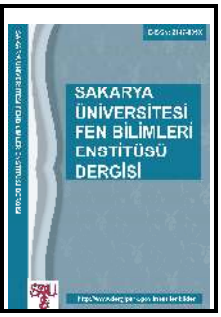
SAKARYA ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ DERGİSİ
SAKARYA UNIVERSITY JOURNAL OF SCIENCE
e-ISSN: 2147-835X
Dergi sayfası: http://www.saujs.sakarya.edu.tr

Geliş/Received
06-01-2017
Kabul/Accepted
11-10-2017

Doi
10.16984/saufenbilder.284473



Determination of triacylglycerol composition of Ayvalık and Memecik olive oils during storage by chemometric methods

Oya Köseoğlu[1], Didar Sevim[*1], Durmuş Özdemir[2]

ABSTRACT

The aim of present investigation is to discriminate two important Turkish olive cultivars (Ayvalık and Memecik) by studying their triacylglycerol (TAG) compositions during storage (15 months) taken from different orchard in Ayvalık and Aydın region which have a significant potential for olive oil production in Turkey, during 2009 and 2010 harvest years. Olives were harvested by hand at 2 different maturation indices and processed by an Abencor system. The olive oil samples were stored at room temperature and they were divided into two groups including exposed to diffused daylight and dark for a period of 15 months. Multivariate classification and clustering were done by the application of unsupervised chemometrics methods such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) based on the TAG profiles of the olive oil samples. PCA and HCA analysis of olive oils showed significant differences according to harvest years and cultivars. PCA scores plot showed that the samples were classified into two main groups with respect to harvest years based on the first principal component (PC1). In terms of storage effect, there was no significant change in TAG compositions among the samples from beginning of storage to 15 months of storage regardless of storage conditions (either in dark or in daylight). In addition, PCA scores plot indicated that the samples were also successfully clustered into two sub-groups according to cultivars in both years based on the second principal component (PC2).

Keywords: Ayvalık and Memecik, Triacylglycerol, OOO, HCA, PCA, Olive oil

Depolama sırasında Ayvalık ve Memecik zeytinyağlarının triaçilgliserol kompozisyonundaki değişimlerin kemometrik yöntemler ile tespiti

ÖZ

Bu çalışmada, Türkiye'de zeytinyağı üretimi için önemli bir potansiyele sahip olan zeytin çeşitleri (Ayvalık ve Memecik) Ayvalık ve Aydın Bölgesindeki farklı zeytin bahçelerinden 2009 ve 2010 hasat yıllarında hasat edilerek edilen yağların depolama boyunca (15 ay) triaçilgliserol (TAG) kompozisyonlarındaki değişimler incelenmiştir. Zeytinler 2 farklı olgunlaşma indeksine elle hasat edilmiş ve Abencor sistemi ile yağ elde edişmiştir. Elde edilen zeytinyağı örnekleri oda sıcaklığında saklanmış ve 15 ay boyunca aydınlık ve karanlığa maruz bırakılmıştır. Zeytinyağı örneklerinin TAG kompozisyonuna çok değişkenli sınıflandırma ve kümeleme analizi olan ana bileşen analizi (PCA) ve hiyerarşik küme analizi (HCA) gibi denetimsiz kemometrik yöntemler uygulanmıştır. Zeytinyağlarının PCA ve HCA analizleri, hasat yılı ve çeşitlerine göre önemli farklılıklar göstermiştir. PCA analizine göre, örneklerin hasat yıllarına göre birinci ana bileşene (PC1) dayalı olarak iki ana gruba ayrıldığı görülmektedir. Depolama etkisine bakıldığında, depolama koşullarından bağımsız olarak (ya karanlıkta ya da aydınlıkta) depolama başlangıcından 15 aylık depolamaya kadar olan sürede örnekler arasında TAG bileşiminde önemli bir değişiklik görülmemiştir. Ayrıca, PCA skoru, çeşitlere göre örneklerin her iki yılda da ikinci ana bileşene (PC2) dayalı olarak iki alt gruba başarıyla kümelendiğini göstermektedir.

Anahtar Kelimeler: Ayvalık ve Memecik, Triaçilgliserol, OOO, HCA, PCA, Zeytinyağı

[1] Turkish Ministry of Food, Agriculture and Livestock, Olive Research Institute İzmir/Turkey, atamete35@hotmal.com
[1]* Turkish Ministry of Food, Agriculture and Livestock, Olive Research Institute İzmir/Turkey, dcengeler@gmail.com
[2] Izmir Institute of Technology, Faculty of Science Department of Chemistry, durmusozdemir@iyte.edu.tr

1. INTRODUCTION

Olive oil is one of the oldest known vegetable oils extracted from fruits of the olive tree, Olea europaea, L. by using only physical methods, which include crushing of olives, malaxation of resulting pastes and separation of the oily phase [1]. Virgin olive oil composition influence by several factors like cultivar, environment, and agronomic practices affect the fruit physiology, and also processing and storage conditions affect the oil composition [2]. Olive oil is composed of triacylglycerols (97-98%) and minor compounds (around 2 %) such as hydrocarbons, alifatic alcohols, sterols, phenolic compounds, tocopherols [3]. The fatty acid composition and triacylglycerol content of virgin olive oil differs considerably depending mainly on latitude, climate, variety and stage of maturity of olives [4]. Olive oils consist predominantly of TAG that generally follows a unique and typical pattern in the glycerol molecule being characteristics in the different oil seeds. TAG composition is immensely useful for the characterization and discrimination, as well authentication of olive oils or its geographical location [5]. Olive oil has a high resistance to oxidative deterioration due to its fatty acid composition, characterized by high monounsaturated-to polyunsaturated fatty acid ration, and to the presence of minor compounds. Despite its antioxidant affect, extra virgin olive oil undergoes oxidative process during storage, which influences its organoleptic properties and the nutritional value [6].

Turkey is an important olive cultivar successfully grown in Mediterranean. And also Turkey is the world's fifth largest producer of olive oil (5.7 %) during 2009/10-2015/16 harvest years [7]. Economically important Turkish olive cultivars are "Memecik" at 45 % and "Ayvalık" at 20 % [8]. Nearly 75-80 % of the total production of olive oil is obtained from the Aegean Region, where "Ayvalık" and "Memecik" are the main varieties [9].

Principal component analysis (PCA) is a factor analysis method which is based on projecting the original data from high dimensional space on to a line, a plane, or a 3D-coordinate system. Simply, PCA decomposes the data matrix into two smaller matrices named as scores and loadings. The projection of higher dimensional data to a lower dimeension is done by using so called scores vectors which are the lineer combinations of the original variables. Since maximum variability of the data is accounted by the first couple of principal component (PC), it is very usefull to plot the first two scores vectors (PC1 and PC2) in order to observe possible subclasses and groups in a given data set resulting from original variables (e.g. TAG composition). Hierarchical cluster analysis (HCA) is another unsupervised classification and clustering method concerning with forming groups of similar objects based on several variables on the objects. The main idea is to examine the interpoint distances between all the samples and represents that information in the form of two dimensional plots as a dendrogram. To generate the dendrogram, HCA forms clusters of samples based on their similarities in space. Several ifferent approaches are used to measure distances between the clusters. Firstly the distances between samples or objects are calculated such as with euclidian distance method and linkage methods (e.g. Ward meethod) are used to form the dendrogram. In a summary, dendrogram shows the closeness of samples in row space in the form of two-dimensional graph. The samples are plotted either against the distances or the similarities/differences between samples without imposing prior information regarding the class membership.

In this study, it was aimed to determine of TAG components of Ayvalık and Memecik extra virgin olive oils during storage (15 months) taken from different orchard in Ayvalık and Aydın region which have a significant potential for olive oil production in Turkey, during two harvest years, 2009 and 2010. Olives were harvested by hand at 2 different maturation index and processed by an Abencor system and the olive oils were divided in two parts in order to observe storage effect in daylight and in dark at room temperature for a period of 15 months. Initial determination of TAG components have been carried out just after the processing the olives (0 months) and then after 15 months of storage both in daylight and in dark according to the High Performance Liquid Chromatography (HPLC) method. Multivariate classification and clustering were done by the application of unsupervised chemometrics methods such as PCA and HCA based on the TAG profiles of the olive oil samples.

2. MATERIALS AND METHODS

2.1. Reagents

HPLC grade solvents of acetone and acetonitrile were obtained from Merck (Darmstadt. Germany). Margins**.**

2.2. Material and Oil Extraction

The research was conducted during the seasons of 2009 and 2010 harvest years. "Ayvalık" and "Memecik" cultivars were harvested from orchard in Ayvalık and Aydın Region, respectively. Olives were harvested by hand at 2 different maturation indices and processed by an Abencor system at the Olive Research Institute of Turkish Ministry of Food, Agriculture and Livestock in Izmir/Turkey. The olive fruits (15 kg) which were washed with tap water crushed immediately to obtain oil by using an Abencor System (MC2 Ingenierias y Sistemas Sevilla, Spain) equipped with fruit crushing, malaxation and centrifuge parts. The malaxation temperature was 30 ˚C for 30 min. Only healthy fruits, without any kind of infection or physical damage, were processed. All oil samples were filtered and stored using transparent glass bottles (100 mL). The olive oils were divided in two parts in order to observe storage effect in daylight and in dark at room temperature for a period of 15 months. Oil samples were analyzed after extraction prior to storage, and after 15 months of storage.

2.3. Maturity Index (MI)

The maturity index was determined according to the method given by International Olive Council [10] based on color of the olive skin and pulp.

2.4. Chromatographic Analysis of Triacylglcerol

The analysis of TAGs was performed according to the official liquid chromatographic method described in Regulation EEC/2568/91 of the European Union Commission [11]. The chromatographic analysis was performed using Agilent 1200 HPLC system consisted by a degasser, quaternary pump, manual six-way injection valve, refractometer detector, and Chemstation Software (3365) package for instrument control, data acquisition, and data analysis. The results were expressed in percentage of total TAG. The column was a Superspher 100 RP-18 HPLC column (Merck, Germany) (250 x 4 mm i.d. x 4 µm temperature 35 °C). A 5% solution of the olive oil was prepared, by weighing 0.5 ± 0.001 g into a 10 ml graduated flask and making up 10 ml with the acetone solvent. A loop of 100 µL capacity was used in which 0.5 µL sample was injected. Acetone (63.6 %)/acetonitrile (36.4 %) were mobile phases with a flow rate linear gradient (1.200 mL min^{-1}) under nebulizer gas pressure 2.00 bar for 45 min.

2.5. Statistical Analysis

Multivariate classification and clustering analysis were done by the application of unsupervised chemometrics methods such as PCA and HCA based on the TAG profiles of the olive oil samples using MINITAB stastical software package version 15. Prior to PCA and HCA analysis, the data were normalized by subtracting the mean and dividing to standard deviation of each variable. Then the averages of three parallel determinations were used for PCA and HCA analysis.

3. RESULTS AND DISCUSSION

MI of the olive oils were determined for each variety. The maturity index of olives were classified into two groups, for Ayvalık olive 2.02 (first harvest-1st) and 2.33 (second harvest-2nd), for Memecik olive 1.97 (1st) and 2.83 (2nd) on the 2009 harvest year. On the 2010 harvest year MI was determined for Ayvalık olive 1.5 (1st) and 2.2 (2nd), for Memecik olive 1.6 (1st) and 2.3 (2nd). Triglycerides composition has also been established as a measurement of the quality and authenticity of vegetable oils. Because of the specificity of the composition of different kinds of fats and oils, it is used increasingly in the food industry to confirm authenticity despite this type of analysis being quite demanding [12]. A typical HPLC chromatogram of the 15 month storage at dark conditions of Ayvalık olive oil analyzed is shown in Figure 1. As seen on the chromatograms of TAG components, the percentage of triolein (OOO) was determined to be the highest. The percentage of OOO was followed by the palmitodiolein (POO) and linoleyldiolein (LOO), respectively. Table 1 and Table 2 show the results of triacylglycerol composition obtained on studying the extra virgin olive oil stored in daylight and dark conditions during 15 months storage in 2009 and 2010 harvest years, respectively with three parallel determinations.

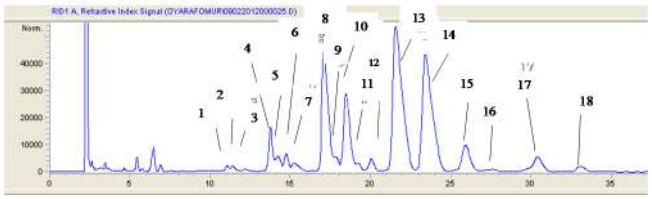


Figure 1. Chromatograms of TAGs Composition of 15 month storage at dark conditions of Ayvalık Olive Oils (2010) 1:LLL (trilinolein), 2: POLL, 3:PLLn, 4:LOL (linoleyloleyllinolein), 5:OLnO: 6:LPL (linoleylpalmityllinolein), 7:POLn, 8:LOO (linoleyldiolein), 9:POO (palmitodiolein), 10:PLO (palmityllinoleylolein), 11:PoOP, 12:PLP (palmityllinoleylpalmitin), 13:OOO (triolein), 14: POO (palmityldiolein), 15: POP (palmityloleylpalmitin) 16:PPP (tripalmitin), 17: SOO (stearyldiolein), 18:SOP (stearyloleypalmitin)

Table 1 Triacylglycerol composition (%) of the studied olive oils at 2009 harvest year

Storage Periods (Months)	Storage Type	MI	Sample	LLL	LOO	PLO	OOO	POO	POP	SOO	ECN42	ECN44	ECN46	ECN 48	ECN 50	PLO/ OOO	LLL/ ECN 42	PLL/O LL	ECN48/ ECN46	LOO/ PLO	OOO/ POO
0	Initial	1st	A1	0,20	14,58	8,32	33,39	24,51	4,10	4,13	0,74	5,76	25,61	62,73	5,20	0,25	0,26	0,38	2,45	15,10	1,36
			A2	0,24	14,47	8,25	33,55	24,33	3,98	4,05	0,80	6,11	25,48	62,52	5,09	0,25	0,30	0,36	2,45	14,47	1,38
			A3	0,23	15,44	9,38	31,36	24,22	4,06	3,88	0,78	6,48	27,67	60,18	4,92	0,30	0,29	0,40	2,17	12,70	1,29
			M1	0,13	13,48	6,87	37,08	24,35	3,83	4,42	0,74	5,03	22,87	65,76	5,61	0,19	0,17	0,31	2,88	23,25	1,53
			M2	0,12	13,60	6,36	39,60	24,04	3,59	3,76	0,61	4,68	22,16	67,85	4,73	0,16	0,19	0,27	3,06	28,95	1,65
			M3	0,24	16,34	8,40	33,74	23,24	3,84	3,21	0,87	6,65	27,14	61,34	4,08	0,25	0,28	0,32	2,26	17,66	1,45
		2nd	A1	0,16	13,60	7,39	36,85	24,46	3,69	4,45	0,63	4,84	23,23	65,72	5,61	0,20	0,25	0,34	2,83	20,00	1,51
			A2	0,20	15,14	8,36	33,69	23,95	3,78	4,09	0,79	5,91	26,08	62,07	5,17	0,25	0,25	0,35	2,38	16,07	1,41
			A3	0,25	16,18	9,47	31,69	22,99	3,83	3,95	0,84	6,97	28,41	59,09	4,91	0,30	0,30	0,40	2,08	11,98	1,38
			M1	0,24	14,16	9,77	28,90	23,86	4,24	5,16	1,05	8,20	26,78	57,38	6,72	0,34	0,22	0,48	2,14	8,58	1,21
			M2	0,16	15,68	6,97	38,38	22,28	3,22	3,73	0,69	5,61	24,63	64,46	4,65	0,18	0,23	0,27	2,62	23,78	1,72
			M3	0,12	16,31	7,42	36,70	22,49	3,35	3,90	0,65	5,62	25,86	63,02	4,88	0,20	0,19	0,27	2,44	24,42	1,63
15	Daylight	1st	A1	0,22	14,45	8,33	33,65	24,78	4,13	3,92	0,78	5,51	25,44	63,26	5,03	0,25	0,28	0,36	2,49	15,81	1,36
			A2	0,25	14,56	8,33	33,37	24,29	3,82	4,09	0,83	5,98	25,86	62,10	5,24	0,25	0,30	0,35	2,40	14,96	1,37
			A3	0,30	15,33	9,28	31,33	24,27	4,18	3,73	0,99	6,23	27,65	60,40	4,74	0,30	0,30	0,38	2,18	13,39	1,29
			M1	0,12	13,80	6,93	37,43	24,10	3,53	4,63	0,66	4,92	23,13	65,54	5,77	0,19	0,18	0,30	2,83	25,10	1,55
			M2	0,13	13,63	6,24	39,77	24,00	3,63	3,64	0,68	4,60	22,12	67,89	4,57	0,16	0,19	0,26	3,07	30,62	1,66
			M3	0,25	16,28	8,44	33,80	23,24	3,76	3,16	0,94	6,52	27,28	61,27	4,03	0,25	0,27	0,30	2,25	18,71	1,45
			A1	0,21	14,34	8,26	33,81	24,73	3,90	4,03	0,70	5,60	25,49	63,02	5,20	0,24	0,30	0,37	2,47	15,67	1,37
			A2	0,26	14,61	8,37	33,55	24,24	3,90	3,96	0,83	6,14	25,71	62,35	4,99	0,25	0,31	0,37	2,42	14,47	1,38
			A3	0,21	15,39	9,31	31,25	24,30	4,26	3,85	0,61	6,53	27,58	60,44	4,88	0,30	0,34	0,40	2,19	12,83	1,29
			M1	0,12	13,64	6,88	37,37	24,17	3,74	4,58	0,63	4,85	22,97	65,82	5,75	0,18	0,19	0,30	2,87	25,02	1,55
			M2	0,10	13,64	6,35	39,85	24,14	3,43	3,69	0,52	4,77	22,17	67,99	4,58	0,16	0,19	0,27	3,07	29,02	1,65
			M3	0,23	16,14	8,29	34,06	23,17	3,83	3,29	0,82	6,53	26,90	61,60	4,20	0,24	0,28	0,30	2,29	18,76	1,47
		2nd	A1	0,16	14,03	7,51	35,91	24,22	3,69	4,33	0,63	5,34	24,08	64,51	5,48	0,21	0,25	0,35	2,68	17,58	1,48
			A2	0,23	15,27	8,35	33,66	23,85	3,71	3,97	0,85	6,18	26,11	61,88	5,01	0,25	0,26	0,34	2,37	15,90	1,41
			A3	0,26	16,42	9,58	31,68	23,00	3,51	3,98	0,87	6,87	28,54	58,68	5,06	0,30	0,29	0,40	2,06	12,00	1,38
			M1	0,26	13,99	9,55	28,79	23,45	4,50	5,21	1,06	8,05	26,50	57,29	7,13	0,33	0,24	0,50	2,16	8,32	1,23
			M2	0,16	15,69	6,98	38,87	21,93	3,03	3,78	0,72	5,61	24,66	64,33	4,70	0,18	0,22	0,25	2,61	25,53	1,77
			M3	0,16	15,83	7,00	39,02	21,97	2,96	3,74	0,68	5,54	24,72	64,45	4,63	0,18	0,24	0,25	2,61	25,53	1,78
			A1	0,16	13,95	7,30	37,47	24,03	3,34	4,38	0,63	4,87	23,60	65,43	5,50	0,19	0,26	0,32	2,77	20,83	1,56
			A2	0,24	15,61	8,51	34,29	23,36	3,34	4,00	0,84	6,12	26,53	61,53	5,00	0,25	0,28	0,34	2,32	16,02	1,47
			A3	0,25	16,19	7,96	31,66	22,84	3,65	3,98	0,78	6,97	26,99	58,80	4,97	0,25	0,32	0,39	2,19	12,36	1,39
			M1	0,23	14,38	9,76	29,17	23,54	3,92	5,15	0,97	8,28	27,06	57,09	6,63	0,33	0,24	0,49	2,11	8,52	1,24
			M2	0,17	15,70	6,89	38,80	21,99	3,09	2,24	0,74	5,52	24,68	64,42	3,16	0,18	0,23	0,26	2,61	24,92	1,76
			M3	0,13	16,34	7,46	36,81	22,39	3,37	3,89	0,66	5,50	25,92	63,08	4,87	0,20	0,19	0,26	2,43	26,57	1,64

MI: Maturity Index, A: Ayvalık M:Memecik, LLL (trilinolein), LOO (linoleyldiolein), POO (palmitodiolein), PLO (palmityllinoleylolein), OOO (triolein), POO (palmitodiolein), POP (palmityloleylpalmitin) SOO (stearyldiolein), ECN_{42} (LLL+LOLn+POLL+PLLn); ECN 44 (OLL+OLnO+PLL+POLn); ECN 46 (LOO+PLnP+PoOO+PLO+SLL+PoOP+PLP); ECN 48 (OOO+SLO+POO+POP+PPP);ECN 50 (SOO+POS)

The analysis of triacylglycerols allows the identification and the quantification of 19 triacylglycerols. Among them, LOO, PLO, OOO and POO account for more than 85% of the total are of the peaks in profile, whereas LLL, POP and SOO were present in low percentages. TAG contents showed variations between samples from different regions (Ayvalık and Memecik) and harvest years (Table 1 and Table 2). In this study, no significant difference was determined for TAG composition of olive oils in terms of maturity index. This can be caused by close maturity index of olive oils. Guiffre (2014) reported that Southern Italy olive oils level of OOO was increased with ripening, and the level of LLL was decreased during ripening. According to their studies the cultivar influenced the TAGs composition each harvest date. When we look at storage conditions, the results indicated that there was no significant difference between the samples stored in dark and in daylight conditions. In the relation to the main TAGs (LOO, PLO, OOO and POO), the level of OOO was remarkably high, ranging from 31.36 % to 33.55 %, from 31.69 % to 36.85 % for Ayvalık and from 33.74 % to 39.60 % from 28.90 % to 38.38 % for Memecik first and second harvest at 2009, respectively. In 2010 the level of OOO was ranging between 28.34 % and 32.91 %, between 27.60 % and 31.15 % for Ayvalık and ranging between 24.65 % and 30.90 %, between 29.05 % and 32.42 % for Memecik first and second harvest, respectively. Yorulmaz et al. (2014) determined Turkish monovarietal olive oils OOO values between 24.72 % and 48.64 %. Our results are compatible with the report. Among the all olive oil samples obtained from fruits of Ayvalık and Memecik the content of LLL did not exceed the maximum limit of 0.5 % determined by European Commission.

İlyasoğlu and Özçelik (2011) has reported that content of POO and LOO of Memecik olive oil ranging from 18.25 % to 25.82 % and from 6.01 % to 9.18 %, respectively. The value of Ayvalık and Memecik olive oils POO and LOO is higher than the reports. Aranda et al. (2004) has reported that the value of OOO, SOO, ECN 48 and ECN 50 of Cornicabra virgin olive oil was 51.7 %, 6.76 %, 74.7 % and 8.68 %, respectively. The value of OOO, SOO, ECN 48 and ECN 50 of samples is lower than Cornicabra virgin olive oil. Ben Temime et al. (2006) has reported that TAG content of Chetoui which is second olive oil variety cultivated in Tunisia depending on pedoclimatic conditions and on the region of cultivation. The value of OOO, POO and LOO were ranging from 29.59 % to 37.38%, from 15.11 % to 18.02% and from 19.03 % to 24.74 %, respectively. The value of LOO of Ayvalik and Memecik c.v. olive oil is lower, the value of OOO and POO are higher than Chetoui c.v. olive oil. Sevim et al. (2013) has expressed the Gemlik olive oil, which was economically important olive for Turkey, TAGs content OOO ranging from 33.05 % to 37.19 %, SOO 4.32 % to 4.59 %, POO 24.56 % to 25.52 %, PLO 6.20 % to 7.38 %, LOO 11.3 % to 12.98 %. The TAGs compositions of the studied olive oils were similar except for PLO and LOO. Ayvalık and Memecik olive oils PLO and LOO percentage were determined higher than Gemlik olive oil. Guerfel et al.(2012) has reported

that level of OOO, POO and POL of Chemlali olive oil was ranging between 28.5 % and 31.7 %, between 30.87 % and 35.34 % and between 3.50 % and 31.77 %, respectively. Fuentes et al. (2015) was reported that the main TAGs (OOO, POO, OLO, PLO+SLE) were for the discrimination of the olive varieties of Morisca and Carrasquena.

Table 2 Triacylglycerol composition (%) of the studied olive oils at 2010 harvest year

Storage Periods (Months)	Storage Type	MI	Sample	LLL	LOO	PLO	OOO	POO	POP	SOO	ECN42	ECN44	ECN46	ECN 48	ECN 50	PLO/OOO	LLL/ECN 42	PLL/OLL	ECN48/ECN46	LOO/PLO	OOO/POO
0	Initial	1st	A1	0,21	14,03	8,45	32,91	25,29	4,24	3,89	0,75	5,79	25,61	62,97	4,91	0,26	0,28	0,39	2,46	14,03	1,30
			A2	0,32	15,83	10,22	28,34	24,15	4,67	3,51	0,98	7,47	29,30	57,79	4,49	0,36	0,33	0,40	1,97	11,15	1,17
			A3	0,28	14,66	10,02	28,88	25,04	4,91	3,43	0,95	7,08	28,01	59,40	4,60	0,35	0,29	0,44	2,12	10,43	1,15
			M1	0,38	15,97	9,68	29,29	24,25	4,83	3,10	1,07	7,49	28,69	58,73	4,02	0,33	0,35	0,35	2,05	12,53	3,60
			M2	0,62	17,22	11,54	24,45	22,57	4,89	2,77	1,64	10,12	31,97	52,51	3,78	0,47	0,38	0,39	1,64	8,77	1,09
			M3	0,19	15,10	8,98	30,90	24,85	4,41	4,34	0,77	6,20	26,91	60,48	5,67	0,29	0,25	0,36	2,25	15,82	1,24
		2nd	A1	0,37	15,74	10,19	28,16	23,75	4,56	3,38	1,11	8,05	29,37	57,10	4,38	0,36	0,33	0,42	1,94	10,15	1,19
			A2	0,35	15,85	10,37	27,60	23,92	4,91	3,20	1,18	7,82	29,68	57,09	4,25	0,38	0,30	0,42	1,92	10,55	1,15
			A3	0,23	15,19	9,31	31,15	24,11	4,25	3,65	0,84	6,70	27,55	60,21	4,72	0,30	0,27	0,41	2,19	12,05	1,29
			M1	0,47	18,15	9,91	29,05	21,89	4,09	2,89	1,32	8,53	30,68	55,59	3,90	0,34	0,35	0,31	1,81	13,20	1,33
			M2	0,20	16,60	8,70	32,42	23,46	4,24	3,61	0,74	6,17	27,79	60,61	4,71	0,27	0,26	0,31	2,18	18,14	1,38
			M3	0,29	16,99	9,57	29,69	22,72	4,35	3,72	0,98	7,58	29,35	57,29	4,80	0,32	0,29	0,33	1,95	14,16	1,31
15	Daylight	1st	A1	0,21	14,17	8,45	33,00	25,36	4,08	3,75	0,69	5,82	25,78	62,87	4,87	0,26	0,31	0,39	2,44	13,83	1,30
			A2	0,32	15,75	10,27	28,55	24,65	4,31	3,41	1,00	7,17	29,47	57,96	4,44	0,36	0,32	0,42	1,97	11,05	1,16
			A3	0,28	14,76	10,12	28,88	24,80	4,72	3,59	0,86	7,06	28,49	58,93	4,67	0,35	0,33	0,44	2,07	10,40	1,16
			M1	0,39	16,09	9,90	28,97	24,10	4,58	2,98	1,15	7,56	29,19	58,07	4,06	0,34	0,34	0,35	1,99	12,38	1,20
			M2	0,65	17,18	11,60	24,60	22,44	4,83	2,88	1,74	9,89	32,13	52,29	3,96	0,47	0,37	0,38	1,63	9,16	1,10
			M3	0,20	14,94	9,01	30,57	24,78	4,54	4,45	0,84	6,09	27,10	60,33	5,66	0,29	0,24	0,36	2,23	15,97	1,23
	Dark		A1	0,19	14,00	8,46	32,70	25,21	4,33	3,85	0,64	5,83	25,67	62,85	5,02	0,26	0,29	0,39	2,45	14,07	1,30
			A2	0,34	15,67	10,29	28,35	24,22	4,61	3,35	0,99	7,55	29,35	57,69	4,45	0,36	0,34	0,41	1,97	10,77	1,17
			A3	0,29	14,75	10,09	28,88	24,74	4,91	3,56	0,89	6,97	28,39	59,16	4,60	0,35	0,32	0,44	2,08	10,68	1,17
			M1	0,38	15,90	9,63	28,81	24,18	5,03	3,17	1,12	7,53	28,68	58,54	4,15	0,33	0,34	0,34	2,04	12,77	1,19
			M2	0,61	17,21	11,46	24,64	22,55	5,13	3,02	1,64	9,83	31,75	52,77	4,03	0,46	0,37	0,38	1,66	9,33	1,09
			M3	0,21	14,95	8,92	30,63	24,81	4,77	4,20	0,89	6,29	26,67	60,67	5,51	0,29	0,23	0,35	2,28	15,99	1,23
	Daylight	2nd	A1	0,35	15,61	10,12	28,24	23,88	4,72	3,27	0,97	8,17	29,12	57,51	4,23	0,36	0,36	0,41	1,98	10,17	1,18
			A2	0,33	15,68	10,49	27,68	24,35	4,89	3,34	0,87	7,67	29,60	57,44	4,45	0,38	0,38	0,41	1,94	10,63	1,14
			A3	0,22	15,22	9,46	31,06	23,98	4,23	3,91	0,69	6,46	27,96	59,84	5,09	0,30	0,31	0,39	2,14	13,02	1,30
			M1	0,46	18,18	9,98	29,16	21,90	4,03	2,96	1,28	8,60	30,82	55,51	3,81	0,34	0,36	0,32	1,80	12,94	1,33
			M2	0,22	16,49	8,77	32,60	23,72	4,26	3,46	0,74	6,09	27,70	61,00	4,50	0,27	0,29	0,30	2,20	18,42	1,37
			M3	0,31	17,13	9,53	30,08	22,72	4,05	3,83	1,05	7,60	29,14	57,27	4,96	0,32	0,29	0,32	1,97	14,27	1,32
	Dark		A1	0,38	15,81	10,26	28,35	23,99	4,55	3,20	1,06	7,86	29,53	57,44	4,13	0,36	0,35	0,41	1,95	10,44	1,18
			A2	0,37	15,95	10,47	27,83	24,05	4,96	3,35	1,11	7,93	29,72	56,88	4,39	0,38	0,33	0,43	1,91	10,10	1,16
			A3	0,24	15,31	9,34	31,42	24,04	4,10	3,84	0,86	6,54	27,60	60,11	4,91	0,30	0,28	0,40	2,18	12,40	1,31
			M1	0,47	18,29	10,02	29,26	21,87	3,92	3,03	1,31	8,66	30,79	55,38	3,88	0,34	0,35	0,31	1,80	12,88	1,34
			M2	0,21	16,58	8,81	32,62	23,62	4,04	3,57	0,77	6,27	27,73	60,65	4,59	0,27	0,27	0,31	2,19	17,46	1,38
			M3	0,30	17,12	9,51	30,09	22,75	4,01	3,93	0,99	7,68	29,05	57,25	5,05	0,32	0,30	0,34	1,97	13,59	1,32

MI: Maturity Index, A: Ayvalık M:Memecik, LLL (trilinolein), LOO (linoleyldiolein), POO (palmitodiolein), PLO (palmityllinoleylolein), OOO (triolein), POO (palmitodiolein), POP (palmityloleylpalmitin) SOO (stearyldiolein), ECN_{42} (LLL+LOLn+POLL+PLLn); ECN 44 (OLL+OLnO+PLL+POLn); ECN 46 (LOO+PLnP+PoOO+PLO+SLL+PoOP+PLP); ECN 48 (OOO+SLO+POO+POP+PPP);ECN 50 (SOO+POS)

According to the results of PCA analysis, the scores plot of PC1 vs PC2 (Figure 2) showed that samples were classified into two main group with respect to the harvest years based on the PC1. In terms of storage effect, there was no significant change in TAG compositions among the samples from beginning of storage to 15 months of storage period regardless of storage conditions (either in dark or in daylight). As it can also be seen from Figure 2, the samples were also successfully clustered into two sub-groups according to cultivars (Ayvalık and Memecik) in both years based on the PC2.

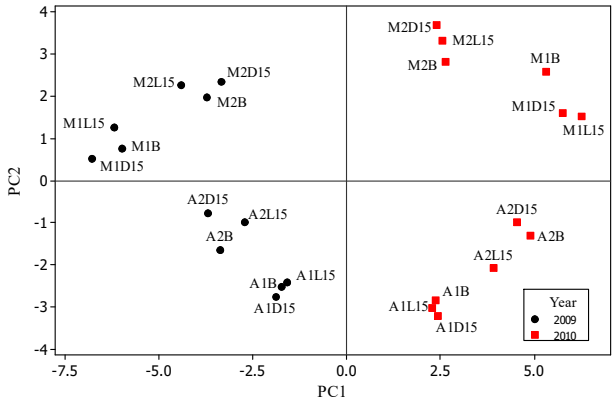


Figure 2. PCA scores plot of the first two principal components (PC1 and PC2) for the TAG composition of Ayvalık and Memecik olive oils stored in daylight and dark conditions in 2009 and 2010 harvest years. The coding for the sample labels is done in the following way. The first letter is the abbreviation of cultivar (A: Ayvalık, M: Memecik), following number is for the harvest time (1: first harvest, 2: second harvest). Third character is for the storage type (L: under light, D: Dark, B:before the storage) and the last number "15" is for the 15 months storage time.

PCA Loadings plot of the first two principal component (PC1 and PC2) shown in Figure 3, indicated that the variables OOO, SOO, PPP, LOO/PLO, SLO+POO, OOO/POO, ECN48,ECN48/ECN46 and ECN50 played an important role in the characterization of the olive oil samples obtained in 2009 harvest years. The scores and loadings bi-plot which is useful to interpret both scores and loadings results on the same plot is shown in Figure 4. As seen in Figures 3 and 4, the Memecik olive oils were essentially characterized by TAG components such as OOO, OOO/POO, OlnO and LOO/PLO while Ayvalık olive oils were characterized by SOO, ECN50, POS, SLO+POO, PoOO, PLL/OLL, PoOP, PLP, POP, PLL,PLO/OO, PLO+SLL, LLL/ECN42 and PLLn contents in both 2009 and 2010 harvest year. Results are in good agreement with researchers [19]. Gokçebağ et al. (2013) were studied a total of 22 domestic monocultivar (Ayvalık and Memecik cv.) virgin olive oil samples taken from various locations of the Aegean region, during two crop years were classified and characterized by well-known chemometric methods on the basis of their TAG composition. The ranges of TAG, namely LOO, OOO, POO, PLO, and SOO, were 13.30–16.08, 37.27–46.36, 21.39–23.24, 4.93–7.03, and 4.72–6.00 %, respectively. Galeano Diaz et al. (2005) reported that for characterization of virgin olive oils the triglycerides composition gives better results than the sterol composition by chemometric methods.

Manai-Djebali et al. (2012) determined a good discrimination between varieties according to triacylglycerol and sterol data with the results of PCA and HCA analyses. Our results are similar to them. Aranda et al. (2004) suggested in their report that with the PCA and discriminant analysis, the TAG variables are more suitable than 2-position fatty acids for optimum classification of commercial samples of analyzed. The results of PCA analysis indicated that there was no significant difference between the samples stored in dark and in daylight conditions. In addition, there were no discrimination among the samples at the initial stage of the storage and after 15 months of storages.

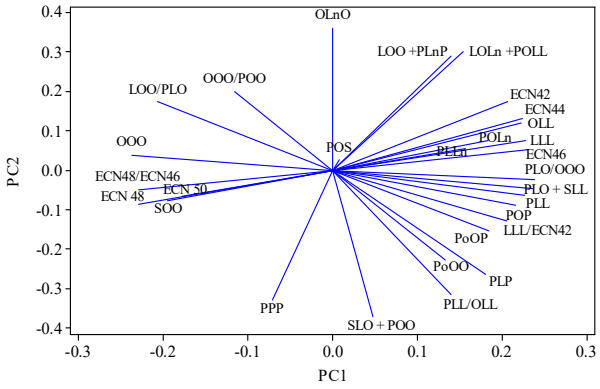


Figure 3. PCA loadings plot of the first two principal components (PC1 and PC2) for the TAG composition of Ayvalık and Memecik olive oils stored in daylight and dark conditions in 2009 and 2010 harvest years.

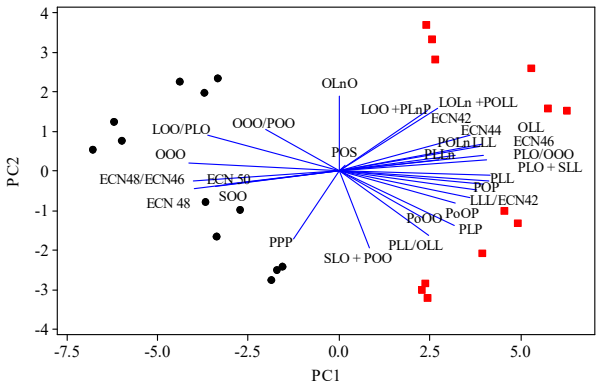


Figure 4. PCA scores and loadings biplot of the first two principal components (PC1 and PC2) for the TAG composition of Ayvalık and Memecik olive oils stored in daylight and dark conditions in 2009 and 2010 harvest years.

The dendrogram obtained from the HCA analysis showed that theolive oils obtained from Ayvalık and Memecik could be divided into two main groups (Figure 5) on the basis of their TAG profile. As can be seen from the dendrogram, samples were first clustered based on their harvest year where 2009 samples were clustered on the right side whereas 2010 samples were clustered on the left side of dendrogram. Each of these clusters was also subclustered into two classes where all of the Ayvalik and Memcik olive oil samples were correctly identified. On the other hand, when HCA analysis was applied to the scores vectors of the first two principal components (PC1 and PC2), the olive oil samples were clustered first into two main groups based on cultivar where Ayvalik olive oil samples placed on the left side and Memecik olive oil samples seen on the right side of the dendrogram as seen in Figure 6. Each of these main groups were then classified into two subclusters where samples on the right side were from 2009 and the samples on the left side from 2010 harvest year. Yorulmaz et al. (2011) reported that main TAGs; OOO, OOL, PLO and POP were influenced by maturation for Memecik and Ayvalık cultivars. Finally, HCA analysis of TAG variables resulted in a dendrogram where the TAG components were clustered into two main groups shown in Figure 7. Gokçebağ et al. (2013) reported that according to the PCA results some TAGs; have an important role in the characterization and geographical classification of 22 monocultivar virgin olive oil samples. The Aegean virgin olive oil samples were successfully classified and discriminated into two main groups as the North and South (growing) subzones or Ayvalık and Memecik olives (cultivars) according to the HCA results based on experimental TAG data and calculated major FA profile.

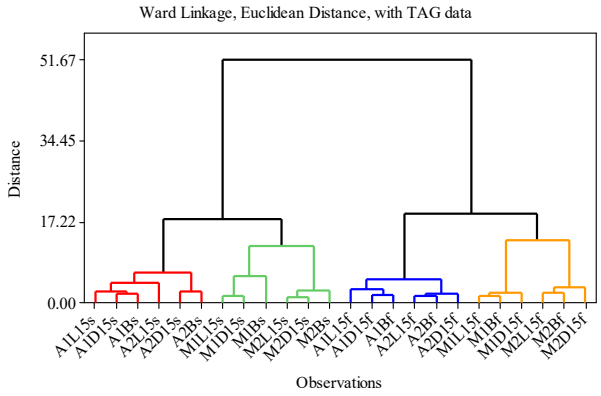


Figure 5. The dendrogram of HCA results based on the TAG composition of Ayvalık and Memecik olive oils stored in daylight and dark conditions in 2009 and 2010 harvest years. The lower case letters (f for 2009 and s for 2010) at the end of the labels corresponds to harvest years.

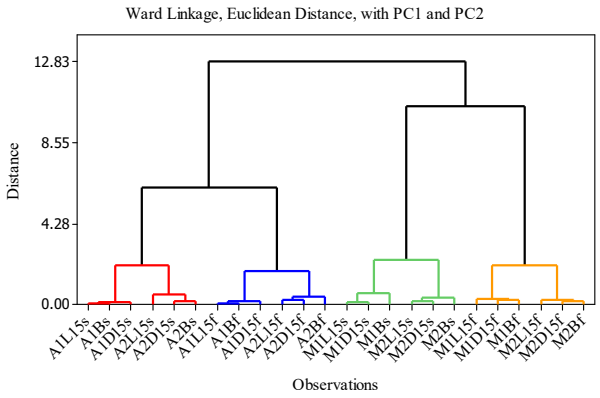


Figure 6. The dendrogram of HCA results based on the first two PCA score vectors of Ayvalık and Memecik olive oils stored in daylight and dark conditions in 2009 and 2010 harvest years. The lower case letters (f for 2009 and s for 2010) at the end of the labels corresponds to harvest years.

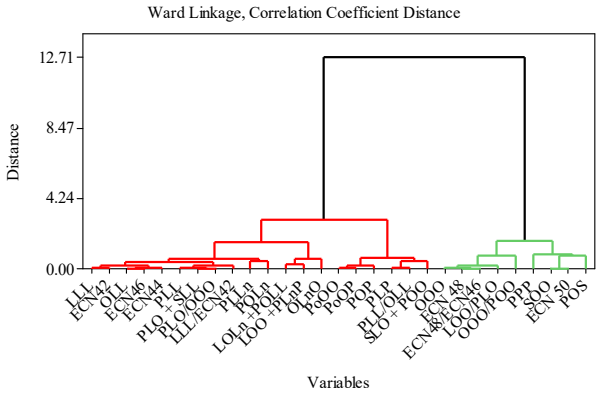


Figure 7. The dendrogram of HCA results for the variables (TAG profiles) of Ayvalık and Memecik olive oils stored in daylight and dark conditions in 2009 and 2010 harvest years.

4. CONCLUSIONS

In this study, no significant difference was determined for TAG composition of olive oils in terms of maturation. This can be caused by close maturity index. The maturity index values are very close for each group. On the other hand, PCA and HCA analysis of olive oils showed significant differences according to harvest years and cultivars. Samples were classified into two main groups with respect to harvest years according to PC1 scores of PCA results. On the other hand they were also classified into two groups as Ayvalik and Memecik olive oils based on the PC2. There was no significant change in TAG compositions among the samples from beginning of storage to 15 months of storage. And also there was no difference observed between daylight and dark conditions. The Memecik olive oils were essentially characterized by TAG components such as OOO, OOO/POO, OlnO and LOO/PLO while Ayvalık olive oils were characterized by SOO, ECN50, POS, SLO+POO, PoOO, PLL/OLL, PoOP, PLP, POP, PLL,PLO/OO, PLO+SLL, LLL/ECN42 and PLLn contents in both 2009 and 2010 harvest years. These components seem to be an effective tool to discriminate between the varieties.

REFERENCES

[1] Angerosa F., Mostallino R., Basti C., Vito R. "Influence of malaxation temperature and time on the quality of virgin olive oils." *Food Chemistry,* vol: 72. pp.19-28. 2001.

[2] Guerfel M., Ben Masour M., Ouni Y., Guido F., Boujnah D., Zarrouk M. "Triacylglycerols composition and volatile compounds of virgin olive oil from Chemlali cultivar: comparison among different planting densities". *The Scientific World Journal,* doi:10.1100/2012/354019, 2012.

[3] Köseoğlu, O., Sevim D., Kadiroğlu, P. "Quality characteristics and antioxidant properties of turkish monovarietal olive oils regarding stages of olive ripening", *Food Chemistry,* vol: 212, pp. 628-634. 2016.

[4] Sevim D., Koseoglu O., Ozturk Gungor F., "Effect of different growing area on triacylglycerol composition of cv. Gemlik olive oil of Turkey", *J Agri Faculty of Uludag University,* vol. 27, no: 1, 49-54. 2013.

[5] Galeano Diaz T., Duran Meras T., Sanchez Casas J., Alexandre Franco, M.F. "Characterization of virgin olive oils according to its triglycerides and sterols composition by chemometric methods", *Food Control,* vol: 16, pp. 339–347.2005.

[6] Bilancia, M.T., Caponio F., Sikorska E., Pasqualone A., Summo C. "Correlation of triacylglycerol oligopolymers and oxidized triacylglycerols to quality parameters in extra virgin olive oil during storage". *Food Research International,* vol: 40, pp.855-861. 2007.

[7] IOC, "http://www.internationaloliveoil.org /estaticos/view/131-world-olive-oil-figures", (12.04.2016). 2016.

[8] Ozturk F., Yalcin M., Dıraman H. "An overview on the olive oil economy of Turkey". *Electronic J Food Technol.* Vol:12,pp. 37-53. 2009.

[9] Sevim D., Tuncay O., Koseoglu O. "The effect of olive leaf addition on antioxidant

content and antioxidant activity of "Memecik" olive oils at two maturity stages", *J Am Oil Chem Soc,* vol: 90, pp.1359-1369, 2013.

[10] International Olive Council, Guide for the determination of the characteristics of oil-olives. COI/OH/Doc.No:1, 2011.

[11] Anonymous, "European Economic Community, E.C. Regulation no 2568/91.Official Journal of European communities L 128/8 15/05/02 2002", Regulation no.796 of 6 May 2002 on change Bruxelles, Belgium. 1991.

[12] Aparicio R., Aparicio Ruiz R. "Authentication of vegetable oils by chromatographic techniques", *J Chromatography A,* vol: 881,pp. 93-104. 2000.

[13] Guiffre, A.M. "Variation in triacylglycerols of olive oils produced in Calabria (Southern Italy) during olive ripening". *Rivista Italiana Delle Sostance Grasse,* vol: 91, pp. 221-240. 2014.

[14] Yorulmaz A., Yavuz H., Tekin A. "Characterization of Turkish olive oils by triacylglycerol structures and sterol profiles". *J Am Oil Chem Soc*, vol: 91.pp 2077-2090. 2014.

[15] İlyasoğlu H. and Özçelik B. "Biochemical characterization of Memecik olive oils". *Food,* vol: 36, no:1, pp. 33-41. 2011.

[16] Aranda F., Gomez-Alonso S., Rivera del Alamo R.M., Salvador M.D., Fregapane G. "Triglyceride, total and 2-position fatty acid composition of Cornicabra virgin olive oil:Comparasion with other Spanish cultivars". *Food Chemistry*, vol: 86. pp.485-492. 2004.

[17] Ben Temime S., Wael T., Bechir B., Leila A., Doua D., Mokhtar Z. "Changes in olive quality of Chetoui variety according to origin of plantation", *J of Food Lipids,* vol: 13, pp. 88-99. 2006.

[18] Fuentes M., De Miguel C., Ranalli A. "Chemical composition and sensory evaluation of virgin olive oils from "Morisca" and "Carrasquena" olive varieties". *Grasas Y Aceites*, vol.66. 2015.

[19] Gokçebag M., Dıraman H., Ozdemir D., "Classification of Turkish Monocultivar (Ayvalık and Memecik cv.) virgin olive oils from North and South zones of Aegean Region based on their triacyglycerol profiles". *J Am Oil Chem Soc*, vol: 90. pp.1661-1671. 2013.

[20] Manai-Djebali H., Krichene D., Ouni Y., Gallardo L., Sanchez J., Osorio E., Daoud D., Guido F., Zarrouk M. "Chemical profiles of five minor olive oil varieties grown in central Tunisia". *J Food Composition and Analysis.* Vol:27. pp.109-119. 2012.

[21] Yorulmaz A., Erin H., Tekin A. "Influence of maturation on triacylglycerol and sterol profiles of virgin olive oil", *AOCS, World conference on oilseed processing, fats & oils processing, biofuels & applications,* 21-23 June 2011 Izmir, Turkey. 36p. 2011.