Evaluation of olive oil quality during the ripening of the organic cultivated olives and multivariate discrimination of the variety with a chemometric approach

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The aim of this study was to evaluate the effect of olive ripening on the oil content of olive fruit, the quality indices, fatty acid composition, sterols, waxes, o-diphenols and oxidative stability of Memecik cv. and Ayvalık cv. olive oil of organic cultivation and to determine the optimum harvesting time for these varieties. The results showed that free fatty acid content and peroxide value increase while MUFA/PUFA ratio, o-diphenols and oxidative stability decrease at more advanced stages of maturity. Statistical analysis demonstrated that fatty acid composition and sterol profile were different according to the olive variety and harvesting periods. Principal Component Analyses showed that the fatty acid composition and sterol profile of olive oils were responsible for the discrimination of olive oils obtained from different olive varieties at different ripening stages. The optimum harvesting time was found to be when the maturity index was determined as 2.09 and 2.6 for Memecik and Ayvalık varieties, respectively. Due to the growing interest in organic olive oil in national and international markets, more researches that investigate the effects of ripening, the olive variety and organic cultivation method on the quality of olive oil, are required.

Keywords: olive oil, sterols, fatty acid composition, o-diphenols, oxidative stability, waxes, principal component analysis.

1. INTRODUCTION

Virgin olive oil is the one of the highest economic added value among vegetable oils, being the main dietary fat in the Mediterranean countries [1]. The dramatic increase in the demand for virgin olive oil cannot be explained only with their health properties, but also with their organoleptic properties [2]. The quality of olive oil is related to several factors, such as agronomic, technological and storage factors [3]. The olive ripening stage is one of the most important factors associated with the quality evaluation of olive oil [4, 5]. The effect of harvesting time on the oil's yield, quality, stability, and sensory characteristics are of interest to the grower [6]. In general, several metabolic processes take place in olives during ripening with subsequent variations on the chemical structure and a concentration on some compounds. These changes are reflected in the quality grade, sensorial characteristics, oxidative stability, and nutritional value of the obtained product [7].

Organic farming is of importance in the agricultural sector of many countries [3]. Organic olive is one of the most important products in the world as well as in Turkey. According to 2013 figures, 7.8% of the olive orchards are managed organically in Turkey. But the production efficiency of Turkish olive orchards is very limited compared to other important olive producing countries like Italy and Spain [8]. Organic cultivation in Turkey is being validated by Control and Certification private bodies according to the Organic Farming Regulation

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(no. 5262/2004). The Turkish Ministry of Food, Agriculture and Livestock accredited control and Certification private bodies.
Many advantages, both from an environmental and social-economic point of view, are offered through the organic cultivations of olive trees. Among them, the most significant are the protection of the environment, protection of the health of producers and consumers and economic advantages [3].
The aim of this study was to investigate the changes in chemical characteristics and minor compounds of monovarietal Memecik cv. and Ayvalık cv. olive oil produced by the organic cultivation method during successive ripening stages and to determine the optimal harvesting time-maturity index of these olive varieties. In order to evaluate these changes, the oil content of olive fruits, several chemical characteristics such as free fatty acid content, peroxide value, K_{232}, K_{270} as well as fatty acid composition, sterol profile, o-diphenols, induction period and wax content were determined. PCA was performed using the fatty acid composition and sterol profile of organic olive oils to discriminate olive variety and harvesting time.

2. MATERIALS AND METHODS

2.1. MATERIALS

This study was carried out by using monovarietal virgin olive oils from the two main Turkish varieties (Memecik cv., Ayvalik cv.). The olive samples were collected from the organic farm in Datça, Muğla, Turkey. The climatic condition in Datça is characterised by a combination of excellent conditions, such as mild winters, extensive hot summer, and long periods of sunlight, optimum rainfall, and winds of moderate intensity, which allow airing the olive groves. Olive fruits were hand-picked at four different harvesting times; 10th September, 10th October, 10th November and 10th December for the 2012/13 crop season. Climatic conditions for these harvesting times are given in Table I.
After harvesting, the olives were washed and crushed with a hammer mill and olive paste mixed at 27°C for 30 min, centrifuged at 1714 G. After centrifugation, oils were filtered, transferred into amber glass bottles, and stored at 4°C. For each olive variety, 30 kg were used for the oil production.

2.2. METHODS

The olive maturity index (MI) was determined according to the method developed by the Agronomic Station of Jaen based on the evaluation of the olive skin and pulp colours. MI was determined on 100 randomly selected olives in each sample to obtain a numerical value. MI values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin) were classified [9].
The oil content was determined by Soxhlet extraction and expressed on a dry weight basis (% fruit DW) [10].
Determination of free fatty acid (FFA) content and peroxide value (PV) was carried out using the analytical methods described in ISI (International Organization for Standardization) 660 and 3960, respectively [11, 12].
UV absorption characteristics (K_{232} and K_{270}) was determined using Agilent Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Inc, Texas, USA) according to COI/T20/Doc.No.19 [13].
For the determination of the fatty acid composition, the methyl-esters were prepared by the vigorous shaking of a solution of oil in n-heptane (0.5 ml in 7 ml) with 1 ml of 2N methanolic potash, and analysed by GC with a Hewlett-Packard (HP 6890) chromatograph equipped with a FID detector. A Supelco Silica capillary column (60 m length × 0.25-0.32 mm i.d.) coated with cynopropylsilicone phase (0.1-0.3 µm thickness) was used for analysis. Supelco 37 Component FAME Mix (CRM47885) was used for the identification of fatty acids. Hydrogen was used as a carrier gas with a flow through the column of 1 ml/min. The temperature of the oven, injector and detector was set at 165°C, 250°C and 270°C, respectively. An injection volume of 1 µl was used (COI/T20/Doc.17) [14].
Sterol profile (%) was determined by gas chromatography, Hewlett-Packard (HP 6890), with a capillary column (20-30 m length × 0.25-0.32 mm i.d.) coated with SE-52 (0.30 µm thickness). 0.2% solution of α-cholestanol (m/V) in chloroform was used for identification of sterols as internal standard. Working conditions were as follows: carrier gas, hydrogen; flow through the column, 1.3 ml/min; injector temperature, 280°C; detector temperature, 290°C; oven temperature, 260°C; injection volume 1 µl (COI/T20/Doc 10) [15].

Table I - Climatic conditions for harvesting times

	September	October	November	December
Relative humidity (%)	58.5	64.0	68.5	73.0
Temperature (°C)	25.8	23.2	18.9	14.1
Soil temperature (10 cm, °C)	30.6	25.8	18.4	13.4
Wind speed (m/sec)	3	3	3.7	4.6
Rainfall height (mm)	0	47.4	79.0	324.0

o-diphenols were determined according to COI/T20-"Colorimetric Determination of o-diphenols in Olive Oils" [16].
Oxidative stability was evaluated by the Rancimat method since it is a fast and reliable analytical procedure [17]. Stability was expressed as the oxidation induction time (h) measured with the Rancimat Model 743 (Metrohm), using an oil sample of 3 g, at 110°C and an air flow of 20 L/h.
Wax samples were prepared using chromatographic column prior to injection. 15 g of silica gel in n-hexane introduced into the glass column and 30 ml n-hexane percolated through packed column to remove any impurities. Exactly 500 mg of the sample was weighted into a 25 ml flask and 0.1 mg internal standard solution of lauryl arachidate at 0.1% (m/V) in hexane was added. Prepared sample was transferred to the chromatographic column. 70 ml of n-hexane was percolated to remove any n-alkanes. Then chromatographic elution carried out by percolating 180 ml n-hexane/ethyl ether mixture (99:1), at a flow of about 15 drops every 10 seconds. Collected fraction evaporated using rotary evaporator until the solvent is removed. After the solvent evaporation, the sample prepared using 4 ml of n-heptane and 1 µl of this solution was injected to gas chromatography. Hewlett-Packard (HP 6890), with a capillary column (10-15 m length × 0.25-0.32 mm i.d.) coated with SE-52 (0.10-0.30 µm thickness) was used for the determination of the waxes (mg/kg).
Working conditions were as follows: carrier gas, hydrogen; flow through the column, 1.2 ml/min; column temperature, 80°C; detector temperature, 350°C; injection volume 1 µl (COI/T20/Doc 18) [18].
Pearson correlation coefficients were determined for significance levels of 0.05 and 0.01. SPSS for windows was used for all statistical evaluations. Principal Components Analysis (PCA) was carried out to discriminate olive oil samples using fatty acid composition and sterol profile in a multidimensional space. The Pearson correlations were followed and only the first two factors were retained for the selection of the number of principal components (PCs). The data was analysed using XL STAT software (Addinsoft Inc., New York, USA).

3. RESULTS AND DISCUSSION

3.1. MATURITY INDEX

During the ripening process, photosynthetic activity decreases and the concentrations of both chlorophylls and carotenoids progressively decrease and the fruit becomes violet or purple due to accumulation of anthocyanins at the end of the maturation process [19]. In this study, MI of the Memecik and Ayvalık olive varieties was increased from September to December with significant linear correlations (Figure 1).

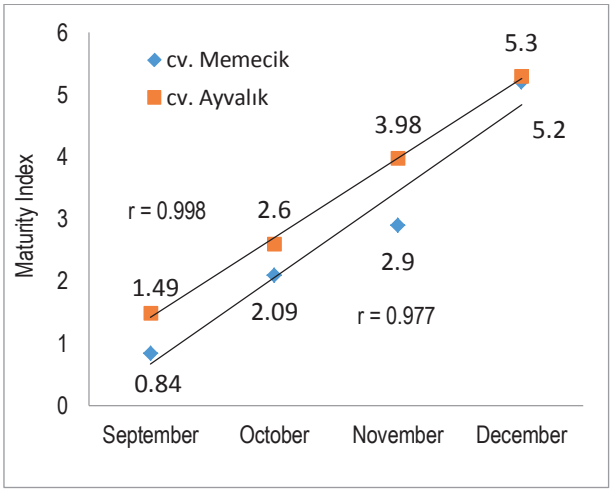

Figure 1 - Changes in MI of olives with harvest time

MI of the Ayvalık olive was measured as slightly higher than Memecik until December while the difference between MI of both varieties reduced in December.

3.2. OIL CONTENT

The oil content of Ayvalık olive generally increased during ripening, as shown in Figure 2. However, at higher MIs (> 2.6) this increase was modest and there was even a slight decrease upon the last MI (5.3). This indicates the contribution of another triacylglycerol-forming biosynthetic pathway that ends 30 weeks after flowering and at higher MI and these compounds probably undergo degradation [20].
While Ayvalık olive has the highest oil content in November, the maximum level was reached in October

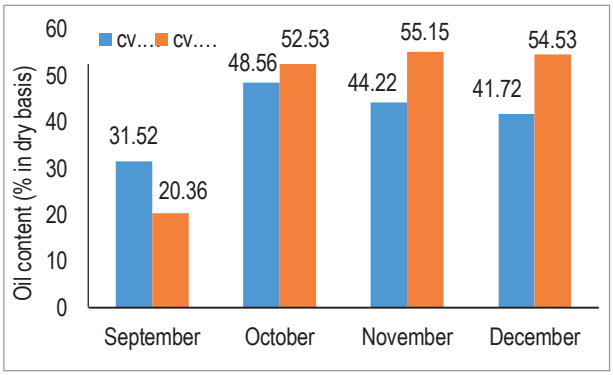

Figure 2 - Changes in oil content of olive fruits during fruit ripening

for Memecik olive. In general, the oil content of Ayvalık olive variety was higher than Memecik.

3.3. QUALITY PARAMETERS

Free fatty acid content, peroxide value, and UV absorption characteristics (K_{232}, K_{270}) are the most important quality parameters of olive oil.

The free fatty acid content of olive oils obtained from both varieties in September and October was much lower than the upper limit of 0.8% (IOOC, 2003) established for the best quality olive oil, named as extra virgin olive oil (Figure 3). However, virgin olive oil (0.8% < free fatty acid < 2.0%) was produced from the both varieties harvested in November and December. Olive fruits at a later stage of maturity undergo an increase in enzymatic activity, especially by lipolytic enzyme and are more sensitive pathogenic infections and mechanical damage [3, 5,17].
The oils obtained from both of varieties at more advanced stages of maturity (MI ≥ 5) showed higher peroxide value (Figure 3). The peroxide value remained below the limits determined for virgin olive oils (< 20 meq O_2/kg oil) [21] with a significant increase in November. High lipoxygenase activity produces an

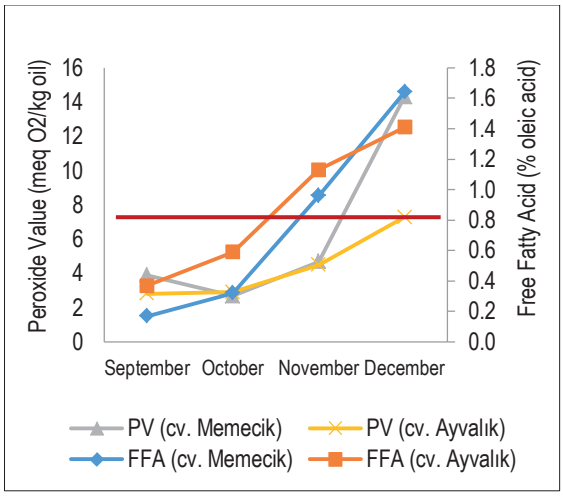

Figura 3 - Changes in free fatty acid content and peroxide value of the oils during fruit ripening

increase in peroxide value. Olive oils extracted at the end of the maturity stage give olive oils that are slightly oxidised compared to those extracted earlier [1].
UV absorption characteristic at 232 nm, K_{232}, is related to the primary oxidation of oil and an indication of conjugation of polyunsaturated fatty acids, whereas K_{270} is an indication of carbonylic compounds such as aldehydes and ketones in olive oil and is related to the secondary oxidation products [22]. The changes in K_{232} and K_{270} were shown in Table II. The same trend as peroxide value was observed with K_{232} that reached the highest value in both varieties from the last MIs (≥ 5). K_{270} values also showed significant differences with MI and show the same trend as peroxide value and K_{232}. The highest K_{270} value was determined in December for both varieties. (MI ≥ 5).

3.4. FATTY ACID COMPOSITION

Table III shows the change in the fatty acid composition of olive oil during fruit ripening. Palmitic, palmitoleic and linolenic acids showed an important reverse correlation with MI. The palmitic acid content decreased during the fruit ripening because of the dilution effect. The stearic acid content reached the maximum level at higher MIs. The oleic acid is the main mono-unsaturated fatty acid and is in higher concentrations. The oleic acid content of Memecik oil slightly increased in October and remained constant during the maturity process. However, the oleic acid level of Ayvalık oil remained unchanged until the end of the fruit development. The linoleic acid content was constant during the ripening period of Memecik variety while it was slightly increased for Ayvalık. The increase in linoleic acid level is due to the fact that, besides the continuing biosynthesis of triglycerides, with the formation of oleic acid, oleate desaturase enzyme is active, transforming oleic acid into linoleic [17]. The changes in the linolenic acid content of both varieties showed fluctuations with MI. The changes in MUFA/PUFA and C18:1/C18:2 of Memecik oil were statistically insignificant and these values reached the maximum level in October. These ratios significantly decreased in Ayvalık oil during fruit ripening. The fatty acid composition of olive oil is an important parameter in shelf life that is quantitatively affected by two main factors that are the olive variety used in the oil production and MI of the olive fruits harvested [23].
PCA was also performed to discriminate the olive oil samples on fatty acid composition according to olive variety and harvesting time. It is observed that the first two principle components were enough to explain the 66.66% of the data variance. Figure 4 shows factor loadings (Figure 4a) and observation scores (Figure 4b) of PCA for the fatty acid composition of olive oils. The observation score plot showed that Memecik and Ayvalık olive oils were clearly discriminated according to the variety and harvesting time. Factor loading plot supplies the necessary information to determine which variables are responsible for the patterns seen

Table II - Changes in K_{232} and K_{270} of the oils during fruit ripening

	Memecik				Ayvalık			
	September	**October**	**November**	**December**	**September**	**October**	**November**	**December**
K_{232}	1.800±0.033 ^{b}	1.800±0.033 ^{b}	1.200±0.037 ^{c}	2.532±0.046 ^{a}	1.927±0.035 ^{b}	1.717±0.030 ^{c}	1.596±0.029 ^{d}	2.392±0.044 ^{a}
K_{270}	0.150±0.003 ^{b}	0.144±0.003 ^{c}	0.138±0.003 ^{d}	0.213±0.004 ^{a}	0.115±0.002 ^{b}	0.010±0.002 ^{c}	0.115±0.002 ^{b}	0.194±0.004 ^{a}

Mean values were represented with ± standard deviations. Superscripted letters show statistical difference between different harvest months in same row for each variety according to Tukey's post-hoc tests.

Table III - Change in fatty acid composition of the olive oils during fruit ripening

	Memecik					Ayvalık				
	September	October	November	December		September	October	November	December	
MI	0.84	2.09	2.9	5.2	Pearson Corr.	1.49	2.6	3.98	5.3	Pearson Corr.
C14:0	0.02±0^{a}	0.02±0^{a}	0.01±0^{b}	0.01±0^{b}	-0.81**	0.02±0	0.02±0	0.02±0	0.02±0	NA
C16:0	15.58±0.28^{a}	14.49±0.26^{b}	13.08±0.24^{c}	13.06±0.24^{c}	-0.84**	16.57±0.3^{a}	15.79±0.29^{b}	15.21±0.28^{c}	15.05±0.27^{c}	-0.87**
C16:1	1.17±0.02^{a}	0.99±0.02^{b}	0.82±0.01^{c}	0.83±0.02^{c}	-0.83**	1.36±0.02^{a}	1.18±0.02^{c}	1.25±0.02^{b}	1.1±0.02^{d}	-0.79**
C17:0	0.13±0^{b}	0.09±0^{d}	0.12±0^{c}	0.14±0^{a}	0.42**	0.12±0^{c}	0.14±0^{b}	0.14±0^{b}	0.15±0^{a}	0.89**
C17:1	0.24±0	0.15±0	0.22±0	0.24±0	0.24	0.21±0^{c}	0.22±0^{b}	0.24±0^{a}	0.24±0^{a}	0.89**
C18:0	2.53±0.05^{b}	2.3±0.04^{c}	2.52±0.05^{b}	2.66±0.05^{a}	0.54**	2.39±0.04^{d}	2.61±0.05^{c}	2.7±0.05^{b}	2.82±0.05^{a}	0.93**
C18:1	64.41±0.77^{b}	69±2.12^{a}	67.21±1.54^{a}	67.45±1.8^{a}	0.33*	67.04±1.48	66.48±1.31	66.14±1.21	67.04±1.48	0.02
C18:2	13.73±0.25	10.79±0.2	13.94±0.25	13.4±0.24	0.15	10.3±0.19^{c}	11.69±0.21^{b}	12.33±0.23^{a}	11.55±0.21^{b}	0.62**
C18:3	1.14±0.02^{a}	0.67±0.01^{d}	0.76±0.01^{b}	0.74±0.01^{c}	-0.61**	0.67±0.01	0.61±0.01	0.68±0.01	0.63±0.01	-0.15
C20:0	0.45±0.01^{b}	0.4±0.01^{d}	0.42±0.01^{c}	0.46±0.01^{a}	0.34*	0.44±0.01^{d}	0.45±0.01^{c}	0.48±0.01^{b}	0.49±0.01^{a}	0.94**
C20:1	0.29±0.01	0.31±0.01	0.3±0.01	0.29±0.01	-0.18	0.29±0.01	0.29±0.01	0.3±0.01	0.29±0.01	0.15
C22:0	0.12±0^{b}	0.11±0^{c}	0.12±0^{b}	0.13±0^{a}	0.66*	0.12±0^{c}	0.12±0^{c}	0.14±0^{b}	0.15±0^{a}	0.95**
C24:0	0.09±0	0.07±0	0.07±0	0.08±0	-0.26	0.07±0	0.07±0	0.08±0	0.07±0	0.25
C18:1/C18:2	4.45±0.13	6.15±0.25	4.67±0.17	4.87±0.19	-0.02	6.28±0.22^{a}	5.54±0.18^{b}	5.22±0.17^{c}	5.64±0.2^{b}	-0.57**
MUFA/PUFA	4.69±0.14	6.4±0.27	4.82±0.17	5.04±0.2	-0.06	6.51±0.23^{a}	5.69±0.19^{b}	5.37±0.18^{c}	5.81±0.21^{b}	-0.57**

Mean values were represented with ± standard deviations. Pearson correlation coefficients show the correlation between MI and each variable. ** and * symbols represents significance level of correlations as P <0.01 and P <0.05 respectively. Superscripted letters show statistical difference between different harvest months in same row for each variety according to Tukey's post-hoc tests.

among the observations. Variables C17:1, C17:0, C20:0, C18:0, C22:0 and C18:1 (variables sorted by their squared cosine values for F1 component) were placed far from the origin, meaning that they have an important effect on the classification of oil samples with respect to harvesting time, while C18:2, C18:3 and C24:0 (variables sorted by their squared cosine values for F2 component) fatty acids have an important effect on the classification of olive oil samples with variety.

3.5. STEROL PROFILE AND TRITERPENE DIALCOHOLS

Sterols are major compounds of the unsaponifiable fraction and their content corresponds to around 20% of the unsaponificable matter of olive oil. Research has shown that each oily fruit has a characteristic

Figure 4 - Plots of factor loadings (a) and observation scores (b) for fatty acid composition

sterol profile which makes it determination an important tool to check the genuineness in an oil [17, 24, 25]. Sterols are also important components for the stability of the oil since they act as inhibitors of polymerisation reactions at high temperatures. In addition, the sterol profile of olive oil is a very useful parameter to detect adulterations or to check authenticity, since it can be considered as a fingerprint [26, 27].

The evaluation of sterols and triterpene dialcohols during ripening was presented in Table IV. Most of these compounds were significantly affected by the maturation stage of Ayvalık variety, particularly campesterol, β-sitosterol, apparent β-sitosterol, and Δ5-avenasterol. Campesterol, β-sitosterol and apparent β-sitosterol decrease during ripening while Δ5-avenasterol significantly increases. Campesterol, Δ5-avenasterol and apparent β-sitosterol decrease during the ripening of the Memecik variety while there is no significant change in β-sitosterol. Stigmasterol is known as the main sterol related to organoleptic quality of olive oils whereas high levels of stigmasterol lowers the organoleptic quality of olive oil [28, 29]. Stigmasterol content of Memecik oil was slightly higher than Ayvalık oil and reached the maximum level at the end of the maturity process. Tiscornia et al. (1978) reported that β-sitosterol is minimal and Δ5-avenasterol maximal when olives are harvested when at their best that would indicate that the quality improved with maturation. This agrees with our results for the month of October for the Memecik variety (MI = 2.09) and December for the Ayvalık variety (MI = 5.3) [30].

Total sterol decreased significantly at the end of maturity of the Memecik variety while it was not significantly affected by the maturation stage of the Ayvalık variety. Total sterol reached the highest level for Ayvalık olive oil obtained in November (Table IV). These results were in good agreement with data reported in a previous study [28].

Triterpene dialcohols (erythrodiol and uvaol), which are also part of the unsaponifiable fraction of olive oil, are usually analysed with the sterol fraction. The sum of erythrodiol and uvaol, another authenticity index established by law, was below the upper limit of 4.5% for the extra virgin olive oil category in all cases [25]. The sum of erythrodiol and uvaol of olive oil samples tends to increase as the maturity index increases for both varieties. This value was very close to 4.5% in Ayvalık oil (4.01%) in December (Table IV).

PCA was also performed to discriminate the olive oil samples on the sterol profile according to olive variety and harvesting time. It is observed that the first two main components were enough to explain the 71.46% of the data variance. Figure 5 shows factor loadings (Figure 5a) and observation scores (Figure 5b) of PCA for the sterol profile of olive oils. Memecik and Ayvalık olive oils at early and middle ripening stages (September, October, and November) were in the same zone of the observation score plot of the

Table IV - Changes in sterol profile and total sterol content of the olive oils during fruit ripening

	Memecik					Ayvalik				
	September	**October**	**November**	**December**		**September**	**October**	**November**	**December**	
MI	0.84	2.09	2.9	5.2	Pearson Corr.	1.49	2.6	3.98	5.3	Pearson Corr.
Cholesterol	0.02±0^{c}	0.03±0^{b}	0.02±0^{c}	0.1±0^{a}	0.89**	0.02±0^{c}	0.03±0^{b}	0.03±0^{b}	0.05±0^{a}	0.92**
24-Methylen cholesterol	0.05±0^{b}	0.03±0^{c}	0.03±0^{c}	0.13±0.01^{a}	0.78**	0.04±0^{b}	0.02±0^{d}	0.03±0^{c}	0.08±0^{a}	0.66**
Campesterol	2.97±0.13^{a}	2.9±0.13^{a}	2.44±0.11^{c}	2.64±0.12^{b}	-0.55**	3.4±0.15^{a}	2.98±0.13^{b}	3.02±0.13^{b}	2.81±0.12^{c}	-0.76**
Campestanol	0.08±0^{a}	0.06±0^{b}	0.05±0^{c}	0.05±0^{c}	-0.81**	0.07±0^{c}	0.05±0^{d}	0.08±0^{b}	0.09±0^{a}	0.70**
Stigmasterol	0.72±0.03^{b}	0.51±0.02^{c}	0.44±0.02^{d}	0.96±0.04^{a}	0.53**	0.38±0.02^{c}	0.3±0.01^{d}	0.43±0.02^{b}	0.58±0.03^{a}	0.81**
Δ7-Campesterol	0.07±0^{b}	0.05±0^{c}	0.05±0^{c}	0.21±0.01^{a}	0.83**	0.06±0	0.02±0	0.05±0	0.06±0	0.25
Clerosterol	0.77±0.03^{b}	0.78±0.03^{b}	0.77±0.03^{b}	1.03±0.05^{a}	0.85**	0.12±0.01^{d}	0.53±0.02^{c}	0.69±0.03^{b}	0.82±0.04^{a}	0.94**
β-sitosterol	87.77±0.54	85.72±0.63	86.53±0.58	87.64±0.55	0.137	85.16±0.65^{a}	84.25±0.69^{b}	84.21±0.7^{b}	82.8±0.76^{c}	-0.74**
Sitostanol	0.75±0.03^{a}	0.45±0.02^{c}	0.49±0.02^{b}	0.37±0.02^{d}	-0.84**	0.52±0.02^{c}	0.55±0.02^{b}	0.65±0.03^{a}	0.27±0.01^{d}	-0.53**
Δ5-Avenasterol	5.24±0.23^{c}	6.82±0.3^{a}	6.39±0.28^{b}	4.93±0.22^{d}	-0.32*	7.05±0.31^{c}	7.47±0.33^{b}	7.33±0.32^{b,c}	8.42±0.37^{a}	0.74**
Δ5-24 Stigmastadienol	0.74±0.03	1.42±0.06	1.05±0.05	0.78±0.03	-0.18	1.82±0.08	2.16±0.1	1.89±0.08	1.83±0.08	-0.21
Δ7-tigmastenol	0.31±0.01^{c}	0.35±0.02^{b}	0.44±0.02^{a}	0.45±0.02^{a}	0.85**	0.43±0.02	0.44±0.02	0.29±0.01	0.47±0.02	-0.05
Δ7-Avenasterol	0.51±0.02	0.88±0.04	1.3±0.06	0.71±0.03	0.19	0.93±0.04^{d}	1.2±0.05^{c}	1.3±0.06^{b}	1.72±0.08^{a}	0.95**
Erythrodiol + Uvaol	2.26±0.1^{a}	1.57±0.07^{d}	2.11±0.09^{c}	2.79±0.12^{b}	0.61**	1.55±0.07^{c}	1.57±0.07^{c}	1.76±0.08^{b}	4.01±0.18^{a}	0.83**
Total Sterol (mg/kg)	1827±80.72^{a}	1796±79.35^{a}	1827±79.37^{a}	1597±70.56^{b}	-0.70**	2109±93.18	2584±114.17	2795±123.49	2141±94.6	0.08
Apparent β-sitosterol	95.27±0.21^{a}	95.19±0.21^{a}	95.23±0.21^{a}	94.75±0.23^{b}	-0.66**	94.67±0.23^{b}	94.96±0.22^{a}	94.77±0.23^{a,b}	94.14±0.26^{c}	-0.55**

Mean values were represented with ± standard deviations. Pearson correlation coefficients show the correlation between MI and each variable. ** and * symbols represents significance level of correlations as P <0.01 and P <0.05 respectively. Superscripted letters show statistical difference between different harvest times in same row for each variety according to Tukey's post-hoc tests.

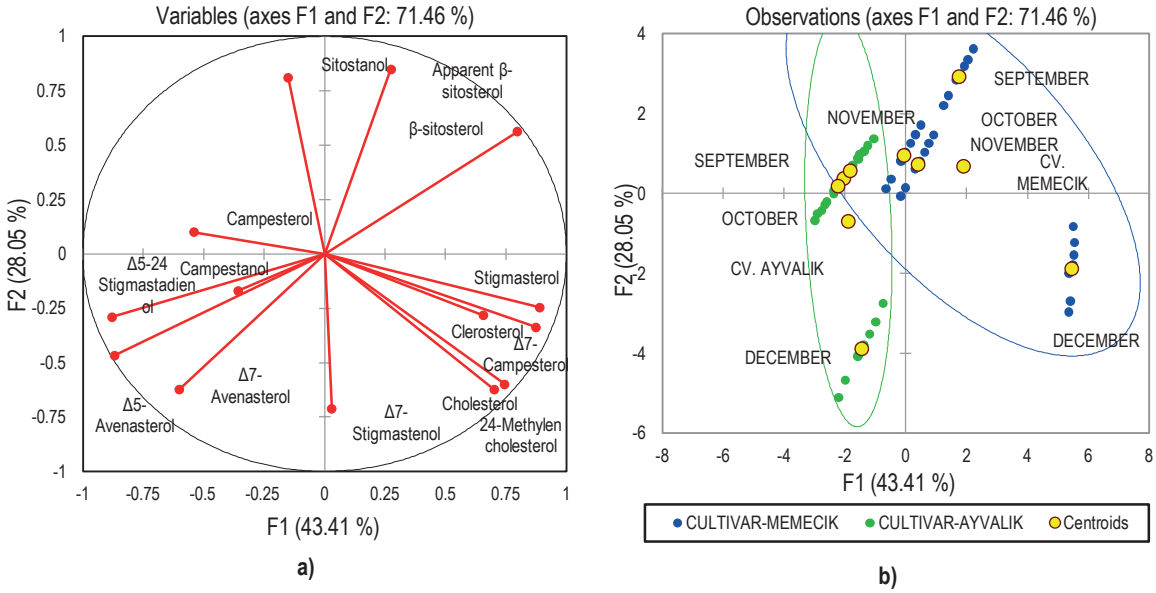

Figure 5 - Plots of factor loadings (a) and observation scores (b) for sterol profile

sterol profile. The observation score plot showed that Memecik and Ayvalık varieties were distinguished clearly in December. Stigmasterol, Δ5-24stigmastadienol, Δ7-campesterol, Δ5-avenasterol, β-sitosterol, 24-methylen cholesterol, cholesterol and clerosterol (variables sorted by their squared cosine values for F1 component) were responsible for discrimination of olive oil samples with respect to olive variety in December. Variables apparent β-sitosterol, sitostanol, stigmastenol and Δ7-avenasterol (variables sorted by their squared cosine values for F2 component) have an important effect on the classification of olive oil samples according to the harvesting time.

3.6. O-DIPHENOLS AND OXIDATIVE STABILITY

The o-diphenols can be identified as the main source contributing to the overall antioxidant activity of extra virgin olive oils and may therefore play a major role ensuring the preservation of the oils and influencing their organoleptic characteristics [7]. The amount of o-diphenols significantly decreased during ripening, as shown in Figure 6. o-diphenols concentration of Memecik oil was significantly higher than Ayvalık oil until November and it reached the minimal level in December for both varieties. These results agree with another data presented in a previous study [31].
Stability to oxidation is an important property of olive oil that is improved by synergistic interactions between various antioxidants present (both phenolic and non-phenolic) and lipid composition [7]. The change in IP of olive oils was presented in Figure 6. There was no significant correlation between o-diphenols and IP of Memecik oil ($r = 0.762$, $P > 0.05$) but IP reached the highest value in the first three harvest month and then decreased drastically in December. The decrease in IP of Ayvalık olive oil showed a linear tendency during ripening and the correlation between IP and o-diphenols was found to be significant ($r = 0.995$, $P < 0.05$).

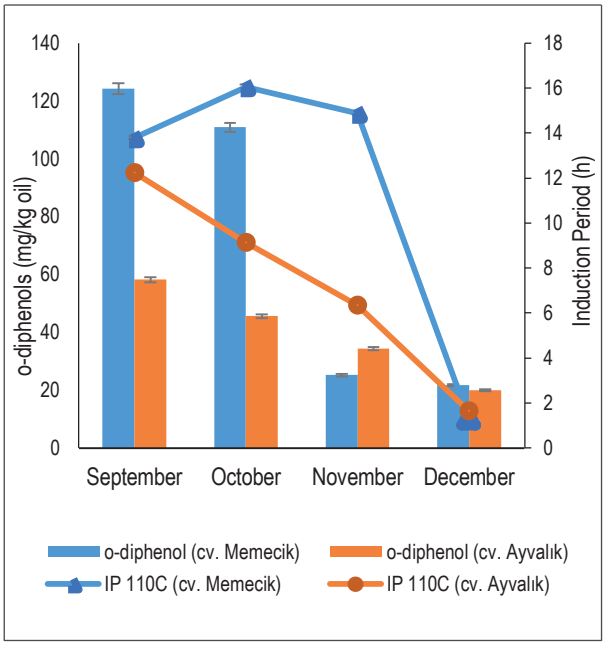

Figura 6 - The changes in o-diphenols and induction period at 110°C during fruit ripening

Oxidative stability is an important quality aspect for virgin olive oils and can be greatly influenced by environment, climate, agronomic techniques, olive variety, ripeness degree, harvest time, extraction technologies, and storage [32]. Although o-diphenols are highly associated with the antioxidative properties of olive oils, other phenolic compounds, tocopherols,

fatty acid composition are also effective on oxidative stability [33]. This clarifies the insignificant correlation between o-diphenols and IP of Memecik oil that can be associated with the existence of different compounds showing that the antioxidative behaviour differs from o-diphenols.

3.7. WAXES

Waxes are a part of the unsaponifiable matter in vegetable oils. Wax ester compositions are not used normally for the characterisation of virgin olive oils. However wax content is employed as a quality parameter and has been used to detect adulteration with lower quality olive oil or pomace oil because solvent-extracted olive oils contain a considerable amount of waxes [34]. The total wax content was significantly affected ($P < 0.05$) by the maturation stage for both varieties. The wax content decreased in October and then it reached its highest value in Memecik oil at the end of the maturation stage (Figure 7). The wax content of Ayvalık oil increased in November and was constant in December. The maximum limit for the total wax content established by the European Legislation is 250 mg/kg for olive oils and the wax contents of all olive oil samples were below legal limits. The results agreed with the results presented with previous studies [35, 36].

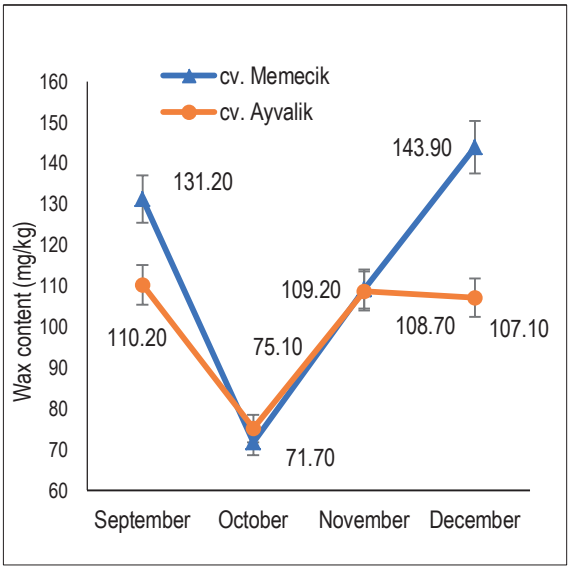

Figure 7 - The change in wax content of the olive oils during fruit ripening

4. CONCLUSION

The results of this study showed that the ripening stage of the olives had a great influence on the oil yield, oil quality and nutritional parameters of the Memecik and Ayvalık varieties grown using the organic method. The results showed that the composition of olive oilvaries greatly during maturation. With delayed harvest, the free fatty acid content increases and MUFA/PUFA ratio, o-diphenols and oxidative stability decrease. Hence, we could recommend that organic olive fruits require an early harvest time to produce extra virgin olive oil containing high levels of phenolic compounds. The optimum harvest time was determined as being the month of October for both varieties in which the maturity index was determined as 2.09 and 2.6 for organic Memecik and Ayvalık olive varieties, respectively. Principal Component Analyses indicated that the fatty acid composition and sterol profile of olive oils were responsible for the characterisation and classification of olive oils obtained from different olive varieties at different ripening stages.

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