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Major fatty acids composition of 32 almond *(Prunus dulcis [Mill.] D.A. Webb)* genotypes distributed in East and Southeast of Anatolia

[Doğu ve Güneydoğu Anadolu'da yayılış gösteren 32 farklı badem *(Prunus dulcis [Mill.] D.A. Webb)* genotiplerinin başlıca yağ asitleri kompozisyonu]

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ABSTRACT

Objective: In this study, the fatty acid compositions of 32 different almond *(Prunus dulcis (Mill.) D.A. Webb)* genotypes seeds that collected from South and South East Anatolia regions in Turkey were studied.

Methods: For lipid extraction of almond genotypes Hara and Radin (1978) method were used. Fatty acids content were determined using gas chromatographic (GC) analysis. The datas were evalulated with SPSS 17.0 statistical program.

Results: In the gas chromatographic analysis, palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) were determined to be 5.34%, 0.70%, 0.85%, 74.46%, 17.89%, and 0.75% respectively. Saturated fatty acids (SFA) 6.19%, unsaturated fatty acids (USFA) 93.81% and a rate of USFA/SFA of 15.40, monounsaturated fatty acids (MUFA) 75.16%, polyunsaturated fatty acids (PUFA) 18.65% and a MUFA/PUFA ratio of 4.32 were found. In the correlation analysis, the highest correlation coefficient (r=-0.988) was detected between oleic acid and linoleic acid. Relationships among almond samples were partially identified with the cluster analysis.

Conclusion: In this study, the variations were found between almond genotypes collected from different locations in terms of fatty acids compositions. Besides, the almond genotypes that have high quality unsaturates fatty acids such as high oleic acids and low linoleik acids content were determened.

Key Words: Almond, seeds fatty acid composition, correlation, cluster analysis

Conflict of Interest: The authors declare no conflict of interest.

ÖZET

Amaç: Bu çalışmada, Doğu ve Güney Doğu Anadolu Bölgesinde yayılış gösteren 32 farklı badem *(Prunus dulcis (Mill.) D.A. Webb)* genotiplerinin tohum yağ asidi kompozisyonu çalışılmıştır.

Metod: Badem genotiplerinin lipit ekstraksiyonu için Hara ve Radin (1978) metodu kullanılmıştır. Gaz kromatografisi (GC) kullanılarak yağ asitleri içeriği belirlenmiştir. Veriler, SPSS 17.0 istatistik programı ile değerlendirilmiştir.

Bulgular: Badem genotiplerine ait tohumların yağ asidi kompozisyonuna göre palmitik asit (16:0), palmitoleik asit (16:1), stearik asit (18:0), oleik asit (18:1), linoleik asit (18:2), linolenik asit (18:3), omega-3 ve omega-6 yağ asitleri yüzde değerleri ortalaması sırasıyla %5.34, %0.70, %0.85, %74.46, %17.89, %0.75, %0.013 ve %18.64 olarak belirlenmiştir. Doymuş yağ asitleri (SFA) %6.19, doymamış yağ asitleri (USFA) 93.81, USFA/SFA oranı 15.40, tekli doymamış yağ asidi (MUFA) %75.16, çoklu doymamış yağ asitleri (PUFA) %18.65 ve MUFA/PUFA oranı 4.32 bulunmuştur. Korelasyon analizinde, en yüksek korelasyon katsayısı (r=-0.988) oleik asit ile linoleik asit arasında tespit edilmiştir. Badem genotipleri arasında akrabalıklar kısmi olarak klaster analizi ile belirlenmiştir.

Sonuç: Bu çalışmada farklı lokasyonlardan toplanan badem genotipleri arasında tohum yağ asitleri kompozisyonları bakımından geniş varyasyona rastlanmıştır. Bunun yanında, yüksek oleic asit ve düşük linoleic asit içeriği gibi yüksek kalitede doymamış yağ asitlerine sahip badem genotipleri belirlenmiştir.

Anahtar Kelimeler: Badem, yağ asitleri kompozisyonu, korelasyon, klaster analizi

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Turkey is one of the world's important sources of genes with its diversity of plant species. The almond is one of the essential species in this gene source. Also, it has considerable economic value with its different usage areas [1-3]. Part of the Rosaceae family, the almond [*Prunus dulcis* (Mill.) DA Webb, syn. *P. amygdalus* Batsch, and *P. communis* (L.)] [4] is divided into two varieties [4] pomologically which are sweet almond (*Prunus dulcis* var. *dulcis*), and bitter almond (*Prunus dulcis* var. *amara*). Almond, which is commonly cultivated for its fruit [5], is grown across a very large part of Turkey. However, there is no certain quality standard for the majority of produced fruits in Turkey.

The consumption and demand for almonds are high due to their impact on human health as a nutrition stabilizer with their high nutrient content and also for their high levels of mono unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) content [1-3,6]. Especially in recent years, the importance of fruit such as almonds which are rich oleic, linoleic and linolenic of unsaturated fatty acid (USFA) content has increased due to the positive effects on cholesterol and cardiovascular disease in humans [7,8]. Almond oil obtained from almond seeds is commonly used in multi-faceted ways in medicine, pharmaceutical and cosmetic industries [8-11].

Chemical composition is usually referred to as the quality characteristic of almond. For this reason, mainly the oil content and composition of almond seed has major importance [12]. A lot of the oil elements in almonds, which are the amounts of oleic acid, oleic/linoleic acid ratio and tocopherol concentration, are used as quality indicators [7,9,12,13]. Also, the oleic/linoleic acid ratio is used in determining the quality of the kernel due to its preventive effect on lipid oxidation [14]. The chemical composition of the various fatty acids which have a wide usage field depends on the genotype, climatic conditions, agriculture and harvest conditions [13,15].

There are wide variations in the morphological and genetic structure in the quality of almond genotypes which have different climates and wide distribution areas in Turkey. The present study will be provide better recognition of almond genotypes by indicating almond oil quality, fatty acid copmositions and its relationships. Nowadays, cytological, anatomical, palynological characters, electrophoresis, isozyme analysis, RAPD (Random amplified polymorphic DNA), DNA Fingerprinting, QTL (Quantitative Trait Loci), RFLP (Restriction Fragment Length Polymorphism), as well as a variety of molecular techniques and some of the secondary products (oil, protein, vitamins, sugar) and derivatives in seed are used in the taxonomic classification of plant species. In addition, the presence of these products provides important information about the quality and breeding of the taxon.

Several characteristics (fruit traits, blooming date, productivity, resistance to pests and diseases, etc.) were taken into account by human selection pressure when looking for the adequate genotypes adapted to local conditions and to the preference of the consumers. The genotypes having incorporated these highly selected traits represent very valuable germplasm for addressing future challenges in almond breeding. As a consequence, these genotypes are preserved, characterized in several almond collections and incorporated into advanced breeding programs [16]. Chemical composition of almond kernels has only been partially studied. This information would be crucial to increase the knowledge of their diversity, the nutritional and healthy value of the kernels, and the possibility of selecting the most adequate parents in a breeding program for increasing kernel quality [9]. In this study, fatty acid composition, correlation and relationship levels of 32 different almond genotypes distributing to East and Southeast Anatolia Regions seeds have been revealed. These findings can be used to identify almond fatty acids quality and selections of almond genotypes for breeding programs and serving to different productions.

Materials and Methods

Almond seed sources

32 different genotypes of *Prunus dulcis* (Mill.) D. A. Webb's mature seeds were collected from different localities of South and Southeast Anatolia Regions (Table 1). From these, the genotypes numbered 37, 42 and 53 are plain, the genotype numbered 43 is bitterish and all other genotypes are composed of sweet almonds.

Chemicals, organic solvents, equipment and tools

The chemicals for fatty acids analyses; palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1 n9), linoleic acid (18:2, n6), linolenic acid (18:3, n3) standards and trace amounts of other fatty acid standards existing in almonds, saturated and USFA methyl esters, methanol, acetonitrile, ethyl alcohol, sulfuric acid, hydrochloric acid, hexane, isopropanol, NaCl, $KHCO_3$ and deionized water.

Auxiliary equipment and devices

Homogenizer, SHIMADZU GC 17 gas chromatography, incubator, pure water apparatus, vortex, precision scale, automatic pipettes, centrifuge, freezer and autosampler vials.

Analysis of Fatty Acids

Preparation of plant extracts

Almond seeds were firstly fragmented with a mixer for two minutes. Then the extraction procedure [17] was performed.

Determination of Fatty Acid Contents

Almond seeds lipid extraction

In this study, Hara and Radin [17] method was used. Also,

Table 1. Localities where Almond seed samples (*Prunus dulcis* (Mill.) D. A. Webb) were taken

Sample number	Region of Turkey	Province	Sample number	Region of Turkey	Province
2	East	Hakkari/ Center	28	Southeast	Adıyaman/ Center
4	Southeast	Şanlıurfa/ Center	30	East	Malatya/ Center
6	Southeast	Şanlıurfa/Bozova	33	East	Malatya/Polat
8	Southeast	Mardin/ Center	35	East	Tunceli/Pertek
11	Southeast	Mardin/ Ömerli	37	East	Tunceli/Pertek
12	Southeast	Mardin/ Center	39	East	Van/Gevaş
13	Southeast	Mardin/ Center	40	East	Van/Gevaş
14	Southeast	Batman/Hasankeyf	42	East	Van/Gevaş
16	Southeast	Batman/Center	43	East	Bitlis/Tatvan
17	Southeast	Siirt/ Aydınlar	44	East	Elazığ/Hankendi
18	Southeast	Siirt/ Bağtepe	46	East	Elazığ/Keban
19	Southeast	Siirt/ Eruh	47	East	Elazığ/Center
21	Southeast	Diyarbakır/ Dicle	49	East	Elazığ/Yarımca
22	Southeast	Diyarbakır/ Hani	52	East	Elazığ/Keban
24	Southeast	Diyarbakır/ Ergani	53	East	Elazığ/Keban
27	Southeast	Adıyaman/ Center	54	East	Elazığ/Keban

Pollard and Stumpf [18] and Ozkaya et al. [19] were used this method in their studies for some plants seeds fatty acids analysis. The extraction of lipids from the almond samples was performed according to [17]. For this, 5 g almond samples were homogenized at a 3:2 (v/v) ratio in a mixture of 15 ml hexane-isopropanol at 11000 rpm for 30 seconds. Then, the homogenized samples were centrifuged at 5000 rpm +4ºC for 10 min and the supernatants were obtained. Thus, supernatants were used for the determination of fatty acids.

Preparation of fatty acid methyl esters from almond seeds

According to Hara and Radin [17], to perform a gas chromatographic analysis of the fatty acids in lipids, methyl esters converted to derivatives. For this, the acid-catalyzed esterification method was used. According to this method; to prepare the methyl ester, the lipid extract in the hexane/isopropanol phase was transferred to 30 ml test tubes. 5 ml of 2% methanolic sulfuric acid was added in and vortexed. This mixture was kept for methylation at 50ºC for 15 hours. At the end of the 15-hour period, the tubes were removed from the oven and cooled to room temperature and stirred thoroughly by the addition of 5 ml of 5% sodium chloride. The fatty acid methyl esters formed in the tubes were extracted with 5 ml hexane. The hexane phase was removed and 5 ml of 2% $KHCO_3$ was added, and allowed to stand for 4 hours to separate the phases. The solvent was evaporated under the nitrogen flow from the mixture containing methyl esters at 45ºC. The residue was dissolved with 1 ml hexane and analyzed by gas chromatography in the autosampler vials.

Gas chromatographic analysis of fatty acid methyl esters

The fatty acids methyl esters were analyzed by gas chromatography SHIMADZU GC 17. Ver. 3 for this analysis a Machery-Nagel BOND (Germany). Gas chromatography was performed with a capillary column (capillary column which was 25 m in length, 0.25 µm inner diameter and at a 25 micron film thickness) using nitrogen as a carrier gas (flow rate 0.8 mL/min.). During analysis, the column temperatures, detector, and injection valve were 120-220, 240, and 280ºC, respectively. Before the fatty acid methyl esters analysis of the samples, the standard mixture of fatty acid methyl esters by injection and each fatty acid retention time were determined. Identification of the individual methyl esters were performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions [20]. The fatty acid content of the almond seed samples was determined according to the standard fatty acid chromatogram (Figure 1). All of the almond genotips gas chromatographic analyses were carried out as shown in Figure 2. The results obtained as averages through triplicate with standard deviation and percentages of genotips values.

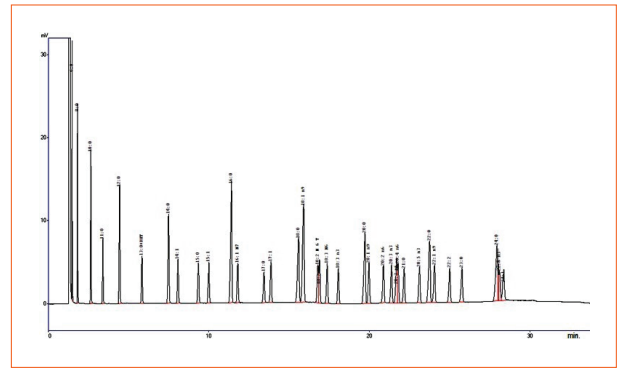

Figure 1. Standard fatty acids chromatogram.

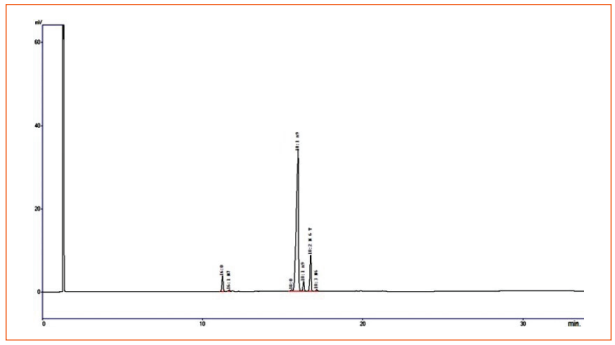

Figure 2. One of the almond fatty acids chromatogram example (1. reputation of 18 numbered genotype).

Statistical analysis of fatty acids

For statistical analysis the SPSS 17.0 software program was used. The analysis was performed in triplicate. As a result of the analysis, to compare differences between samples $p<0.05$, $p<0.01$ and $p<0.001$ probability level analysis of variance (ANOVA) and Duncan tests were performed. The mean values of the genotypes were detected by standard deviation and standard errors of averages. In analysis, fatty acid compositions, % rates and correlations were performed by comparing the averages. Furthermore, each fatty acid composition (palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid) in the seed except for existing trace amounts of almond fatty acids was combined and identified using cluster analysis.

Results and Discussion

In this study, in material and methods, while column temperature was set between 4ºC /min 200ºC to 220ºC with 35 minutes (in this study 34 minutes were used) analysis Sabudak [21], with starting 130ºC of column temperature and termal expansion applied 215-230ºC at 4ºC/min gradually. Sabudak [21] and Turan et al. [22] were used

Table 2. The average, minimum (bold written values)-maximum (bold and taken into boxes values), percentage and standard deviation values of the main fatty acids of almond seeds samples.

Almond Samples	Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	Omega-3	Omega-6
2	5.97±0.27	0.76±0.21	0.67±0.05	71.42±1.97	20.10±2.06	1.08±0.03	0.00	21.18±2.05
4	5.18±0.09	0.79±0.02	0.86±0.09	75.74±0.16	16.73±0.19	0.70±0.01	0.00	17.43±0.18
6	4.92±0.26	0.71±0.14	0.74±0.04	77.26±1.65	15.67±1.88	0.70±0.02	0.00	16.38±1.88
8	5.94±0.38	0.70±0.19	0.90±0.01	76.91±1.54	14.99±1.71	0.55±0.01	0.00	15.54±1.70
11	**4.42±0.16**	0.48±0.15	1.34±0.04	77.46±2.18	15.50±2.20	0.81±0.03	0.00	16.31±2.19
12	5.77±0.96	0.78±0.19	0.44±0.76	70.47±2.99	21.64±2.45	0.90±0.16	0.00	22.54±2.61
13	5.41±0.34	0.66±0.22	1.03±0.07	79.92±1.52	12.67±1.46	**0.32±0.28**	0.00	12.99±1.74
14	6.70±0.16	**0.95±0.02**	1.14±0.04	66.50±1.57	23.98±1.01	0.73±0.63	0.00	24.71±1.62
16	4.87±0.11	0.55±0.01	0.35±0.61	74.16±2.09	19.24±1.73	0.83±0.01	0.00	20.07±1.73
17	4.65±0.09	**0.42±0.14**	1.78±0.01	79.88±1.63	12.78±1.75	0.49±0.00	0.00	13.27±1.75
18	4.63±0.05	0.53±0.12	0.71±0.01	79.92±1.75	13.70±1.70	0.51±0.00	0.00	14.21±1.70
19	5.73±0.05	0.72±0.02	1.32±0.02	77.86±1.99	13.74±2.02	0.62±0.02	0.00	14.37±2.01
21	5.20±0.05	0.71±0.01	0.44±0.76	73.34±0.93	19.46±0.21	0.86±0.01	0.00	20.32±0.22
22	6.02±0.28	0.63±0.19	0.94±0.01	71.38±1.13	20.22±1.12	0.80±0.05	0.00	21.02±1.11
24	5.03±0.21	0.60±0.17	1.21±0.04	76.29±1.53	16.21±1.72	0.66±0.00	0.00	16.86±1.72
27	5.19±0.03	0.70±0.01	0.34±0.59	75.15±0.66	17.83±0.25	0.78±0.01	0.00	18.61±0.25
28	5.08±0.28	0.69±0.07	0.30±0.52	77.28±0.24	15.95±0.25	0.63±0.01	0.00	16.65±0.28
30	5.73±0.27	0.93±0.02	0.30±0.52	78.59±0.18	13.71±0.21	0.49±0.00	0.00	14.46±0.36
33	5.20±0.08	0.63±0.01	0.83±0.04	75.92±0.03	16.70±0.11	0.72±0.00	0.00	17.42±0.11
35	5.53±0.10	0.75±0.01	1.00±0.04	**64.05±2.12**	**27.00±2.09**	**1.67±0.04**	0.00	**28.67±2.13**
37	5.26±0.50	0.79±0.09	0.57±0.99	71.70±0.55	20.38±0.64	1.30±0.32	**0.30**	21.38±0.70
39	5.14±0.06	0.67±0.00	0.83±0.10	68.09±1.68	24.08±1.76	1.18±0.02	0.00	25.26±1.75
40	5.46±0.15	0.83±0.03	0.27±0.46	71.55±0.31	20.85±0.12	1.05±0.10	0.12	21.78±0.12
42	5.42±0.12	0.86±0.18	0.67±0.01	71.67±1.56	20.77±1.21	0.61±0.53	0.00	21.38±1.73
43	5.06±0.02	0.53±0.15	0.92±0.03	75.44±1.73	17.56±1.36	0.50±0.43	0.00	18.06±1.79
44	5.87±0.08	0.81±0.01	1.01±0.01	66.56±1.79	24.48±1.80	1.28±0.01	0.00	25.75±1.79
46	4.68±0.05	0.82±0.12	**0.26±0.45**	79.46±2.59	14.32±2.03	0.46±0.40	0.00	14.78±2.43
47	4.97±0.09	0.83±0.42	0.75±0.03	78.92±1.73	14.19±1.29	0.34±0.29	0.00	14.53±1.58
49	4.65±0.09	0.82±0.02	0.84±0.03	**80.68±2.08**	**12.65±1.81**	0.36±0.31	0.00	**13.01±2.12**
52	5.30±0.69	0.65±0.17	1.09±0.02	76.19±6.32	16.20±5.22	0.57±0.24	0.00	16.77±5.46
53	5.71±0.66	0.52±0.04	**1.67±0.50**	71.94±6.93	19.51±5.64	0.65±0.19	0.00	20.16±5.83
54	**6.25±0.39**	0.61±0.18	1.60±0.38	70.89±2.51	19.76±2.72	0.82±0.05	0.00	20.66±2.56
General Average	**5.34±0.58**	**0.70±0.17**	**0.85±0.51**	**74.46±4.74**	**17.89±4.13**	**0.75±0.35**	**0.013**±0.068	**18.64**±4.38

Table 3. Samples of fatty acid composition of almond seeds and for the mean of % rates with standard deviation and minimum maximum values.

Almond Samples	SFA	USFA	USFA/SFA	MUFA	PUFA	MUFA/ PUFA	Oleic/ Linoleic
2	6.64±0.24	93.36±0.24	14.07±0.54	72.18±1.90	21.18±2.05	3.43±0.40	3.58±0.44
4	6.04±0.01	93.96±0.01	15.54±0.02	76.53±0.18	17.43±0.18	4.39±0.06	4.53±0.06
6	5.66±0.30	94.34±0.30	16.70±0.93	77.96±1.61	16.38±1.87	4.81±0.61	4.98±0.66
8	6.84±0.38	93.16±0.38	13.64±0.83	77.61±1.43	15.54±1.70	5.04±0.68	5.19±0.74
11	5.76±0.16	94.24±0.16	16.38±0.48	77.94±2.12	16.31±2.19	4.84±0.72	5.07±0.80
12	6.21±0.20	93.79±0.20	15.12±0.53	71.25±2.80	22.54±2.61	3.20±0.53	3.30±0.54
13	6.43±0.41	93.57±0.41	14.58±1.02	80.58±1.58	**12.99±1.74**	6.30±1.04	6.38±0.91
14	7.84±0.11	92.16±0.11	**11.76±0.19**	67.45±1.58	24.71±1.62	2.74±0.25	2.78±0.18
16	5.22±0.51	94.78±0.51	18.29±1.77	74.71±2.09	20.07±1.73	3.75±0.45	3.88±0.48
17	6.43±0.10	93.57±0.10	14.56±0.25	80.30±1.70	13.27±1.75	6.14±1.01	6.35±1.08
18	5.34±0.05	94.66±0.05	17.73±0.18	80.45±1.68	14.21±1.70	5.73±0.86	5.91±0.92
19	7.05±0.04	92.95±0.04	13.18±0.07	78.58±2.01	14.37±2.01	5.56±1.00	5.77±1.08
21	5.64±0.72	94.36±0.72	16.92±2.12	74.05±0.93	20.32±0.22	3.65±0.08	3.77±0.09
22	6.97±0.27	93.03±0.27	13.36±0.58	72.01±1.11	21.02±1.11	3.43±0.24	3.54±0.25
24	6.25±0.25	93.75±0.25	15.03±0.64	76.89±1.61	16.86±1.72	4.60±0.60	4.75±0.63
27	5.53±0.58	94.47±0.58	17.20±1.79	75.86±0.65	18.61±0.25	4.08±0.08	4.22±0.08
28	5.38±0.25	94.62±0.25	17.61±0.83	77.97±0.22	16.65±0.28	4.68±0.09	4.85±0.09
30	6.03±0.26	93.97±0.26	15.61±0.69	79.52±0.17	14.46±0.36	5.50±0.15	5.73±0.10
33	6.03±0.11	93.97±0.11	15.60±0.29	76.55±0.03	17.42±0.11	4.39±0.03	4.55±0.03
35	6.53±0.06	93.47±0.06	14.32±0.14	**64.80±2.12**	**28.67±2.13**	**2.27±0.25**	**2.39±0.27**
37	5.83±0.50	94.17±0.50	16.23±1.40	72.49±0.46	21.68±0.95	3.35±0.17	3.52±0.14
39	5.97±0.16	94.03±0.16	15.76±0.44	68.77±1.68	25.26±1.75	2.73±0.26	2.84±0.29
40	5.73±0.32	94.27±0.32	16.50±0.93	72.37±0.33	21.90±0.03	3.30±0.02	3.43±0.03
42	6.10±0.13	93.90±0.13	15.41±0.35	72.52±1.67	21.38±1.73	3.41±0.37	3.46±0.28
43	5.98±0.03	94.02±0.03	15.73±0.08	75.96±1.82	18.06±1.79	4.24±0.55	4.32±0.45
44	6.88±0.07	93.12±0.07	13.54±0.16	67.37±1.78	25.75±1.79	2.63±0.26	2.73±0.28
46	**4.93±0.40**	**95.07±0.40**	**19.35±1.58**	80.28±2.68	14.78±2.43	5.56±1.21	5.65±1.07
47	5.71±0.06	94.29±0.06	16.50±0.19	79.75±1.54	14.53±1.58	5.54±0.75	5.60±0.66
49	5.48±0.06	94.52±0.06	17.23±0.18	**81.51±2.10**	13.01±2.12	**6.41±1.33**	**6.49±1.19**
52	6.39±0.69	93.61±0.69	14.75±1.61	76.84±6.15	16.77±5.46	4.94±1.67	5.07±1.71
53	7.37±1.16	92.63±1.16	12.81±2.38	72.46±6.98	20.16±5.83	3.94±1.78	4.04±1.83
54	**7.85±0.64**	**92.15±0.64**	11.80±1.10	71.49±2.54	20.66±2.56	3.51±0.56	3.65±0.64
General Average	**6.19±0.78**	**93.81±0.78**	**15.40±1.98**	**75.16±4.71**	**18.65±4.39**	**4.32±1.28**	**4.45±1.29**

SFA: Saturated fatty acid; USFA: Unsaturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

injection temperature at 250ºC close to our work.

Mainly fatty acids amounts were determined in almond genotips. The average amounts of fatty acids of almond genotypes with minimum-maximum values and standard deviation are shown as percentages in Table 2 and Table 3. The averages of palmitic acid (16:0) 5.34%, palmitoleic acid (16:1) 0.70% stearic acid (18:0) 0.85%, oleic acid (18:1) 74.46%, linoleic acid (18:2) 17.89% and linolenic acid (18:3) 0.75%, omega 3 and omega 6 were found. In addition, eicosenoic acid (20:3), docosahexaenoic acid (22:6) and tridecanoic acid (13:0) were encountered in very small amounts (<0.5%). Apart from these, SFA 6.19%, USFA 93.81% and a rate of USFA/SFA of 15.40, MUFA 75.16 %, PUFA 18.65 % and a MUFA/PUFA ratio of 4.32 were found. The ratio of oleic/linoleic acid was found to be 4.45 and the sum of oleic + linoleic acid was found to be between 90.48% - 93.78%. Omega-3 fatty acids were found in very low amounts in the majority of genotypes. According to Table 2, overall average of omega-3 was detected as 0.013% with 0.068 standard deviations, 0.005 variance and 0.007 standard error of average. The overall mean of omega-6 of genotypes was found as 18.64% with 4.38 standard deviations, 19.16 varience and 0.447 standard error of mean was detected. Despite the fact that low levels of omega-3 in total in almond genotypes, omega 6 fatty acids were detected in high values. It cannot be produced in human body, omega-3, and omega-6 hold important place in every period of human life and deficiency of its can cause disease in humans body. Therefore, with respect to omega-3 fatty acids, 35 and 40 numbered genotypes and with respect to omega-6 fatty acids, primarily 35 numbered genotype, 44, 39, 14 and 12 numbered genotypes are important. From these, the 35 numbered genotype have the highest degrees terms of

valuable fatty acids that the oleic, linoleic and linolenik acids.

Substantial differences were found between almond genotypes at $\alpha = 0.05$ significance level and the $p<0.001$ probability level according to the proportion of fatty acids. Different sub-groups were found between the genotypes to which the Duncan test was applied. Palmitic acid between 4.42% (genotye number 11) - 6.70% (genotyp number 14), palmitoleic acid between 0.42% (genotyp number 17) - 0.95% (genotyp number 14), stearic acid between 0.26% (genotyp number 46) - 1.78% (genotyp number 17) and oleic acid between 64.05% (genotyp number 35) – 80.68% (genotyp number 49) were found. Linoleic acid was found to be between 12.65% (genotyp number 49) - 27.00% (genotyp number 35) and linolenic acid was found to be between 0.32% (genotyp number 13) - 1.67% (genotyp number 35) which are essential PUFA. The genotyp numbered 35 was one of the important genotypes among all almond genotypes in terms of linoleic and linolenic acid.

A higher proportion of USFA is prefered to SFA because of its benefical effect on human health for almond seed. While the lowest amount of SFA was detected to be 4.93% in the genotype numbered 46, the highest SFA rate was found to be 7.85% in the almond genotype numbered 54. USFA was found to be between 92.15% (genotype number 54)- 95.07 (genotype number 46) in terms of average values of each of the genotypes. The ratio of USFA to SFA (USFA/SFA) is an important feature of oil quality in almond seeds. According to this feature, the almond genotype had rates between 11.76 (genotype number 14) and 19.35 (genotype number 46). Unsaturated fatty acids to saturated fatty acid ratio (USFA/SFA) were higher of overall average of the 32 almond genotypes. It will provide important quality improvement as weel as the effort intensified on these almond genotypes especially 46 numbered genotype that has USFA ratio 95.07% and USFA/SFA ratio 19.35, 16 (USFA/SFA ratio 18.29) and 18 numbered genotype (USFA/SFA ratio 17.73). In terms of MUFA, relative to USFA, the genotypes had ratios between 64.80% (genotype number 35) - 81.51% (genotype number 49). Also, PUFA received between 12.99% (genotype number 13) - 28.67% (genotype number 35). The MUFA/PUFA ratio was evaluated because MUFA is more high quality than PUFA. Therefore, the MUFA/PUFA ratio was found to be between 2.27 (genotype number 35) and 6.41 (genotype number 49) in the genotypes. Likewise, a high oleic/linoleic acid ratio is also prefered. In this respect, the highest rate (6.49) was seen in the genotype numbered 49 numbered. In addition to these, USFA which is based on oleic and linoleic acids is also known as the dominant fatty acid. In terms of sum USFA percentage, the genotypes numbered 46, 18, 16, 28, 49, 27, 47 and 43 have more than 94% (94.02-95.07%) which have high rates while the genotypes numbered 54, 14, 53 and 19 have low values (92.15% and 92.95%).

According to the evaluation of the most important fatty acid compositions such as PUFA, oleic acid, MUFA, USFA/PUFA and oleic/linoleic acids of the almond genotypes, the highest levels were identified in the genotypes numbered 46, 49, 18, 16, 28 and 27. We can say that these genotypes are suitable for agriculture in terms of the quality of the fatty acid. SFA, which has less desirable content such as palmitic and stearic acids, was identified in the genotypes numbered 54, 14, 53 and 19 at high rates. In other words, the genotypes that have low rates of total USFA can be eliminated for breeding programs.

Some important results have been obtained in terms of the fatty acid composition, and the rates and correlations of almond genotypes (Table 4). The highest negative

Table 4. Significant positive and negative correlations between fatty acids and their proportions

Fatty Acids	Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	SFA	USFA	USFA/ SFA	MUFA	PUFA	MUFA/ PUFA	Oleik/ Linoleik
Palmitic	1.000												
Palmitoleic	0.362	1.000											
Stearic	0.022	-0.400	1.000										
Oleic	-0.579	-0.212	-0.070	1.000									
Linoleic	0.481	0.191	-0.017	-0.988	1.000								
Linolenic	0.274	0.077	-0.094	-0.758	0.757	1.000							
SFA	0.759	0.009	0.668	-0.477	0.347	0.143	1.000						
USFA	-0.759	-0.009	-0.668	0.477	-0.347	-0.143	-1.000	1.000					
USFA/SFA	-0.725	-0.005	-0.686	0.466	-0.336	-0.158	-0.986	0.986	1.000				
MUFA	-0.570	-0.177	-0.086	0.999	-0.988	-0.760	-0.480	0.480	0.469	1.000			
PUFA	0.477	0.188	-0.026	-0.988	0.999	0.790	0.338	-0.338	-0.329	-0.988	1.000		
MUFA/PUFA	-0.468	-0.152	0.046	0.951	-0.963	-0.760	-0.318	0.318	0.309	0.952	-0.965	1.000	
Oleic/Linoleic	-0.472	-0.167	0.046	0.955	-0.969	-0.737	-0.321	0.321	0.310	0.955	-0.968	0.998	1.000

SFA: Saturated fatty acid; USFA: Unsaturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

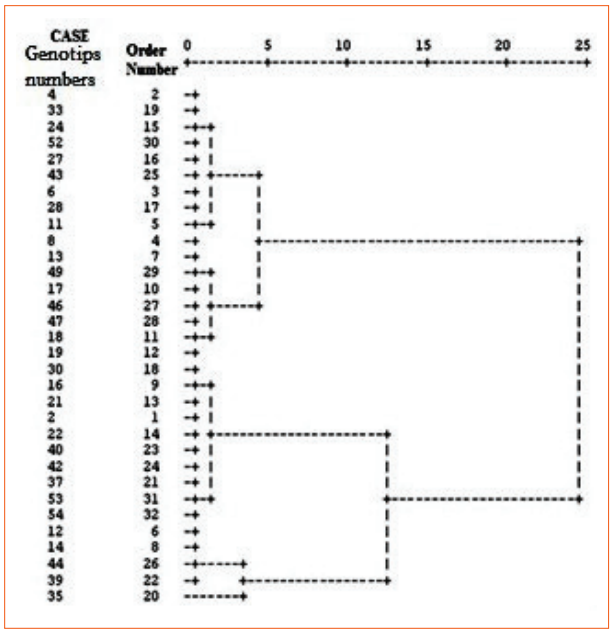

Figure 3. Dendrogram created according to the combination and rate of fatty acids and their smilarities of genotips.

correlation (r=-0.988) was identified between oleic acid and linoleic acid according to the correlation analysis of fatty acid compositions of almond genotypes. Kodad et al. [23], Abdallah et al. [24] stated that there is a negative correlation between oleic and linoleic acids. This result confirmed our findings. Also, a positive correlation (r=0.757) was found between linoleic and linolenic acid. Both fatty acids tend to increase or decrease. Likewise, a negative correlation (r=-0.758) was determined between oleic and linolenic acid as well. In the other words, when oleic acid increased, linoleic and linolenic acid decreased, and vice-versa. A negative correlation coefficient (r=-0.579) was found at a lower level between palmitic acid and oleic acid. In the same way, there were lower positive correlations between palmitic acid and linoleic acid (r=0.481) and between palmitic acid and linolenic acid (r=0.274) contrary to between palmitic acid and oleic acid. The corelations between stearic acid and the other fatty acids generally had very low values. However, we can say that there is a negative correlation between stearic acid and palmitoleic acid at a low-level (r=-0.400). Generally, except for this correlation coefficient, positive correlations (r=0.95 and above) were found with the oleic acid/linoleic acid ratio and oleic acid, MUFA, MUFA/PUFA ratio. The existance of a high ratio of specific fatty acids has a significant effect on correlation coefficients.

The presence of variations and relationships between different origins of almond were exhibited by the dendrogram created using the composition of fatty acids of the genotypes (Figure 3). According to this, two major groups of origins occurred. Also, one of groups was divided into two sub-groups. From these, while the genotypes numbered 44, 39, and 35 numbered created a distinct subgroup, the genotypes numbered 4, 33, 24, 52, 27 and the

Table 5. The comparison of almond fatty acid compositions according to some different sources.

References	Palmitic Acid (16:0)	Palmitoleic Acid (16:1)	Stearic Acid (18:0)	Oleic Acid (18:1)	Linoleic Acid (18:2)	Linolenic Acid (18:3)	Oleic/Linoleic	SFA	USFA	USFA/SFA	MUFA	PUFA	MUFA/ PUFA
Karatay [25]	5.34	0.70	0.85	74.46	17.89	0.75	4.45	6.19	93.81	15.40	75.16	18.65	4.32
	(4.42-6.70)	(0.42-0.95)	(0.26-1.78)	(64.05-80.68)	(12.65-27.00)	(0.32-1.67)	(2.39-6.49)	(4.93-7.85)	(92.15-95.07)	(11.76-19.35)	(64.80-81.51)	(12.99-28.67)	(2.27-6.41)
Piscopo et al. [26]	4.97-7.28	0.38-0.79	1.10-2.03	74.12-81.07	11.01-16.77	0.02-0.06	4.44-7.36			10.24-14.17			4.47-7.41
Kodad et al. [23]	5.96-6.61	0.45-0.54	1.68-2.31	67.19-74.34	16.55-22.72		2.96-4.49						
Mexis et al. [2]	5.31		2.69		19.04			8.15			72.81	19.04	
Kodad and Socias [13]	5.0-7.1	0.3-0.8	1.1-2.8	63.1-78.7	12.1-27.1								
Sathe et al. [30]	5.07-6.78			57.54-73.94	19.32-35.18								
Socias et al. [9]				63.10-75.60	16.55-27.70		2.46-4.53						
Sabudak [21]	5.97	0.03	1.85	68.63	21.77	0.06	3.15	8.1	90.7	11.20			

SFA: Saturated fatty acid; USFA: Unsaturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

other genotypes that existed in other groups had high relationship affinites. In the dendrogram, the bitter almonds which were the genotypes numbered 37, 42 and 53 were in the same group and were seen to be close relatives. Some of the genotypes that were collected from similar geographic regions were involved in the same sub-groups as double or triple genotypes. Relationships of almond samples were partially identified in the cluster analysis. Furthermore, some almond genotypes having essential fatty acids and its ratios such as 46, 16 and 18 numbered were determined for using breeding programs. The dendrogram that consisted of fatty acids composition shows a lower level relationship than the dendgrogram consisting of the sum of the band profiles of SDS-PAGE protein subfractions [25].

The wider variations were identified in the almond genotypes distributed in Eastern and Southeastern Anatolia regions than the other research results surveyed in the different regions according to some USFA such as oleic and linoleic acids. Of them, the higher rate USFA that are useful for human health were identified than the other studies conducted outside our country. Because of these important quality features, the identified almond varieties can be evaluated in breeding programs. Furthermore, there are low levels SFA which reduce the oil quality such as palmitic acid and stearic acid in this study especially.

Almond genotypes having different fatty acid composition brings about alternatives to use them for different purposes. Fatty acids are particularly important in the cosmetic and pharmaceutical making industry. There have been many studies of the fatty acid composition of almonds. Some results of studies on the fatty acids of almond taxons in the world are given in Table 5. In this study, in general, a large number of genotypes and more comprehensive fatty acid compositions of almond genotypes were studied. Our findings were found to be similar to [26] and especially [13] results. Kodad and Socias [13] showed that genotypes' oleic/linoleic acid ratio is an important factor in determining the stability of almond oil. In addition, they indicated that this rate can be used as distinction of genotypes because this rate does not change over the years and linoleic acid is less stable and less saturated than oleic acid. In our study, the oleic acid ratio was found to be 64.05-80.68% and the linoleic acid ratio was found to be 12.65-27.00%, similar to [13] results. Previous studies [24,27] indicated that synthesis of fatty acids may vary according to genetic, ecological, morphological, physiological and cultura factors. But, resently studies [23,28] revealed that oil composition the mostly depend on the genetic factor. Oil content in almond kernel shows a high heritability value of 57% [28], confirming that the genetic factor is the most determinant for oil content in almond kernels. This trait appears to be under polygenic control with a clear environmental effect [24,29-30]. Kodad et al. [23] indicated that the magnitude of the effect of the external factors such as the climatic condition of the year probably depends on the genetic background of each cultivar, explaining the significant effect of the interaction genotype X year.

According to the information received from the studies in Europa (Table 5), maximum USFA/SFA was seen as 14.17 and minimum as 10.24 in Piscopo et al. studies in certain cultivars selected from Italy, France and Spain. In this study, this ratio is quite high determined with an average of 15.40 and maximum and minimum value of 19.35 and 11.76 respectively. Therefore, it can be say that almond genotypes seeds collected Eastern and Southeasten Anatolia have high USFA ratio have more quality features.

High oleic and low linoleic acid leads to increase of the kernel oil stability and nutritional value (Kodad et al. [23]. That is rich in terms of USFA; almond oil reduces the risk of heart diseases [31]. In our study, high USFA values were obtained from the almond genotypes numbered 46, 16, 18, 28 and 49. In addition to these, MUFA consumption can be arranged according to low-density lipoprotein, cholesterol, and total cholesterol levels [14,26,31,32]. Among the works of many fatty acids nuts, the MUFA and PUFA content of almond seeds were found to be higher than in other popular foods such as walnuts, peanuts, pine nuts, Brazil nuts and olives [33,34]. This is one of the important factors of almond seed. With regard to our study, the genotype numbered 49 can be recommended for its MUFA percentage (81.51%). High MUFA and PUFA ratio (MUFA/PUFA) is an important parameter for the stability of USFA [13]. The average MUFA/PUFA ratio is found at the highest rate as 4.32 in our study. Kodad and Socias [13] with Nanos et al. [35] found the oleic acid level to be between 69-78% and 72-80% in wild and cultivated almonds, respectively. Also, similarly, we found the oleic acid values to be between 64.05-80.68% and the average to be 74.46%. In our study, as mentioned above, the almond genotypes that were found to be at higher rates in terms of USFA may be subject to breeding of almond seeds for human nutrition.

Locally, in many regions of Turkey, almonds pomologic characters, especially the hull percentage (hull wt/fruit wt x 100), kernel percentage (kernel wt/nut wt x 100), kernel length, widh, thickness, dual rate, protein, ash and total fat content were studied. Of these, one of the breeding by through the selection study carried out on the naturally grown almond tree in the district of Kemaliye – Erzincan. In this study, only mentioned of the total amount of fat content were given in almond fruits [36]. However, amount of fatty acids and its compositions in the oil have been emphasized in the world generally. In this composition, desired substanstially USFA such as oleic, linoleik, linolenic, omega-3 and omega-6 have become important. Because of its importance, proportions of fatty acids were focused on in almond genotypes oil instead of the total amount of fat content. In this way, the almond genotypes

that have rich for most important fatty acids can be selected and used for breeding programs in Turkey. Thus, it will be opened patway of high quality almond production extended to the world countries.

The USFA/SFA of almond genotypes collected from Eastern and Southeastern Anatolia were detected higher than the almond varieties values detected by Sabudak in Çorlu region [21]. Likewise, in this study, general average of oleic acid and linolenik acid value that are desirable for high quality fat were found as 74.46% and 0.75%. These proportions are higher than Çorlu region [21] almond varieties values that are 68.63% and 0.06% respectively. High percentage of SFA in the oil is undesirable for quality oil. In addition, low amounts of SFA (palmitic and stearic acid) were found in East and Southeastern regions than Çorlu. This situation shows that the almond genotypes collected our working areas have more quality fatty acids contain.

Conclusion

In this study, the variations were found between almond genotypes collected from different locations in the Eastern and Southeastern Anatolia regions in terms of fatty acids compositions. Almond genotypes collected from these regions were determined in terms of high USFA values and more quality features. For genes conservation and development, two almond genes conservation areas were established in 2008 in Elazığ/Gözeli and Keban districts. Some of these genotypes were used in this study for identify fatty acids compositions. As a result, almond genotypes having essential properties such as high oleic acid and low linoleic acid or high value USFA were determined through this study. It can contribute to the better quality almond production, cultivation and promotion by revealed to this study and new genotypes using similar studies will be carried out.

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Conflict of Interest

There are no conflicts of interest among the authors.

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