25 μm film thickness of 50% cyanopropyl, J&W Scientific, Folsom, CA, USA). The oven temperature ranged

Scheme 3 The processing steps of the Abencor extraction method



from 170 to 210 °C with an increase of 2 °C/min and then was held at 210 °C for 10 min. The carrier gas was helium (0.5 ml/min) and the injector and detector (FID) temperatures were 250 °C. The spilt ratio was 1:100 and the injected volume was 0.2 µl. The identification of FAMEs was performed by comparing each sample to a standard FAME reference mixture (Sigma-Aldrich Chemicals 189–19). The Squalene analysis was determined from the squalene that appeared in the fatty acid chromatogram. All fatty acid peak areas were calculated by the HP 3365 Chemistation program and recorded as the peak area percentage [8, 19].

Iodine values (IV) were calculated from fatty acid percentages by using the following formula [22]:

$$\begin{aligned} \mathrm{IV} = {} & (\%\ \text{Palmitoleic} \times 1.001) + (\%\ \text{Oleic} \times 0.899) \\ & + (\%\,\text{Linoleic} \times 1.814) + (\%\,\text{Linolenic} \times 2.737) \end{aligned}$$

Statistical Analysis

Data of early harvest olive oil groups were presented as means ± standard deviation (SD) and were subjected to analysis of variance (ANOVA). Among groups, significant means were compared by Tukey's multiple range tests at $\alpha = 0.05$ level (n-1 = 23). Statistical analyses were performed using the SPSS 12 (SPSS Inc., Chicago, IL, USA) statistics software [23].

Multivariate Analysis

Characterization and classification of early harvest olive oils from different locations in Turkey were carried out using chemometric methods, Principal Component Analysis (PCA, Ward Method) [24] and hierarchical cluster

Table 1 Varieties, locations and crop years of olives used for the oil extracting

Sample code no	The location	Geographical region	Crop year	Extraction system	Olive variety
NA-1	Ayvalik-1 (Balıkesir)	NA	2001	Continuous	Ayvalik
NA-2	Ayvalik-2 (Balıkesir)	NA	2002	Continuous	Ayvalik
NA-3	Ayvalik-3 (Balıkesir)	NA	2002	Continuous	Ayvalik
NA-4	Burhaniye (Balıkesir)	NA	2002	Continuous	Ayvalik
NA-5	Ayvalik-4 (Balıkesir)	NA	2004	Continuous	Ayvalık
NA-6	Edremit (Balıkesir)	NA	2004	Continuous	Ayvalık
NA-7	Çesmealti (İzmir)	IP	2006	Continuous	Gemlik
SA-1	Yesilyurt-1 (Muğla)	SA	2001	Continuous	Mostly Memecik and some Domat, Ayvalik
SA-2	Yesilyurt-2 (Muğla)	SA	2002	Continuous	Mostly Memecik and some Domat, Ayvalik
SA-3	Yesilyurt-3 (Muğla)	SA	2003	Continuous	Mostly Memecik and some Domat, Ayvalik
SA-4	Kemalpaşa (Izmir)	SA	2002	Continuous	Mostly Memecik and some Memeli, Ayvalik
SA-5	Seljuk (Izmir)	SA	2003	Super Press	Memecik
SA-6	Seljuk (Izmir)	SA	2004	Super Press	Memecik
MB-1	Akhisar-1 (Manisa)	EA	2001	Continuous	Mixture (Domat, Uslu, Gemlik)
MB-2	Olive Res. Inst.	EA	2003	Abencor	Uslu
MB-3	Orhangazi (Bursa)	MR	2003	Continuous	Gemlik
MB-4	Akhisar-2 (Manisa)	EA	2004	Continuous	Mixture (Gemlik, Uslu, Ayvalik)
MB-5	Akhisar-3 (Manisa)	EA	2004	Continuous	Mixture (Gemlik, Uslu, Ayvalik)
MB-6	Salihli (Manisa)	EA	2005	Continuous	Gemlik
SEA-1	Nizip-1 (Gaziantep)	SEA	2003	Continuous	Nizip Yaglik
SEA-2	Nizip-2 (Gaziantep)	SEA	2003	Continuous	Nizip Yaglik
SEA-3	Hatay	MeR	2003	Continuous	Gemlik
SEA-4	Alanya (Antalya)	MeR	2006	Continuous	Erkence
SEA-5	Northern Cyprus ***	MeR	2004	Abencor	Gemlik

NA North Aegean, *IP* Izmir Peninsula, *SA* South Aegean, *EA* East Aegean, *SEA* South Eastern Anatolia, *MR* Marmara region, *MeR* Mediterranean region

analysis (HCA, Euclidian distance) [24]. Multivariate analysis was performed using the Matlab 7.5.0 (R2007b) [25].

Results and Discussion

The results for free acidity, peroxide number, UV coefficients, oxidative stability, chlorophyll, pheophytin-α, color scale and squalene are shown in Table 2. The FFA values of samples were between 0.25 and 2.90% oleic acid (Table 2). The FFA test has traditionally been used as a basic commercial criterion for grading olive oil. According to the distribution of FFA values, early harvested oils were classified as extra virgin (11/24 or 45.8%), virgin (11/24 or 45.8%) and ordinary (2/24 or 8.3%) using the official FFA maximum values of 0.8, 2, and less than 3.3%, respectively, as guidelines.

The peroxide values (PV) ranged from 1.13 to 26.02 mequiv O_2/kg oil where 16.7% of the total samples had values below 20 mequiv O_2/kg oil the acceptable limit of the IOOC [26] and the Turkish Food Codex [27]. When olives were stored for 2–5 days in jugs or plastic sacks at 20–25 °C prior to oil extraction, the FFA and PV values exceeded the recommended limits.

The ultra violet (UV) absorption values at K_{232} and K_{270}, ranged from 1.31 to 3.02 and 0.09 to 0.27, respectively. The UV absorption extinction coefficient at 232 nm (K_{232}), is related to the primary oxidation of oil and is indicative of the conjugation of PUFAs. K_{270} is an indication of carbonylic compounds, aldehydes and ketones and is related to secondary oxidation products [1, 2]. The UV absorption values allow an approximation of the oxidation of unsaturated oils to be made. The UV absorption extinction coefficients, particularly K_{270}, were all below the limits established for extra virgin olive oils by IOOC [26] and the Turkish Food Codex [27]. The early harvest oils were classified as extra virgin (21/24 or 87.50% with $K_{270} \leq 0.22$), virgin (1/24 or 4.16% with $K_{270} \leq 0.25$), and ordinary (2/24 or 8.33% with $\leq$0.30) olive oils. The

Table 2 Information on basic quality parameters and selected chemical properties of olive oils and color scala*

Olive oil groups	Free acidity (oleic acid) (%)	Peroxide number (mequiv O_2/kg)	232 nm[a]	270 nm[a]	Peroxide number (mequiv O_2/kg) at 98 °C 24 h	Oxidative stability (as changing % in PV)	Total chlorophyll content (mg/kg)	Pheophytin-α (mg/kg)	Color scala
NA	0.62–1.26	9.91–26.02	1.69–3.02	0.09–0.21	13.82–72.36	181.55–17.32	2.56–11.46	7.84–39.78	2/1–3/6
$n = 7$									
Mean	0.85 a ± 0.20	15.56 c ± 5.97	2.24 c ± 0.45	0.16 a ± 0.05	29.15 ab ± 20.18	76.36 a ± 50.87	5.28 b ± 3.08	17.74 b ± 10.76	–
SA	0.25–2.50	4.21–22.60	1.37–2.30	0.09–0.27	10.90–30.43	32.26–578.19	2.88–8.78	10.09–29.24	2/2–3/2
$n = 6$									
Mean	0.98 a ± 0.86	9.13 a ± 7.05	1.69 a ± 0.33	0.17 a ± 0.07	23.00 a ± 8.25	236.83 b ± 204.40	5.11 b ± 1.96	17.13 b ± 6.45	–
MB	0.28–1.63	1.13–20.60	1.31–2.18	0.12–0.25	18.02–121.30	87.21–1567.73	0.84–6.93	2.92–24.17	2/1–3/1
$n = 6$									
Mean	0.95 a ± 0.57	10.56 a ± 7.18	1.86 b ± 0.35	0.16 a ± 0.05	49.47 c ± 45.66	527.36 d ± 544.12	2.32 a ± 2.30	14.66 a ± 16.36	–
SEA	0.28–2.90	6.80–19.27	1.31–2.16	0.12–0.30	14.49–258.70	59.93–1476.54	0.38–13.71	1.32–47.81	2/1–3/10
$n = 5$									
Mean	1.00 a ± 1.08	12.37 b ± 4.80	1.85 b ± 0.33	0.19 b ± 0.07	20.95 a ± 104.79	391.70 c ± 606.49	5.52 b ± 5.35	21.62 c ± 16.40	–

[a] UV coefficients

* The mean values in the same column with different letters show statistically significant differences ($P < 0.05$)

differences in UV absorptions, K_{232} and K_{270} coefficients, may be affected by cultivar, fruit quality, climatic and ecological conditions, harvest time, altitude, crop season, growing location and storage conditions [28–30].

Quality indices, FFA, PV, extinction coefficients (K_{232} and K_{270}), of early harvest oils were in agreement with those of commercial oils collected from different locations of Turkey [7, 11, 12] and the analytical results of the survey on regional characterization of Turkish olive oil varieties including Ayvalik, Memecik, Gemlik, Uslu, Nizip Yaglik and Erkence [10, 14–16]. There was a wide distribution in FFA, PV and UV absorption, K_{232} and K_{270} values for samples and groups. The variation and significance ($P < 0.05$) differences were determined among parameters PV, K_{232} and K_{270} of the oil groups, according to the results of the Tukey multiple range test (Table 2). The variation in quality parameters may be due to agronomic and post-harvest conditions. Low FFA, PV, K_{232} and K_{270} values, in the virgin olive oils depend on high quality fruit and the small scale extraction systems.

Although there is no agreed-upon standard method for oxidative stability analysis in virgin olive oils, the oxidative stability tests are recommended. Table 2 shows that the oxidative stability (OS) of oil samples ranged from 7.32 to 1567.54% according the changing peroxide value (CPV%). Oxidative stability by growing location and cultivar from high to low stability was North Aegean (Ayvalik variety) > South Aegean (Memecik variety) > South Eastern Anatolia (Nizip Yaglik, Erkence and Gemlik varieties) > Bursa – Manisa (Domat, Uslu, Gemlik varieties). The cultivars having the highest and lowest values for oxidative stability were found in Ayvalik (NA) and Uslu (MB), respectively. Our data agree with widely held beliefs of the national olive oil sector that location and cultivar play significant roles in oil quality. Similar changes on oxidative stability were reported in oils of Arbequina olive variety, Spanish origin, in different regions of Tunisia [31] and single variety olive oils from new productive zones in Argentina [30]. There were large differences ($P < 0.05$) among groups in terms of oxidative stability (Table 2) perhaps due to cultivar characteristics and ecological factors. Oxidative stability depends on the olive variety and quality, location, maturity stage, oleic/linoleic or MUFA/PUFA ratio, antioxidants (phenolic substances, tocopherol content, volatile components), oil extraction systems (Schemes 1, 2, and 3) and storage conditions [28, 29, 31–33]. The results of the oxidative stability of the early harvested olive oil are in agreement with the findings on commercial oils [12] and domestic varieties [16] taken from different geographic locations in Turkey.

The total chlorophyll contents in samples were between 0.84 and 11.46 mg/kg and the range of pheophytin-α

Table 3 The concentrations of *cis–trans* isomers of fatty acid in the early harvest olive oil samples*

Fatty acids and their codes	North Aegean $n = 7$	Mean	South Aegean $n = 6$	Mean	Manisa–Bursa $n = 6$	Mean	South Eastern Anatolia $n = 5$	Mean
14:0 MA	0.01–0.02	0.02 ± 0.00 a	0.01–0.02	0.01 ± 0.00 a	0.01–0.02	0.01 ± 0.00 a	0.01–0.02	0.02 ± 0.00 a
16:0 PA	9.00–13.90	12.39 ± 1.97 a	11.63–13.47	12.21 ± 0.89 a	10.57–13.89	12.62 ± 1.15 a	13.71–18.20	15.73 ± 1.74 b
16:1 PoA	0.76–1.00	0.87 ± 0.08 a	0.73–0.96	0.84 ± 0.09 a	0.74–1.23	0.95 ± 0.17 a	1.20–1.74	1.39 ± 0.22 b
17:0 Mg	0.03–0.17	0.13 ± 0.05 b	0.03–0.11	0.05 ± 0.03 a	0.09–0.20	0.16 ± 0.03 bc	0.10–0.13	0.12 ± 0.01 b
17:1 MgO	0.06–0.25	0.20 ± 0.06 b	0.04–0.17	0.09 ± 0.04 a	0.24–0.29	0.26 ± 0.02 c	0.16–0.21	0.18 ± 0.02 b
18.0 SA	2.36–3.54	2.84 ± 0.46 ab	1.84–3.15	2.48 ± 0.47 a	1.61–3.63	2.92 ± 0.73 ab	2.64–4.25	3.53 ± 0.69 bc
18 :1*t*	0.00–0.02	0.01 ± 0.01 a	0.005–0.01	0.007 ± 0.00 a	0.00–0.01	0.01 ± 0.00 a	0.01–0.02	0.01 ± 0.00 a
18:1 OA	70.02–74.30	72.00 ± 1.70 b	73.18–76.36	74.06 ± 1.71 c	68.59–76.15	72.21 ± 2.43 b	64.91–72.95	68.56 ± 3.15 a
18:2 LO	7.43–10.98	9.92 ± 1.27 b	7.45–9.19	8.69 ± 0.64 a	6.95–11.40	9.31 ± 1.48 ab	6.87–11.02	8.86 ± 1.52 a
18:2*t* + 18:3*t*	0.03–0.08	0.06 ± 0.01 a	0.02–0.09	0.05 ± 0.02 a	0.05–0.09	0.07 ± 0.01 a	0.04–0.05	0.05 ± 0.00 a
18: 3 LnO	0.48–0.62	0.55 ± 0.05 a	0.55–0.71	0.64 ± 0.06 b	0.55–0.70	0.63 ± 0.07 b	0.53–0.77	0.63 ± 0.09 b
20:0 AA	0.39–0.57	0.46 ± 0.07 a	0.33–0.52	0.42 ± 0.06 a	0.24–0.49	0.43 ± 0.08 a	0.42–0.59	0.53 ± 0.08 b
20:1 (G)	0.22–0.35	0.29 ± 0.04 ab	0.29–0.35	0.32 ± 0.02 b	0.21–0.30	0.27 ± 0.03 a	0.21–0.27	0.23 ± 0.02 a
22:0 B	0.09–0.16	0.13 ± 0.02 a	0.13–0.10	0.11 ± 0.01 a	0.06–0.12	0.10 ± 0.00 a	0.10–0.15	0.12 ± 0.02 a
24:0 Lg	0.03–0.07	0.06 ± 0.01 a	0.05–0.06	0.06 ± 0.01 a	0.03–0.06	0.04 ± 0.01 a	0.06–0.10	0.07 ± 0.02 a
Squalene (SQ)	0.38–0.78	0.59 ± 0.12 a	0.59–0.95	0.73 ± 0.15 b	0.35–1.01	0.56 ± 0.26 a	0.44–1.22	0.83 ± 0.28 b
TFA	0.05–0.09	0.07 ± 0.01 a	0.03–0.10	0.05 ± 0.02 a	0.05–0.09	0.07 ± 0.01 a	0.05–0.07	0.06 ± 0.00 a
SFA	13.18–18.01	16.02 ± 1.67 ab	13.30–16.54	15.35 ± 1.30 a	14.95–17.73	16.29 ± 1.07 b	16.91–21.94	19.58 ± 2.48 c
MUFA	71.40–75.47	73.35 ± 1.65 b	73.65–77.35	75.31 ± 1.70 c	68.89–77.43	73.26 ± 2.79 b	65.13–75.16	69.81 ± 3.79 a
PUFA	8.01–11.49	10.46 ± 1.26 b	8.07–9.79	9.34 ± 0.65 a	7.52–12.05	9.95 ± 1.52 ab	7.64–11.60	9.93 ± 1.47 ab
PUFA / SFA	0.49–0.87	0.66 ± 0.13 b	0.58–0.70	0.61 ± 0.05 b	0.50–0.70	0.61 ± 0.08 b	0.44–0.53	0.47 ± 0.04 a
MUFA / PUFA	6.21–9.44	7.11 ± 1.11 a	7.52–9.58	8.11 ± 0.77 c	5.72–10.30	7.56 ± 1.54 ab	5.61–9.84	7.98 ± 1.76 c
C18:1 / C18:2 OA/LO	6.38–10.00	7.39 ± 1.24 a	7.87–10.25	8.57 ± 0.88 c	6.02–10.96	7.97 ± 1.66 b	5.89–10.62	7.98 ± 1.76 b
C16:0 / C18:2 PA/LO	0.83–1.81	1.28 ± 0.33 a	1.27–1.56	1.41 ± 0.10 ab	1.22–1.52	1.37 ± 0.1 ab	1.51–2.00	1.79 ± 0.2 b
C18:2 / C18:3 LO/LNo	12.81–21.54	18.11 ± 3.09 c	12.10–16.25	13.33 ± 1.42 a	12.19–17.54	14.68 ± 1.94 b	8.92–19.00	14.45 ± 3.69 ab
Iodine value (IV)	82.73–88.56	85.11 ± 2.15 b	84.26–86.70	85.07 ± 0.61 b	77.51–86.13	83.22 ± 2.75 a	79.57–88.43	82.41 ± 3.10 a

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *TFA* total *trans* fatty acids, *SQ* squalene

*Each value is an average of three determinations, and values in the same row with different letters show statistically significant differences ($P < 0.05$). Each value is expressed as wt% total fatty acid methyl esters

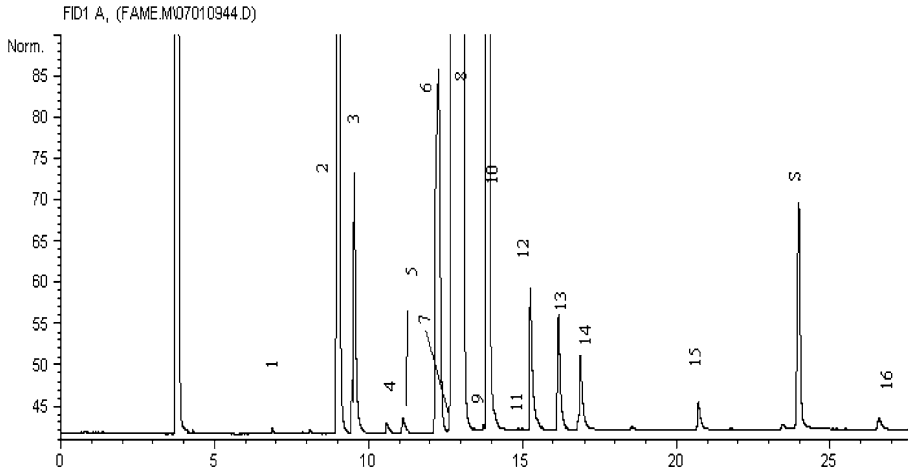


Fig. 1 A typical chromatogram olive oil extracted from early harvested olives, Memecik variety and by dry super press technique in Selcuk–İzmir (Sample no: 13). **1** 14:0, **2** 16:0, **3** 16:1, **4** 17:0, **5** 17:1, **6** 18:0, **7** 18:1*trans*, **8** 18:1, **9** 18:2*trans*, **10** 18:2, **11** 18:3*trans*, **12** 18:3, **13** 20:0, **14** 20:1, **15** 22:0, **16** 24:0, *S* Squalene

(pheo-α), among chlorophylls in the virgin olive oil predominant fraction, was 2.92–39.78 mg/kg (Table 2). The distribution of the pheo-α in samples was similar to that of chlorophyll. There is no official standard for total chlorophyll and pheophytin-α contents. Chlorophyll has always been considered a characteristic pigment of virgin olive oil. Chlorophyll decreases the oxidative and flavor stability of oil. Therefore, the chlorophyll content should be between 1 and 20 ppm [1, 2]. There was a wide variation and significant ($P < 0.05$) difference among the chlorophyll and pheophytin-α values of the oil groups (Table 2). The total level of chlorophyll pigments in olive oil depend on genetic factors, the stage of fruit ripeness, fruit quality, the altitude, the extraction process (Schemes 1, 2, and 3) and storage conditions [9, 20, 30]. Oils rich in chlorophyll were rapidly oxidized when exposed directly to light. Therefore, early harvest oils must be stored in the dark, such as in a green bottle, in order to preserve the quality. The results of total chlorophyll and pheophytin-α contents agreed with the findings of others [9, 20, 28, 30].

The color scale changes of oil samples were between 2/1 and 3/10. No important color differences in single variety oils have been noted between Ayvalik and Memecik olive varieties in two maturity stages [34] nor in commercial virgin olive oils taken from different locations [9]. The data on the color changes were generally similar to the results of these researchers. The color of olive oil is a visual quality indicator for consumers. It may vary among olive oils extracted at different times. The pigments (chlorophylls, pheophytins, xanthophylls and carotenes), mostly present in the olive fruit at harvest time, are mainly responsible for the color of olive oil. As maturity advances, the color of olive oil turns from light green to golden yellow and overripe fruit gives olive oil green to light brown color due to the presence of pheophytins. Also, the oil extraction systems (Schemes 1, 2, and 3) affect the color of virgin olive oil [2].

Olive oil contains large amounts of squalene, a terpenoid hydrocarbon, a phytosterol and having antioxidant properties. The amounts of squalene determined in oil samples ranged from 0.38 to 1.22%. The differences in squalene levels in olive oils may depend on the specific cultivar and the altitude at which the trees are grown [2]. These findings are generally in accordance with French olive oils [35] and domestic-foreign olive cultivars [13] in which the values range from 0.74 to 1.14% in French olive oils and from 0.35 to 1.03% in the National Olive Genetic Bank samples [1]. Squalene levels differed significantly between the oil groups (Table 3).

The fatty acid composition is a quality parameter and authenticity indicator of virgin olive oils. As shown in Table 3, numerous *cis–trans* isomers of fatty acids were detected in the early harvest oil samples produced from important domestic olive varieties. A typical chromatogram of an early harvest extra virgin oil sample [represented by dry super pres extracted (Scheme 2) oil from Memecik] is shown in Fig. 1.

The MUFAs are of great importance because of their high nutritional value and positive effect on oxidative stability of oils. Oleic acid (18:1), the predominant fatty acid in virgin olive oil, was between 64.91 and 76.36%. The oil groups from the southern part (SEA) of Turkey had the lowest oleic acid and MUFAs levels compared to the other district groups (NA, SA and MB) (Table 3).

Linoleic acid, which is much more susceptible to oxidation than MUFAs ranged from 6.87 to 11.40%. Palmitoleic, stearic, linolenic and arachidic (20:0) varied between cultivars, however, the actual contents were small (Table 3). The linolenic acid level of Turkish virgin olive oil samples was below the maximum value fixed by the IOOC (1.0%) and by the Turkish Codex and the EU (0.9%) [26, 27]. These findings are in agreement with the results found in various olive oil producing countries of the Mediterranean basin [8, 10, 11, 13, 14, 28, 29, 32, 35, 36].

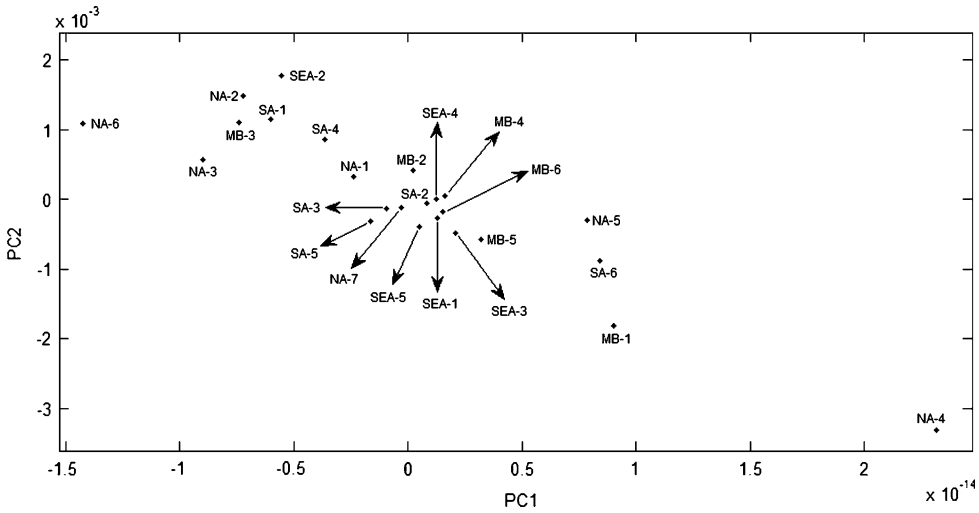


Fig. 2 The principal component analysis (PCA) results concerning the distribution of early harvest virgin olive oil samples from different geographical locations of Turkey based on their fatty acid profiles

The percentages of saturated fatty acids, MUFAs and PUFAs of early harvest olive oils were also calculated (Table 3). The SAE group mean had the highest total SFAs (21.94%) essentially due to the higher palmitic acid content, which represents the major fatty acid in the SFA fraction. The SA group, specifically Memecik, contained the highest percentage (75.31%) of total MUFAs. The NA group had the highest total PUFAs (10.46%) due to the high linoleic acid content.

Virgin olive oils are classified into two types based on their fatty acid compositions. The first type of olive oil is characterized by low linoleic and palmitic and high oleic acid contents. The second type has high linoleic and palmitic and low oleic acid contents. Turkish virgin olive oils (like Spanish, Italian and Greek) are of the first type, while Tunisian oils are of the second type [1].

The fatty acid composition of the early harvest olive oil groups from different locations of Turkey has been shown to be different with regard to the ratios of oleic to linoleic acid and MUFAs to PUFAs which are an important indicator of oxidative stability (Table 3). The nutritional fatty acid ratios of oil groups ranged from 13.33 (SA group) to 18.11 (SA group). Thus, the ratios were greater than 12, the minimum standard for Turkish olive oils. According to the results of the Tukey's multiple range tests (Table 3), there were wide variations and significant ($P < 0.05$) differences among the fatty acid profiles of the early harvest virgin oil samples. The variations in fatty acid profiles of early harvest olive oils differ depending on the zone of olive production. Primary factors affecting fatty acid composition, especially oleic acid content, in virgin olive oils may originate from latitude, climate, olive cultivar and/or stage of fruit maturity during harvest [1, 2, 8, 18, 28, 29, 36].

The distribution of fatty acids in early harvest oils was in agreement with those of commercial oils collected from different locations of Turkey [5–7, 9, 10] and other authors working on Turkish olive oil varieties [12–16]. Also, these values were similar to those reported for foreign olive oil varieties [18, 30, 31, 35, 36].

Palmitic (16:0), oleic (18:1),linoleic (18:2) and stearic (C18:0) were measured as major fatty acids. Palmitoleic (16:1), linolenic (18:2) and arachidic (20:0) acids were present in small amounts. All the values of fatty acids during six harvest years were in conformity to those of the IOOC's regulation [26], the EU [19] and the Turkish Food Codex standards [27].

The level of palmitic acid (16:0), the major fraction of SFA in olive oil, ranged from 9.00 to 18.20% and the range of stearic acid levels (18:0) in samples were 1.61–4.25%. The differentiation between structural isomers of these two fatty acids would bring a better knowledge of the chemical composition of olive oil and can be of great interest in their nutritional impact.

Elaidic acid and total *trans* isomers of linoleic and linolenic acids in early harvest groups ranged from 0.00 to 0.02% and 0.05 to 0.07%, respectively. The oils contained small amounts (0.05–0.07%) of total *trans* fatty acids. The total amount of *trans* fatty acids in virgin olive oils should be not exceed 0.1%. The all *trans* fatty acid isomers C18:1*t* and C18:2*t* + C18:3*t* were generally within acceptable limits of the IOOC's regulation [26], the EU [18] and the Turkish Food Codex standards [27]. This distribution of the *trans* fatty acids were similar to those reported in Turkish virgin olive oils [6–8, 10, 14, 15, 17].

Iodine values (IV) were calculated according to their fatty acid compositions. The IV values were within the limits specified by the Turkish Food Codex standard [27]. The saturated (SFAs) and unsaturated (MUFAs and PUFAs) levels of olive oils affected the IV.

Fatty acid profiles (Figs. 2 and 3) played a role in the characterization of virgin olive oil from different geographical locations of Turkey. The chemometric analysis

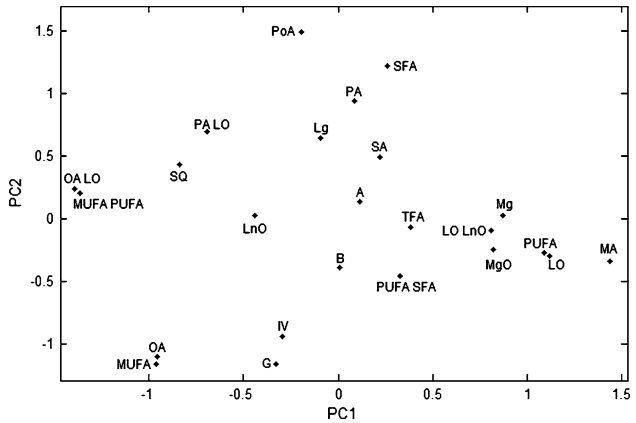


Fig. 3 The principal component analysis (PCA) results concerning the distribution of early harvest virgin olive oil samples the fatty acid profiles from different geographical locations of Turkey

(PCA) showed that palmitoleic acid, PA/LO and squalene were responsible in the classification of SEA-2, NA-2 and NA-6 samples. Palmitic, lignoceric, stearic and arachidic acids and SFAs were discriminative in classification of MB-6, NA-7, SEA-1, SA-5, SEA-5, SEA-3, SEA-4 and MB-4 samples. The TFA and behenic acid aided in the characterization of SA-6, MB-5 and NA-5 samples. The MB-1 sample was characterized with PUFA/SFA. The variance levels explained by the PCA were 33.18 and 56.30% for PC1 and PC2, respectively. The fatty acid profiles were determined as 28.94 and 52.48% for PC1 and PC2, respectively.

In the present study, the early harvest virgin olive oils from different geographic locations of Turkey were classified by chemometric methods (Principal component analysis, PCA and Hierarchical cluster analysis, HCA). The dendrogram (Fig. 4) based on the HCA results (Euclidian method) of early harvest virgin olive oil samples could be separated into four groups based on their fatty acid profiles.

Group 1 NA-3, NA-4, NA-2, NA-1 (Ayvalik), MB-1, MB-4 (the mixture with Uslu, Domat, Gemlik olive cultivars) MB-2 (Gemlik), NA-5, NA-6 (Ayvalik)

Group 2 SEA-1, SEA-2 (Nizip Yaglik), SEA-5 (Gemlik)

Group 3 NA-7 (Gemlik), SA-5, SA-6 (Memecik), SA-2, SA-4, SA-3 (the mixture with domestic Memecik)

Group 4 SA-1 (the mixture domestic Memecik), MB-3 (Gemlik), MB-5 (the mixture with domestic Gemlik), SEA-4 (Gemlik), MB-6 (Gemlik), SEA-3 (Gemlik)

The HCA results indicated a predominance in most of the samples from the same source (sub region or the geographical location). The oil samples were classified based on the olive growing zone according to the HCA results.

Fifteen fatty acids and 11 chemical parameters were used to identify early harvest virgin olive oil samples from different geographical locations of Turkey (Fig. 5). The linoleic acid, PUFA and linolenic acid of the oil samples grouped to the left in the dendrogram. The oleic acid, from characteristic MUFAs in olive oil, was grouped together with MUFA and Iodine value (IV) in the same cluster (Fig. 5). The palmitic acid and SFA appeared together with PUFA/SFA, PA/LO, Squalene, MUFA/PUFA on middle dendrogram. Some SFAs, Stearic, Arachidic, Behenic and Lignoceric, grouped to the right of dendrogram. Similar investigations based only on fatty acid compositional data for French [35] and Turkish [11, 13, 17] oils resulted in a few defined regions and crop years. However, the previous studies were carried out using different software programs (SAS, SPSS, SIMCA, Matlab), including PCA, HCA and DA than those used here.

These data provide evidence of the variation in olive oil quality, especially the early harvest, and *cis–trans* isomers of fatty acids resulting from Turkey's diverse

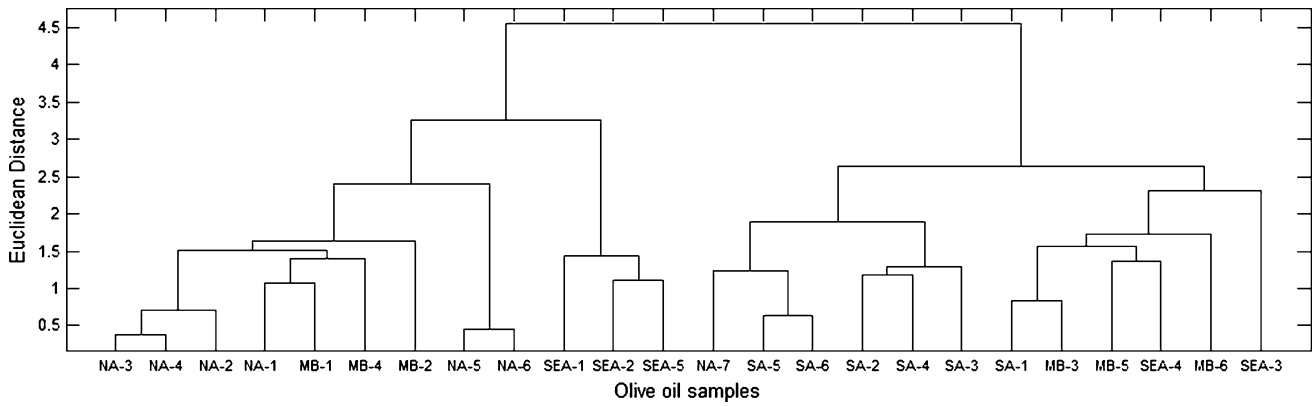


Fig. 4 The dendogram on hierarchical cluster analysis (HCA) results concerning the classification of early harvest virgin olive oil samples from different geographical locations of Turkey based on their fatty acid profiles

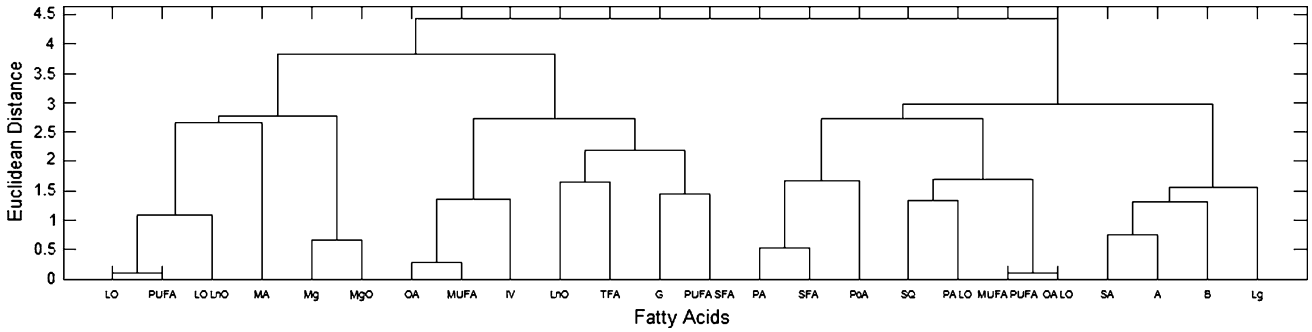


Fig. 5 The dendogram on HCA results concerning the distribution of the fatty acid profiles of early harvest virgin olive oil samples from different geographical locations of Turkey

ecological environments and important olive cultivars. In conclusion, the results (FFA, PV and the UV absorption values) presented here indicate that the improvement and protection of the early harvest oil quality depend on some specific conditions such as favorable fatty acid profile, rapid transportation, fresh extraction and storage in steel containers.

Early harvesting could be an effective way of preventing damage due to climate and insects and to improve the virgin olive oil quality in Turkey. In light of the results obtained in this study, a more detailed study is required to establish whether the differences in the chemical properties of early harvest olive oils are mainly due to agronomic and climate variables or, to the processing practice employed by the olive oil plants.

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