



Comparison of some chemical parameters of a naturally debittered olive (*Olea europaea* L.) type with regular olive varieties



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ABSTRACT

Some olives grown in Karaburun peninsula in the west part of Turkey and mostly coming from Erkence variety lose their bitterness while still on the tree and are called Hurma among locals. This olive type does not require further processing to remove the bitter compounds. In this study, sugar, organic acid and fatty acid profiles of Hurma, Erkence (not naturally debittered) and Gemlik (commonly consumed as table olive) olives were determined throughout 8 weeks of maturation period for two consecutive harvest seasons, and the results were analysed by principal component analysis (PCA). PCA of sugar and organic acid data revealed a differentiation in terms of harvest year but not on variety. Hurma olive is separated from others due to its fatty acid profile, and it has higher linoleic acid content compared to others. This might be an indication of increased desaturase enzyme activity for Hurma olives during natural debittering phase.

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1. Introduction

Olive fruit which could be consumed as table olive or used in oil production has significant economic importance especially for the Mediterranean countries. Olive and its products have become even more valuable since their health benefits have come under light. There are reports of various health benefits of consuming table olives such as prevention of coronary heart disease, some cancer types, and inflammation, due to its highly monounsaturated fatty acid profile and phenolics content. It has been claimed that consuming 5–10 table olives a day might cover the daily intake of polyphenols (Boskou et al., 2006).

Erkence olive variety, mostly cultivated in Karaburun peninsula in the west part of Turkey, goes through a natural debittering phase during its maturation period. As a result, olive becomes ready to eat as a table olive while still on the tree and known by the name of Hurma among locals in the Karaburun region. Hurma type of olive does not require processing steps used in table olive preparation to remove bitter tasting phenolic compounds of olive. Similar sweet olives were also reported in studies from Greece and Tunisia (Jemai, Bouaziz, & Sayadi, 2009; Zoidou et al., 2009). Since natural debittering of olives is confined to only certain areas in a few countries it is generally associated with factors such as climate and/or soil. However, there are a few studies in literature about the

changes in olive composition during this natural phase. A study about sweet Thassos olive which is grown in Thassos Island of Greece showed that oleuropein responsible for bitter taste is hydrolysed to hydroxytyrosol and its derivatives by β -glucosidase enzyme, which is produced by fungi and bacteria during ripening (Zoidou et al., 2009). Same trend was also observed in a study about Dhokar olives which are cultivated in southern region of Tunisia (Jemai et al., 2009). In addition, it was found out that Hurma olive has lower phenolic content compared to regular olive types (Aktas, Ozen, Tokatli, & Sen, 2014). Despite these studies, knowledge about natural debittering phenomenon is limited mostly to phenolic changes and further study is needed to identify the other chemical changes that take place in olive composition during maturation.

Sugars, organic acids and fatty acids are significant components of olive fruit. Sugars not only provide energy for metabolic changes that take place in the fruit but also are related to textural properties of the olive. In addition, sugars are the precursor for fatty acid biosynthesis and they act as carbon sources for microorganisms during table olive processing (Marsilio, Campestre, Lanza, & De Angelis, 2001). Major soluble sugars in olive are reported as glucose, fructose, sucrose, xylose, rhamnose and mannitol (López-López, Jiménez-Araujo, García-García, & Garrido-Fernández, 2007). In sweet Thassos olives, glucose and mannitol were detected as the main sugar and sugar alcohol, respectively and concentrations of these compounds were very close to each other (Marsilio et al., 2001). In naturally debittered Dhokar olives,

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glucose and mannitol reach to their highest level of concentration at the last stage of ripening, and their concentrations in naturally debittered Dhokar olives are higher compared to regular Chemlali olives (Jemai et al., 2009). Organic acids which are approximately 1.5% of the flesh part (Cunha et al., 2001) of an olive, are produced during the formation and degradation of the other components like carbohydrates in olive play an important role in metabolic activity (Cunha et al., 2001). According to a study about Turkish olives, succinic, malic and citric acids are determined as major organic acids in Memecik and Domat varieties (Ergönül & Nergiz, 2010). Citric, succinic and galacturonic acids were identified as the main organic acids in another study which determined the organic acid profile of olives during maturation (Arslan & Özcan, 2011).

Aim of this study is to determine the changes in important chemical parameters (sugars, organic acids and fatty acids) of Hurma olives during natural debittering phase on the tree in comparison to Erkence (same variety as Hurma but not naturally debittered) and Gemlik (olive variety commonly consumed as table olive) olive types.

2. Materials and methods

2.1. Olive samples

Three different types of olives were used in the analyses. These types are Hurma (H) (naturally debittered Erkence), Erkence (E) and Gemlik (G) olives. Hurma and Erkence olives were hand-picked from an olive orchard (latitude: 38°54'07"N, longitude: 26°57'24"E) which is located in Karaburun Peninsula of Izmir, while Gemlik type was obtained from another orchard located in Izmir Institute of Technology campus area (latitude: 38°19'30.84"N, longitude 26°37'48.87"E) which is 30 km south of the first orchard.

For the two harvest years (2011 and 2012), all olives were picked during 8 weeks of maturation period from the end of October till the beginning of December. This is the period that appearance of Hurma becomes wrinkly on the surface while Erkence has a smooth skin; therefore, they can be differentiated with respect to their appearance. Every week approximately half a kilogram of olives were picked from the all sides of three trees for each type.

After harvesting kernels of olives were removed from the fruit immediately. For the storage, olives were first immersed into liquid nitrogen, then dried with a freeze-dryer (Labconco, USA). All chemical analyses were completed within 2–3 months after harvesting.

2.2. Chemicals

Reagents used in the chemical analysis were obtained from Riedel-de Haën (Germany) and Sigma–Aldrich (Germany) and they are either HPLC or analytical grade.

2.3. Organic acid and sugar analysis

Sugar and organic acid analyses of the samples were performed according to a method in the literature (López-López et al., 2007). Same extraction procedure was followed up for sugar and organic acid analyses. 5 g lyophilised olive was weighed. 5 mL of 1000 ppm sorbitol solution was added to the sample as an internal standard and the solution was completed to 50 mL by adding ultra-pure water at 60 °C. The mixture was mixed for 30 min. Then, it was centrifuged at 9000 rpm (Sigma-2-16KC Centrifuge, The United Kingdom) for 15 min. Supernatant was collected and filtered through 0.45 µ syringe filter into vials and injected into HPLC. Extraction and analysis was applied at least twice for each sample.

Chromatographic analyses were performed with an HPLC (Agilent 1200), equipped with a RI detector, a column oven and an auto-sampler. For sugar determination, 50 µL of the extract was

injected into the equipment. Column was HPX-87C (Bio-rad, USA) with 300 × 7.8 mm dimensions. 0.05 M H₂SO₄ was used as the mobile phase and the flow rate was set to 0.7 mL/min. Column temperature was kept at 65 °C. Sugar standards used in the analysis were glucose, fructose, lactose, mannose, mannitol and sucrose.

For organic acid analysis, external standard method was used. Mobile phase was 0.05 M H₂SO₄. Flow rate was kept at 0.7 mL/min. HPX-87H column (300 × 7.8 mm, Bio-rad, USA) was used and injection temperature was set to 65 °C. Organic acid standards used in this analysis were lactic, acetic, malic, citric and succinic acids. Organic acid and sugar compositions were expressed in terms of mg/kg dw (dry weight) of olive samples.

2.4. Fatty acid analysis

First, extraction of oil was done. For this purpose, 5 g lyophilised olive was extracted with *n*-hexane at 180 °C by an automatic Soxhlet extraction unit (Gerhard Multistat, Germany). In order to determine the fatty acid profile, fatty acid methyl esters were formed. European Official Methods of Analysis (European Union Commission, 1991) was used to prepare methyl esters. 100 mg oil was weighed into 25 mL centrifuge tubes. The samples were dissolved in 10 mL *n*-hexane and saponified to their methyl esters with the addition of methanolic potassium hydroxide solution. The sample solution was vortexed for 30 s and centrifuged at 5000 rpm for 15 min. Supernatant was collected and filtered through 0.45 µ syringe filter into vials and injected to a gas chromatography (GC) equipment. Extraction and analysis was applied at least twice for each sample.

Chromatographic analyses were performed with a GC (Agilent 6890) equipped with an auto-sampler, a split/splitless (1:50) injector and an FID detector. An HP 88 capillary column (100 m × 0.25 mm ID × 0.2 µm) was used. The carrier gas was helium with 2 mL/min constant flow rate. Injection and detector temperatures were 250 and 280 °C, respectively. The oven temperature program was run at 120 °C for 1 min, varied at 3 °C/min to 220 °C and held at this temperature for 5 min. Peak quantification is expressed as percentage of FAME using FAME standards and sample chromatograms. A 37 component mixture of FAME (Sigma) was used as the standard.

2.5. Statistical analysis

The multivariate data matrix consists of observations represented by samples from three different olive types for two harvest years and variables represented by individual sugar, organic acid or fatty acid concentrations. Data were auto-scaled before multivariate analysis. The data matrix was analysed by principal component analysis (PCA). The multivariate analyses were performed by SIMCA-P v.11.5 (Umetrics, Umea, Sweden). Results of PCA are visualised by scores and loading plots. Scores plots were constructed to observe principal groupings among observations. Loadings indicate the importance of each variable for the model and loading plots are used to interpret the relations among variables and clusters observed in the score plots. The same analyses were also performed for each harvest year by separating the data into two to observe the differences between olive types more clearly.

3. Results and discussion

3.1. Sugar composition of olive varieties

Glucose, fructose, sucrose, mannose and mannitol are the sugars that were detected in Hurma, Erkence and Gemlik olive types. Concentrations of these sugars for different varieties with respect to harvest time and year are listed in Table 1.

For the first harvest year, glucose and mannitol were detected for all olive types as the main sugars as in sweet Dhokar olives (Jemai et al., 2009). Sucrose disappeared after the second week of sampling in Hurma olive. At the same time, fructose and glucose concentrations increased implying a conversion of sucrose to these sugars. For all olive types, there is a slight increase in the amounts of all sugars for the last 3 weeks of ripening except mannitol. Mannitol was not detected after 5th week of maturation for all olive types.

The highest concentration of glucose was mostly detected in Hurma type and it varied between 21,256 and 296,787.05 mg/kg dw in the first harvest year. While glucose concentration changed between the ranges of 30,700–163,449.44 mg/kg dw for Erkence, it was between 39,160 and 88,883.15 mg/kg dw in Gemlik type. For Dhokar variety, glucose was determined as the main sugar with concentrations of 40,830 mg/kg dw followed by fructose (45,170 mg/kg dw). These sugars were in higher concentrations compared to a regular olive variety grown in the same region (Rigane, Salem, Sayadi, & Bouaziz, 2011). The lowest concentrations of mannitol in the first harvest year were detected in Gemlik variety and its range is between 7360 and 9700 mg/kg dw. Erkence type had comparably higher concentrations of mannitol and it varied between 4386.3 and 18,971.63 mg/kg dw. While mannitol reached to 79,800 mg/kg at the end of ripening in Dhokar olives (Jemai et al., 2009), it increased to 11,681.49 mg/kg in Hurma and did not show any linear increasing trend as opposed to Dhokar.

In contrast to the first harvest year, sucrose was detected throughout the ripening period and mannitol did not disappear after 5th week of maturation in the second year. Olives had higher concentrations of mannose than the first harvest year. The highest concentration of glucose was observed in Erkence type, and it varied between 13,218.97 and 53,439.29 mg/kg dw. Similar to glucose, the highest concentrations of fructose (11,075.2–50,872.33 mg/kg dw) were found in Erkence type until the last 2 weeks of sampling. Gemlik variety had the lowest amounts of mannitol (3587.83–11,853.94 mg/kg dw) as in the first harvest year.

The total sugar content of olive varieties investigated in this study is listed in Table 1. The total sugar content in the first season increased significantly in the last 3 weeks of harvesting. Other than

that there is no significant trend regarding the total sugar content. There are increases and decreases in sugar concentrations throughout the sampling period. These changes are associated with the continuous synthesis of sugar during the ripening period and its use in the fatty acid biosynthesis (Menz & Vriesekoop, 2010). Although some studies reported a decrease in total sugar content during ripening (Ergönül & Nergiz, 2010; Menz & Vriesekoop, 2010; Nergiz & Engez, 2000) an increase in reducing sugar content was observed for Chemlali and Dhokar varieties in another study (Jemai et al., 2009). While another study also reported an up and down trend for a certain olive variety (Uslu) as it was observed in our study a decreasing trend was reported for another variety (Nergiz & Ergonul, 2009).

In order to investigate the relation between the sugar profile and the parameters of olive variety, harvest time and harvest year statistically, PCA was used. PCA model for the data containing two harvest years is made from 2 PCs and R^2 value of this model is 0.716. Score plot (Fig. 1a) shows that there is a difference in terms of sugar composition of olive samples between harvest years although some samples are placed within the other class. While the first year samples are mostly placed in the right part of the plot, second year samples are in the left part of it. According to loading plot (Fig. 1b), sucrose and glucose are the sugars that cause differentiation of the first year from the second year. Sucrose has lower concentration in the first year samples and mannitol, fructose and mannose which are the differentiating parameters in the second year have more regular distribution throughout sampling period compared to the first year. However, a separation based on olive types was not observed according to the score plot. As a result, it can be concluded that sugar profile do not provide much differentiation between the olive varieties while the effect of harvest year is identified as an important factor determining the sugar concentrations of olive varieties investigated in this study.

3.2. Organic acid composition of olive varieties

Organic acid compositions of Hurma, Erkence and Gemlik olive types during 8 weeks of maturation in 2011 and 2012 harvest years are provided in Table 2. For both harvest years, main organic

Table 1
Sugar concentrations (mg/kg dw) of Hurma (H), Erkence (E) and Gemlik (G) olive types for two harvest years.

2011/12							2012/13						
Olive ^a	Glucose	Fructose	Sucrose	Mannose	Mannitol	Total	Olive ^a	Glucose	Fructose	Sucrose	Mannose	Mannitol	Total
H11	21256	nd	3371.21	nd	4227.2	28854.41	H21	8009.55	11036.15	5631.45	4997.84	8556.30	38231.29
H12	20546.1	nd	2156.9	1291.78	2942.6	26937.38	H22	18828.36	7047.26	5709.69	3361.8	3534.64	38481.75
H13	21859.1	1785.3	nd	1124.6	5770.7	30539.7	H23	23122.95	13137.24	4955.6	4411.99	14748.41	60376.59
H14	46305.5	5780.1	nd	5000.24	11681.49	68767.33	H24	12879.09	9475.97	5426.96	3598.35	9259.45	40639.82
H15	14399.7	2003.7	nd	nd	4564.4	20967.8	H25	13378.57	7569.46	3223.61	nd	8083.40	32255.04
H16	296787.05	1723.77	nd	nd	nd	298510.82	H26	20619.04	14885.54	6177.33	2587.45	10851.11	55120.47
H17	197919.6	nd	nd	nd	nd	197919.6	H27	14663.89	15827.61	5409.96	2333.7	10934.98	49170.13
H18	118058.6	4180.35	nd	nd	nd	122238.95	H28	11677.49	9690.07	3861.60	3682.48	7348.53	36260.17
E11	19364.4	nd	4284.44	5026.7	4386.3	33061.84	E21	13457.78	24904.36	9172.23	6530.98	9817.16	63882.51
E12	26777.4	5385.4	4573.1	2838.8	18971.63	58546.33	E22	53439.29	33284.23	8037.53	21459.07	22882.42	139102.54
E13	30700	5780.1	5070	1729.6	7970	51249.7	E23	33454.02	15464.92	16040.07	1278.03	14962.48	81199.51
E14	42440	3220	4580	2700	10990	63930	E24	13218.97	50872.33	16287.21	nd	22627.95	103006.46
E15	41400	5960	14110	5590	14110	81170	E25	23352.15	15889.83	3541.45	23714.03	3541.45	70038.91
E16	135115.61	7148.49	26978.66	nd	nd	169242.76	E26	38161.43	31263.91	5542.18	2847.77	17594.42	95409.71
E17	163449.44	7743.52	32613.53	15953.92	nd	219760.41	E27	21440.40	11363.67	7001.10	2099.71	6545.73	51450.61
E18	153751.42	12382.12	50059.92	14654.35	nd	230847.81	E28	19500.65	11075.20	4390.96	2797.72	7680.71	45445.24
G12	35620	5740	4950	nd	8740	55050	G21	13163.60	13858.91	8365.76	nd	6058.68	43446.95
G13	39160	4351	4580	1321.8	7360	56772.8	G22	18368.63	16477.97	6061.72	6711.77	4778.42	52368.51
G14	33333.8	4530	2720	4770	9700	55053.8	G23	29216.18	16478.14	23581.62	14742.02	9737.25	93755.21
G15	40160	6890	nd	nd	9110	49270	G24	30741.38	19786.03	6616.78	nd	11853.94	68998.13
G16	88883.15	15346.75	9313.69	8483.03	nd	122026.62	G25	8933.01	14465.70	4693.02	1970.83	4103.99	34166.55
G17	33807.88	nd	5768.05	nd	nd	39575.93	G26	8628.53	5946.45	3575.75	2714.38	4274.32	25139.43
G18	73598.39	5624.01	21733.53	12260.71	nd	113216.64	G27	4701.81	6109.90	3379.29	1467.76	3587.83	19246.59
							G28	11118.79	12205.61	2383.07	1643.02	4609.45	31959.94

^a First number after olive type represents the harvest year and second number shows the harvest time (1st week, 2nd week, etc.), nd: Not detected.

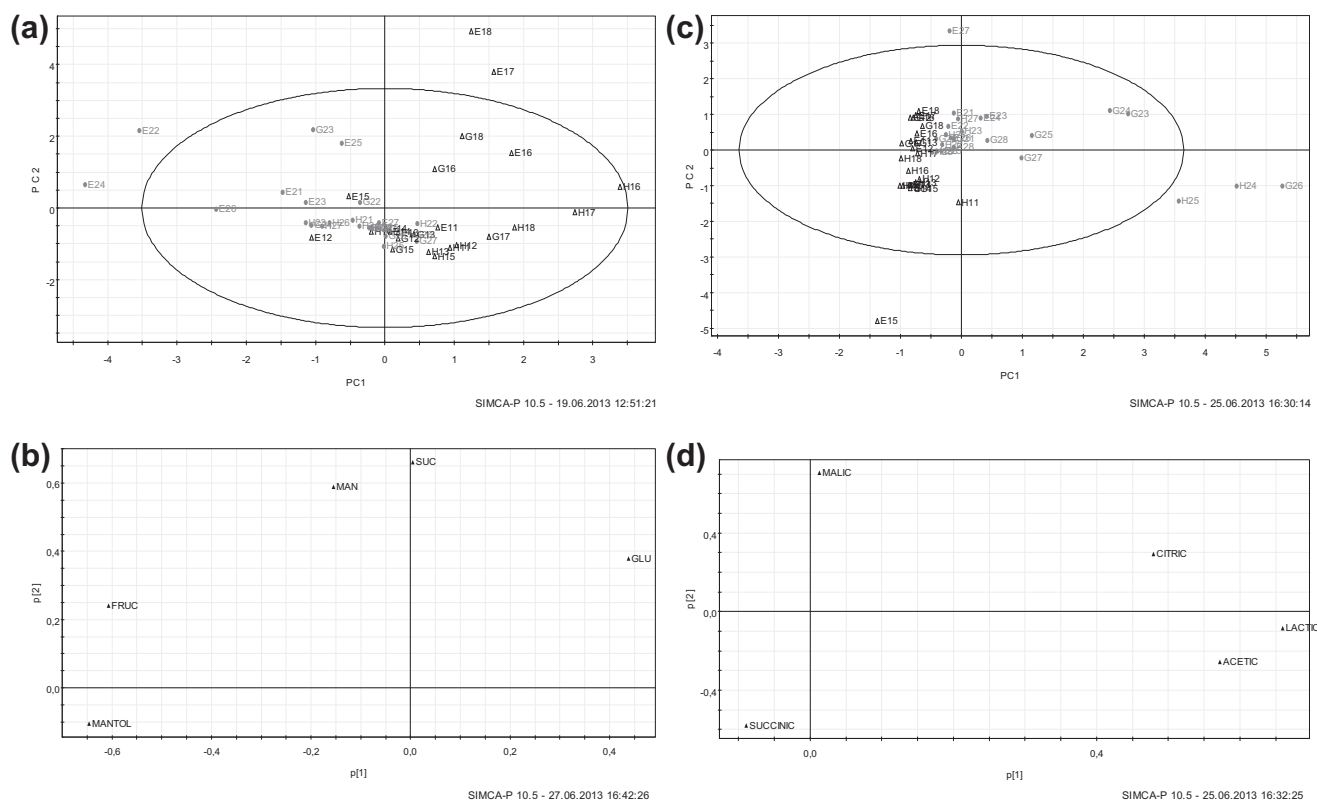


Fig. 1. (a) Score and (b) loading plots obtained with PCA of sugar compositions; (c) score and (d) loading plots obtained with PCA of organic acid compositions of Hurma, Erkençe and Gemlik types of olives during 8 weeks of maturation for both harvest years (H: Hurma, E: Erkençe, G: Gemlik, first number after the letter is the harvest year and second number is the harvest week).

acid was citric acid for all olive types. Citric acid is detected in all olive samples during whole maturation period. Malic and succinic acids were also found in olive samples. According to some studies about Turkish olives, malic acid was determined as the dominant organic acid with high concentrations (Ergönül & Nergiz, 2010; Nergiz & Ergonul, 2009) while another study reported citric acid as the main organic acid followed by succinic acid in olives grown in several locations of Turkey (Arslan & Özcan, 2011).

Higher concentrations of citric acid were measured in Gemlik variety between the ranges of 6907.85–16,412.88 mg/kg dw in the first harvest year. Malic acid was also a significant organic acid for Erkençe and Gemlik types. Although malic acid was detected until 6th week of maturation in Hurma, it increased and reached to the highest concentration (6390.74 mg/kg dw) at the last week of the first year. Succinic acid was not detected after 5th week in Hurma olive. In Turkish Domat and Memecik olives, succinic acid was detected at lower concentrations of 539–614 mg/100 g (Ergönül & Nergiz, 2010).

In the second harvest year, almost all individual and total organic acid concentrations were higher than the first year (Table 2). Similar to the first year, Gemlik, in general, had the highest amounts of citric acid between the ranges of 26,055.86–86,098.68 mg/kg dw. Citric acid was measured in the concentration range of 702–1024 mg/100 g dw in Memecik and Domat in another study (Ergönül & Nergiz, 2010). Contrary to the first year, malic acid existed in all olive types during maturation and it was found in higher amounts in Erkençe (4583.79–12,935.2 mg/kg dw). Succinic acid was not detected in Erkençe for the second year. In addition, it disappeared from Hurma after 5th week as in the first harvest year. Disappearance of succinic acid during maturation period was also observed for certain varieties in another study (Nergiz & Ergonul, 2009). While lactic acid was not detected in

the first harvest year it was present mainly in Gemlik variety in the second year.

In order to investigate the organic acid results with respect to olive variety, harvest time and harvest year statistically, PCA analysis was used. PCA model has R^2 of 0.966 and 4 components. As it is shown in the score plot (Fig. 1c), there is a good separation between harvest years in terms of organic acids. Most of the first harvest year samples are located on the left side of the plot. First year samples are more closely clustered while there is more spreading out in the second year samples. Another study also reported the significant effect of harvest year on organic acid concentrations of Turkish olives (Arslan & Özcan, 2011). According to loading plot malic and succinic acid concentrations are the differentiating parameters for the first year (Fig. 1d). Malic acid was observed throughout maturation period in the second year while in the first year it was on and off form depending on the variety. On the other hand, organic acid profiles of investigated olive varieties do not provide separation with regard to variety as in the sugar profile according to the score plot (Fig. 1c).

3.3. Fatty acid composition of olive varieties

Fatty acid compositions of olive varieties during maturation period for two harvest years are listed in Table 3. As would be expected oleic acid is the major fatty acid for all olive types over the whole sampling period. According to the previous studies, the oleic acid (18:1n9c) content rises throughout the maturation period of olives (Issaoui et al., 2008). However, a linear increasing trend for oleic acid content was not observed in Hurma, Erkençe and Gemlik olives during ripening in this study. Oleic acid content in the second season was lower than the first season for all olive varieties. As it is shown in Table 3, the highest percentage of oleic acid (66.85%)

Table 2
Organic acid concentrations (mg/kg dw) of Hurma (H), Erkence (E) and Gemlik (G) olive types for two harvest years.

2011/12							2012/13						
Olive ^a	Citric a.	Malic a.	Succinic a.	Lactic a.	Acetic a.	Total	Olive ^a	Citric a.	Malic a.	Succinic a.	Lactic a.	Acetic a.	Total
H11	11546.2	nd	3644.08	690.67	743.71	16624.66	H21	34306.53	9651.37	1873.02	nd	nd	45830.9
H12	13402.05	nd	103.45	nd	39.36	13544.86	H22	32140.03	7702.11	2223.12	nd	nd	42065.25
H13	9859.43	nd	288.32	nd	59.49	10207.24	H23	46080.78	9644.55	2780	nd	nd	585053.25
H14	1170.4	nd	403.616	nd	nd	1574.016	H24	52511.74	5168.42	837.65	1989.42	2171.4	62678.62
H15	1134.87	nd	148.81	nd	462.63	1329.94	H25	27357.3	3549.53	1030.82	1850.78	1952.74	35741.16
H16	7087.48	2655.4	nd	nd	nd	9742.88	H26	27252.19	5088.22	nd	nd	nd	32340.41
H17	13145.27	6055.09	nd	nd	nd	19200.36	H27	41919.85	11129.53	nd	nd	nd	53049.38
H18	1343.9	6390.74	nd	nd	nd	7734.64	H28	23880.62	5519.26	373.77	nd	nd	29773.65
E11	8113.63	9701.52	nd	nd	nd	17815.15	E21	38514.92	12935.2	nd	nd	nd	51450.12
E12	9715.76	7778.39	nd	nd	nd	17494.15	E22	34925.36	10146.73	nd	nd	nd	45072.09
E13	8644.42	nd	1300.49	nd	nd	9944.91	E23	62574.99	9191.86	nd	nd	nd	71766.85
E14	10036.08	nd	1612.19	nd	nd	11648.27	E24	58357.97	9424.42	nd	nd	nd	67782.39
E15	11872.44	nd	47636.79	nd	nd	59509.23	E25	30521.56	4583.79	nd	nd	nd	35105.35
E16	12240.11	11077.65	nd	nd	nd	23317.76	E26	36880.2	7183.56	nd	nd	nd	44063.76
E17	10932.49	15701.11	nd	nd	nd	26633.6	E27	33711.43	33711.43	nd	nd	nd	67422.86
E18	13185.96	16432.87	nd	nd	nd	29618.83	E28	39251.79	4666.87	nd	nd	nd	43918.66
G12	7071.67	15594.01	nd	nd	nd	22665.68	G21	39145.16	6669.53	nd	nd	nd	45814.69
G13	12143.49	8996.2	nd	nd	nd	21139.69	G22	26055.86	8243.273	nd	nd	nd	34299.13
G14	10091.89	nd	2663.6	nd	nd	12755.49	G23	73957.76	10667.35	nd	1931.45	nd	84625.11
G15	15489.05	nd	3541.31	nd	nd	19030.36	G24	86098.68	9515.324	nd	1392.26	nd	97006.26
G16	9294.58	15279.37	nd	nd	nd	24573.95	G25	47637.01	7500.662	nd	1020.28	nd	56157.95
G17	1387.66	9938.1	nd	nd	nd	11325.76	G26	58393.23	6454.433	1424.26	2234.46	2526.67	71033.05
G18	16412.88	12338.86	nd	nd	nd	28751.74	G27	33768.14	5164803	1548.96	1182.50	nd	41664.404
							G28	64755.05	4366.905	1837.04	nd	nd	70958.995

^a First number after olive type represents the harvest year and second number shows the harvest time (1st week, 2nd week, etc.), nd: Not detected.

was observed in the 3th week of the maturation for Hurma type for 2011 season and it reached to the highest level at the 6th week of maturation (64.95%) in 2012. In the first harvest year, Erkence type had the highest oleic acid among other types and the amount of this fatty acid varied between the ranges of 68.75–71.83%. However, in the second year its range decreased to 60.5–66.82%. In Gemlik type, similar amounts of oleic acid were detected for both harvest years and it was between the ranges of 61.86–65.87% in the first season and 63.05–66.79% in the second season.

Palmitic acid (C16:0) was the other fatty acid that was detected in higher concentrations. Palmitic acid was found between the ranges of 8.55–18.94% in Gemlik in 2011 season. In Erkence type, its concentration decreased from 13.81% to 11% throughout the ripening period. This decrease had been observed before by other researchers during maturation (Ayton, Mailer, Haigh, Tronson, & Conlan, 2007; Beltrán, del Río, Sánchez, & Martínez, 2004). While palmitic acid content of Hurma was observed between the ranges of 14.17–15.94% in the first harvest year, it changed between 12.55% and 14.28% in the second year.

Higher concentrations of linoleic acid (C18:2n6) were observed in Hurma type in both harvest years. In the first year, it followed an up and down trend during sampling period. Linoleic acid content of Hurma reached to a maximum of 17.19% in the first year and 22.47% in the second year at the end of the maturation. In sweet Dhokar variety, linoleic acid content (22.29%) was also higher compared to other varieties (Rigane, Ayadi, Boukhris, Sayadi, & Bouaziz, 2013). In Gemlik olives, linoleic acid increased at the last 3 weeks of maturation and it reached to the highest concentration (15.97%) at the last week of ripening in the first season. Similar levels of the same fatty acid (11.78–16.11%) were also observed for Gemlik type in the second year. Stearic acid had been found in lower values between 1.66% and 4.39% for all varieties in both harvest years. Then, very low amounts some fatty acids such as palmitoleic (16:1n9), linolenic (18:3n3), behenic (22:0), arachidic (20:0), arachidonic (20:4n6), lignoceric (C24:0) and heptadecanoic (C17:0) acids were observed depending on olive type and harvest year. Cis-10-heptadecenoic acid (C17:1) is detected only in the second harvest year in all olives.

It was observed that oleic to linoleic acid ratio of Hurma olives were generally lower compared to Erkence and Gemlik for both harvest year and Hurma had higher linoleic acid content compared to other types for two years (Fig. 2a). This ratio was also lower for sweet Dhokar olive compared to another regular olive variety (Rigane et al., 2013). Therefore, this might be an indication for increased desaturase activity for the conversion of oleic acid to linoleic acid during debittering. Fatty acid desaturases are the enzymes which catalyse the formation of double bonds. Lower MUFA/PUFA ratio of Hurma compared to Erkence and Gemlik olives for both harvest years also strengthen this hypothesis (Fig. 2b).

To see the differences between varieties, harvest time and harvest year, PCA, was applied to the data. Although R^2 values of the models obtained are not very high PCA plots are still helpful in visualising the differences regarding the olive type, harvest time and harvest year.

For the whole data, a model with 4 principal components and R^2 of 0.611 was obtained (plot is not shown). According to this plot, a differentiation could be observed between the first and the second harvest year olives with respect to their fatty acid profiles. First 5 week samples from Hurma type of 2011 harvest year are placed in the left lower quartile of the plot. The later weeks (6–8 weeks) of the first harvest year are totally separated from the rest of the samples and are on the right upper quartile of the plot. There is a clear separation between the first and the second year samples. Higher concentrations of oleic acid were detected in 2011 season. Therefore, both oleic acid and the first harvest year samples are located on the left side of the loading plot (plot is not shown). Gemlik type for the 2012 season had higher concentrations of palmitic acid. Therefore, both palmitic acid and the second year Gemlik samples are located in the right side of loading plot. Furthermore, palmitic, stearic and linolenic acids which had higher percentages in the second year are also located on the same side in the loading plot with the second year oil samples.

To better understand the differences between each type of olive, PCA was run separately for each harvest year and score and loading plots are shown in Fig. 3. PCA constructed for the first harvest year

Table 3
Fatty acid composition (%) of Hurma (H), Erkençe (E) and Gemlik (G) olive types for two harvest years.

Olive ^a	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1n9c	C18:2n6c	C18:2n6t	C18:3n3	C20:0	C20:1	C20:4n6	C20:5n3	C21:0	C22:0	C24:0	C24:1
H11	14.65	0.78	0.13	nd	2.72	62.80	17.01	0.04	0.30	0.41	0.85	0.04	0.11	nd	nd	0.07	nd
H12	15.33	0.97	0.11	nd	2.67	60.38	18.04	nd	0.28	0.43	0.87	0.03	0.37	0.02	0.11	0.06	0.05
H13	14.17	0.76	0.14	nd	2.96	66.85	19.97	nd	0.33	0.47	0.87	0.04	0.43	0.03	0.26	0.07	nd
H14	13.60	0.67	0.13	nd	3.01	64.20	16.26	0.01	0.32	0.47	0.80	0.04	0.25	0.02	0.13	0.06	nd
H15	15.94	1.19	0.13	nd	3.02	62.80	14.79	nd	0.27	0.46	0.68	0.03	0.27	0.03	0.13	0.06	nd
H16	14.69	0.55	nd	nd	2.63	66.53	15.04	nd	nd	nd	0.56	nd	nd	nd	nd	nd	nd
H17	14.90	0.65	nd	nd	2.86	63.63	16.98	nd	nd	0.37	0.69	nd	nd	nd	nd	nd	nd
H18	15.43	0.66	nd	nd	3.10	62.86	17.19	nd	nd	nd	0.66	nd	nd	nd	nd	nd	nd
E11	13.81	0.58	0.14	nd	2.66	66.50	14.30	0.04	0.31	0.43	0.74	0.05	0.08	nd	0.12	0.06	0.03
E12	13.53	0.59	0.14	nd	2.75	66.38	14.18	nd	0.32	0.45	0.65	0.07	0.19	nd	0.12	0.08	0.21
E13	13.89	0.56	0.16	nd	3.08	65.78	14.37	nd	0.30	0.38	0.63	nd	nd	nd	0.11	0.07	0.46
E14	12.13	0.47	0.14	nd	2.62	68.60	14.15	0.05	0.31	0.41	0.70	0.02	0.12	nd	0.12	0.14	0.04
E15	12.35	0.50	0.15	nd	2.63	69.01	13.44	0.04	0.32	0.41	0.68	0.02	0.06	nd	0.12	0.07	0.04
E16	11.60	0.28	nd	nd	2.67	71.83	12.80	nd	nd	0.30	0.59	nd	nd	nd	nd	nd	nd
E17	11.00	0.34	nd	nd	2.87	72.00	13.00	nd	nd	0.31	0.52	nd	nd	nd	nd	nd	nd
E18	11.81	0.38	0.12	nd	2.33	70.59	13.59	nd	0.21	0.30	0.58	nd	nd	nd	0.09	nd	nd
G12	16.80	1.18	0.13	nd	3.33	61.86	12.87	nd	0.21	0.47	0.60	nd	nd	nd	nd	nd	nd
G13	16.79	1.40	0.13	nd	2.91	63.91	13.43	nd	0.26	0.41	0.59	nd	nd	nd	0.11	nd	nd
G14	15.71	1.29	0.13	nd	2.83	65.87	12.79	nd	0.27	0.43	0.59	nd	nd	nd	0.12	0.10	nd
G15	8.56	1.18	0.12	nd	3.16	65.34	12.79	nd	0.25	0.46	0.55	nd	0.14	nd	0.12	nd	nd
G16	15.56	0.99	0.11	nd	3.60	64.80	13.63	nd	0.19	0.41	0.51	nd	nd	nd	0.08	0.03	nd
G17	15.88	1.24	0.09	nd	2.28	64.59	14.57	nd	0.23	0.33	0.55	nd	nd	nd	0.09	0.03	nd
G18	15.49	0.95	0.01	nd	2.68	63.89	15.97	nd	0.20	0.56	0.56	nd	nd	nd	0.07	0.04	nd
H21	14.28	0.54	0.20	0.16	4.40	61.01	17.64	0.05	0.24	0.37	0.65	0.39	nd	nd	0.14	0.06	nd
H22	13.84	0.55	0.22	0.22	3.71	62.82	17.24	nd	0.23	0.31	0.56	0.16	nd	nd	0.15	nd	nd
H23	13.65	0.53	0.17	0.35	2.76	60.66	2nd	nd	0.21	0.42	0.64	0.23	0.31	nd	0.38	0.06	nd
H24	13.77	0.46	0.28	0.42	2.75	60.30	20.24	nd	0.32	0.32	0.62	0.38	nd	0.01	0.14	0.05	nd
H25	12.80	0.44	0.18	0.27	2.31	63.32	18.36	nd	0.24	0.36	0.69	0.30	nd	0.15	nd	0.21	nd
H26	12.28	0.46	0.20	0.54	3.15	64.95	16.99	nd	0.23	0.33	0.61	0.51	nd	0.07	nd	nd	nd
H27	12.55	0.45	0.30	0.37	2.93	62.99	18.65	nd	0.42	0.33	0.71	0.31	nd	0.25	nd	nd	nd
H28	13.08	0.51	0.15	0.17	2.70	62.45	22.47	nd	0.29	0.28	0.72	0.33	nd	nd	nd	nd	nd
E21	13.68	0.48	0.21	0.20	2.46	62.50	18.28	nd	0.21	0.32	0.58	0.39	nd	nd	nd	nd	nd
E22	15.18	0.59	0.30	0.34	5.22	60.50	16.03	nd	0.47	0.31	0.53	0.54	nd	nd	nd	nd	nd
E23	12.59	0.57	0.31	0.38	2.69	66.82	15.02	nd	0.23	0.34	0.60	0.30	nd	nd	0.28	nd	nd
E24	13.03	0.45	0.60	0.43	2.87	65.10	16.48	nd	0.19	0.33	0.59	0.24	nd	nd	nd	nd	nd
E25	12.96	0.46	0.21	0.23	2.58	66.36	15.70	nd	0.21	0.34	0.54	0.25	nd	nd	0.33	nd	nd
E26	15.29	1.24	nd	nd	2.42	63.24	13.56	nd	0.16	0.34	0.47	0.36	nd	nd	nd	nd	nd
E27	12.60	0.60	nd	0.19	2.47	64.49	16.24	nd	0.27	0.33	0.52	0.30	nd	nd	nd	nd	nd
E28	12.57	0.49	0.20	0.36	2.48	64.17	16.11	nd	0.22	0.33	0.52	0.33	nd	nd	0.36	nd	nd
G21	16.46	1.42	0.23	0.17	3.00	63.49	12.99	nd	0.20	0.34	0.52	0.39	nd	nd	0.79	nd	nd
G22	16.66	1.46	0.10	0.09	2.65	64.37	12.27	nd	0.48	0.33	0.46	0.66	nd	nd	0.48	nd	nd
G23	15.57	1.15	0.14	0.32	2.55	64.92	13.60	nd	0.26	0.40	0.61	0.32	nd	nd	0.16	nd	nd
G24	16.24	1.36	0.13	0.22	1.67	64.30	14.34	nd	0.28	0.33	0.62	0.37	nd	nd	0.15	nd	nd
G25	15.69	1.25	0.26	0.18	2.48	64.01	14.28	nd	0.31	0.36	0.57	0.47	nd	nd	0.14	nd	nd
G26	12.75	0.48	0.14	0.24	2.83	66.79	15.18	nd	0.25	0.37	0.58	0.28	nd	nd	0.11	nd	nd
G27	14.63	1.19	0.20	0.30	2.34	66.63	11.78	nd	0.23	0.35	0.51	0.28	nd	nd	0.13	nd	nd
G28	15.42	1.10	0.12	0.22	2.40	63.05	16.11	nd	0.22	0.36	0.54	0.33	nd	nd	0.17	nd	nd

^a First number after olive type represents the harvest year and second number shows the harvest time (1st week, 2nd week, etc.), nd: Not detected.

consists of 3 principal components with R^2 of 0.679. Gemlik samples are mostly located around the ellipsoid center. There is a clear separation between Hurma and Erkençe and between Gemlik and Erkençe with respect to their fatty acid profiles (Fig. 3a). According to loading plot (Fig. 3b), palmitic, palmitoleic and stearic acids are the fatty acids that cause gathering of Gemlik samples at the center of the score plot. Actually, Gemlik type generally had the higher concentrations of these fatty acids compared to others throughout sampling period. Main fatty acid causing separation of Erkençe from Hurma and Gemlik is oleic acid (Fig. 3b). Oleic acid content of Erkençe (66.38–72.19%) was the highest among the others during the ripening and it increased with ripening and reached to the highest level at the 7th week. Linolenic and gondoic (20:1n9c) acid contents of Erkençe are comparable and higher than Gemlik; therefore, these fatty acids are located between Hurma and Erkençe in the loading plot. First 5 week samples of Hurma are located separately from the last 3 weeks since fatty acids such as eicosapentaenoic (20:5n3) and heneicosanoic (21:0) acids existed in small amounts only in Hurma in early period and disappeared later. Another fatty acid that causes separation of Hurma from the rest is linoleic acid which is observed in this olive in higher amounts (14.79–18.45%).

PCA model for the second harvest year has R^2 of 0.475 and 2 principal components. PCA, in this case, provided better classification for Hurma and Gemlik olive types (Fig. 3c). Other than the first week sample of Erkençe, Hurma and Erkençe samples separated from each other with respect to their fatty acid profiles. Some samples of Erkençe and Gemlik types are located away from their groups but it could be still concluded that there is a differentiation between these types of olives. According to loading plot (Fig. 3d), palmitic and palmitoleic acids are the main parameters causing separation of Gemlik as in the first year. Hurma can be differentiated from other olives mainly owing to its higher content of stearic and linoleic acid content. Hurma also had higher linoleic acid in the first harvest year. Erkençe and Gemlik have comparable levels of oleic acid in the second year; therefore this fatty acid is located in between Erkençe and Gemlik in the loading plot.

As a result, it can be concluded that the fatty acid profile provides differentiation with respect to olive varieties and harvest year. The effect of harvest year is identified as an important factor determining fatty acid profile of olive varieties investigated in this study.

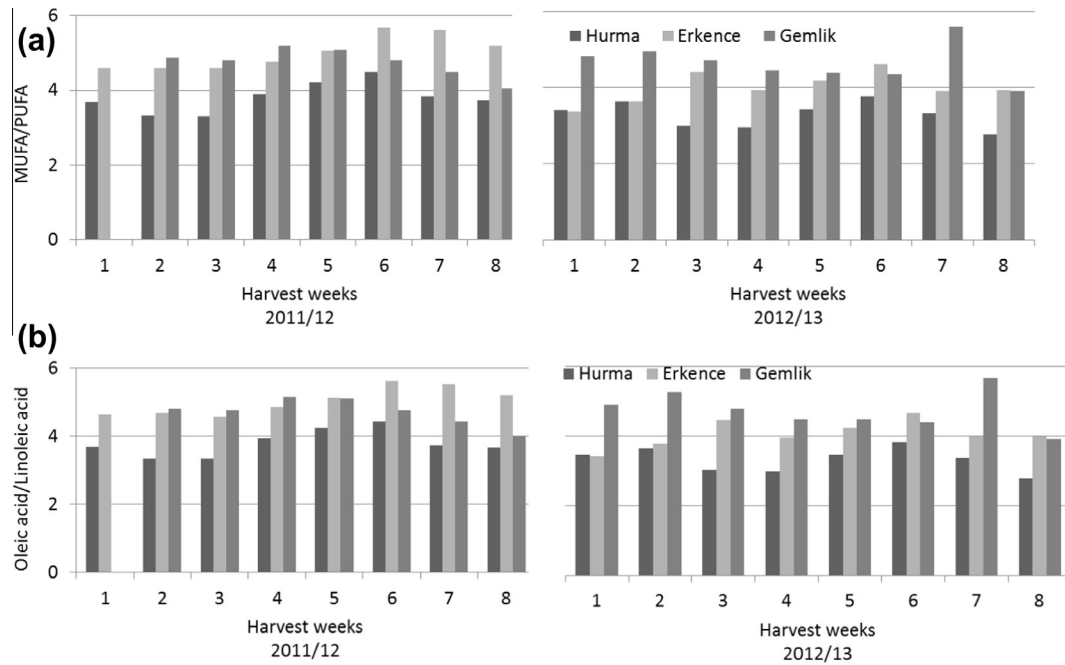


Fig. 2. (a) Oleic acid/linoleic acid and (b) MUFA/PUFA ratios of Hurma, Erkence and Gemlik olives during 8 weeks of maturation for both harvest years.

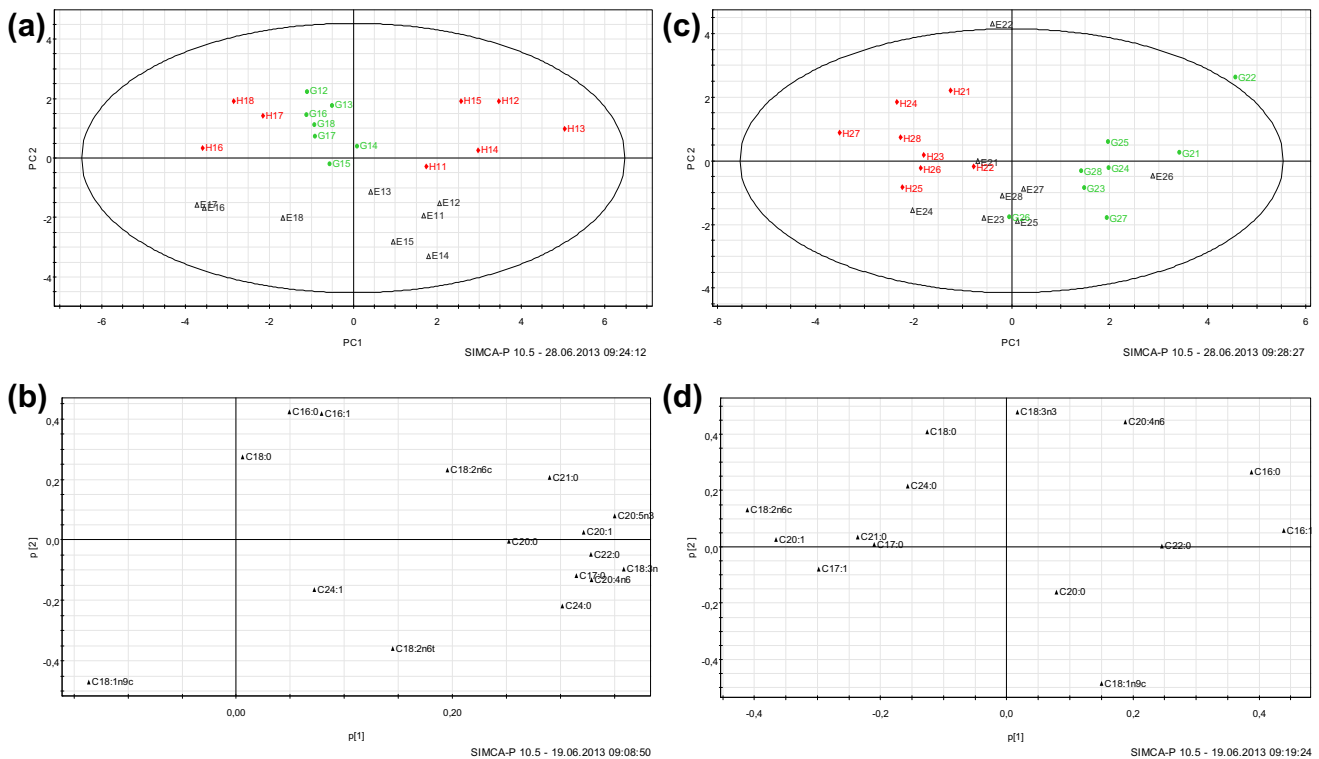


Fig. 3. (a) Score and (b) loading plots obtained with PCA of fatty acid compositions of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for 2011 harvest year; (c) score and (d) loading plots obtained with PCA of fatty acid compositions of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for 2012 harvest year (H: Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week).

4. Conclusions

Glucose and mannitol were detected as the main sugar and polyol for all olive types, respectively and the main organic acids for the investigated olives were the citric and malic acids. Gemlik

variety had the highest citric acid content compared to other types for both harvest years. Total organic acid composition of olives in the second harvest year was significantly higher compared to the first year. There was no differentiation between olive types depending on their organic acid and sugar contents; however,

organic acids provided a clear separation and sugars allowed some differentiation in terms of harvest years. Oleic acid was identified as the main fatty acids for Hurma (naturally debittered Erkençe), Erkençe and Gemlik (regular olive) as expected. Hurma had higher content of linolenic acid in both harvest years compared to other types. Fatty acid profile allowed a differentiation with respect to variety and also harvest year according to PCA. Differences between harvest years with regard to compositional parameters might be related to periodicity of the fruit or climatic conditions. However, more data need to be collected to reach a definite conclusion on this matter. It was hypothesised that the changes during natural debittering of olives could be related to the activities of β -glucosidase and esterase enzymes and cause a decrease in phenolic compounds (Aktas et al., 2014; Jemai et al., 2009). However, as this study shows not only phenolic compounds but also fatty acids are affected from natural debittering process since there is a separation between Erkençe and Hurma olives depending on their fatty acid profiles. This difference could be associated with the esterase activity. In addition, lower oleic acid to linoleic acid and MUFA/PUFA ratios might be an indication of increased desaturase activity. Therefore, to increase the understanding of natural debittering phenomenon further research concentrating on these aspects will be beneficial.

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