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The Effect of Microwave Pre-Treatment on Fatty Acid and Triacylglycerol Composition of Ayvalık and Memecik Olive Oils

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Abstract

The objective of the current study was to assess the influence of microwave (MV) pre-treatment of olive oils. Extraction of olive oil from Ayvalık and Memecik olives was carried out by using an Abencor System (MC2 Ingenierias y Sistemas Sevilla, Spain). In the study, the fatty acid (FA) and triacylglycerol (TAG) composition of Ayvalık and Memecik olive oils were determined. Oils were heated in a microwave oven for 0, 3, 5, 10 and 15 minutes microwave oven MARSXpress,2450 Hz at medium power 800W). FA and TAG analyses were performed with Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) systems, respectively. . At the study ANOVA analysis showed that there were significant differences between the olive oil samples in terms of FA (p<0.05). According to TAG analyses it was determined that Memecik and Ayvalık olive oils significantly affected from the MV heating time (p <0.05).

Keywords: Microwave, Olive oil, TAG, FA, Ayvalık and Memecik.

Mikrodalga Uygulamasının Ayvalık ve Memecik Zeytinyağlarının Yağ Asidi ve Triaçilgliserol Kompozisyonu Üzerine Etkisi

Özet

Bu çalışmanın amacı, mikrodalga ön uygulama işleminin zeytinyağı kalitesi üzerine etkisini değerlendirmektir. Ayvalık ve Memecik zeytinlerinden zeytinyağlarının ekstraksiyonu Abencor Sistemi (MC2 Ingenierias y Sistemas Sevilla, İspanya) kullanılarak gerçekleştirilmiştir. Çalışmada Ayvalık ve Memecik zeytinyağlarının yağ asidi kompozisyonu (YAK) ve triaçilgliserol (TAG) kompozisyonları belirlenmiştir. Yağlar mikrodalga fırınında 0, 3, 5, 10 ve 15 dakika (mikrodalga fırın MARSXpress, 2450 Hz orta güçte 800W) ısıtılmıştır. YAK ve TAG analizleri sırası ile Gaz Kromatografisi ve Yüksek Performanslı Sıvı Kromatografi sistemleriyle gerçekleştirilmiştir. yapılmıştır. ANOVA analizinde, zeytinyağı örnekleri arasında YAK açısından anlamlı farklar olduğu görülmüştür (p <0.05). TAG analizlerine göre ise Memecik ve Ayvalık zeytinyağlarının mikrodalga ısıtma süresinden önemli ölçüde etkilendiği belirlenmiştir (p <0.05).

Anahtar Kelimeler: Mikrodalga, zeytinyağı, TAG, YAK, Ayvalık ve Memecik

Introduction

Turkey is the one of the countries where the olive oil production has increased day by day, making it the largest producer after Spain, Italy and Greece. There are 91 native olive varieties in Turkey. These olives have been received under protection in National Gene Bank in Kemalpasa Production and Experimental Area in İzmir Olive Research Institute. Each olive oil has its own orginal characteristic of chemical composition and aroma. When olives are harvested under ideal conditions and with an optimum maturity index and subjected to appropriate extraction, olive oils are obtained which give a delicate and unique flavor (Kesen et al., 2013).

The Turkey olive grove lands, is dominated by three major varieties, ‘Ayvalık’, ‘Memecik’ and ‘Gemlik’. The origin of Ayvalık olive variety is Edremit district of Balıkesir province. It constitutes 25% of the tree in the Aegean region. The fruit is mature in the early period. The origin of Memecik olive variety is Mugla province. It forms a large part of the tree in the Aegean region. The origin of Gemlik olive variety is Gemlik

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district of Bursa province. It constitutes the vast majority of the tree existence in the Marmara Region. Considering the intensive seedling production in recent years is spreading rapidly in Turkey in general. It is the most important variety considered as a black table. The fat percentage in these three varieties is more than 22% (Kaya, 2017). In Turkey olive oil consumption is approximately 2 liters of this value is increasing with each passing day. This is due to the positive effects of antioxidant compounds in olive oil on human health. The content of these compounds is related to the olive variety, geographical origin, seasonality, agronomic factors and technological conditions in the production of olive oil and olive oil storage conditions.

MV heating is a common and fast producer for food preparation and manufacturing. The effect of MV heating on the micro and macro components in foods can differ significantly from those produced by heating in a conventional oven (Abd El-Moneim Mahmoud et al., 2009). Recent technological developments and modern lifestyles have led to procedural changes in food and cooking process technology. Since the development of the microwave oven, the benefits of convenience, ease of use and the ability to transmit heat quickly have increased, and the use of the MV oven has increased steadily both in domestic and industrial sectors (Yahyaoui et al., 2014). Extra virgin olive oil (EVOO) consumption has increased due to the increased attention of consumers for its health beneficial biological activity on human health, because of presence of chemical composition (Köseoğlu et al., 2016). The content of these compounds is related to the cultivar, geographical origin, seasonality, agronomical and technological conditions of oil production and storage (Guerfel et al., 2012). In the kitchens, olive oil is used in pastry and pie making, deep frying, frying in frying, roasting, MV cooking etc. are known to be used.

Several studies have been performed for determination of the effect of MV heating on the degradation of quality parameters and chemical characteristics of virgin olive oils. Brenes et al. (2002) researched on Spanish cultivars, Chiavaro et al. (2009) studied on commercial categories of olive oil, Abd El-Moneim Mahmoud et al. (2009) evaluated on Italian and Egyptian olive oils, Malheiro et al. (2009) evaluated on Portuguese PDO olive oils, Cossignani et al. (1998) investigated in Italian olive oil, Yahyaoui et al. (2014) researched on Tunisian olive.

According to our knowledge there are no investigation regarding the effect of MV heating on the degradation of FA and TAG composition of Turkish olive oils obtained from Memecik and Ayvalık cultivars. In this sense, the objectives of this study were to determine the effect of different MV (MARSXpress, 2450 Hz at medium power 800W) heating times (0, 3, 5, 10, 15 min) on FA and TAG composition of Memecik and Ayvalık olive oils.

Materials and Methods

Olive oil samples

Ayvalık (Figure 1) and Memecik (Figure 2) olive varieties were harvested from the North Aegean and the South Aegean in 2015/16 crop season, respectively. A representative 10 kg sample of healthy olives were extracted to oil using a laboratory scale mill (Abencor , Mc2 Ingenieria y Sistemas, Sevilla, Spain) equipped with fruit crushing, malaxation and centrifuge parts in the Olive Research Institute of Ministry of Food, Agriculture and Livestock in Izmir/Turkey. The malaxation temperature and duration was 30 ˚C, 30 min respectively. All of the oil samples were filtered and stored in the amber glass bottles without headspace at +4˚C until analysis (Each oil samples contains 250 mL).

Figure 1. Ayvalık variety

Figure 2. Memecik variety

Maturity Index (MI)

The olives were collected at the same maturity index. The olive maturity index was determined according to the method given by International Olive Council (IOC, 2011) based on the evaluation of the olive skin and pulp colors.

MV heating treatment

A laboratory MV oven was used for sample treatment (MARSXpress, CEM, USA). Two aliquots (90 mL) of each oil were placed in opened 150 ml flasks on the rotatory turntable plate of the oven at equal distance and exposed at a frequency of 2450 Hz at medium power (800W). The oil samples were subjected to MV for 0 , 3, 5, 10 and 15 min. The two 90 mL aliquots of each oil were combined after microwaving, in order to obtain a homogeneous sample used for analysis. Unheated olive oil was used as control.

Fatty Acid Composition

FAs of olive oil samples were determined using gas chromatography system (HP 6890, USA) equipped with flame ionization detector (FID) described by International Olive Council (IOC, 2015-a). The capillary column (DB-23, 30m *0.25 mm*film thickness: 0.250 µm, Agilent J&W GC Columns, USA) was used for analyses. The temperature of the detector and injector was set to 250 ˚C. The oven temperature was programmed from 170 to 210˚C with an increment of 2 ˚C/min. The analysis was ended by maintaining the temperature to 210˚C for 10 min.

In a 5 mL screw-top test tube weighed approximately 0.1 g of the oil sample. Added 5 mL of hexane, and shaked. And than 0.5 mL of the methanolic potassium hydroxide solution was added and shaked vigorously for 30 seconds. Later leaved to stratify until the upper solution becomes clear. Than decanted the upper layer containing the methyl esters. The hexane solution (1µl) was injected into the gas chromatograph.

Determination of the Difference Between Actual and Theoretical Content of Triacylglycerols with ECN 42

The analysis of TAGs was performed according to the official liquid chromatographic method described in Regulation EEC/2568/91 of the European Union Commission (Anonymous, 1991). The analysis was performed using an Instrument Agilent 1200 HPLC system (USA). 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). A loop of 100 µL capacity was used in which 0.5 µL of sample was injected. Acetone (63.6 %) / acetonitrile (36.4 %) were mobile phases with a flow rate linear gradient (1.200 mL/ min) under nebulizer gas pressure 2.00 bar for 45 min. Determination of the absolute difference between the experimental values of TAGs with equivalent carbon number 42 ($ECN42_{HPLC}$) obtained by determination in the oil by HPLC and the theoretical value of TAGs with an equivalent carbon number of 42 ($ECN\ 42_{theoretical}$) calculated from the fatty acid composition (IOC, 2010).

Chemical Reagents

Potassium hydroxide, n-hexan (for gas chromatography ECD and FID), methanol (for gas chromatography), acetone (for liquid chromatography) and acetonitrile (for liquid chromatography) were purchased from Merck (Germany).

Statistical Analysis

Results of the analytical determinations were expressed as mean ± standard deviation (SD) of measurements. Statistical differences were calculated with JMP (Version 5.0.1, SAS Institute Inc, Cary, NC, 1989-2007). JMP was used to perform one-way analysis of variance (ANOVA, employing the Student's t-test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Maturity Index (MI)

MI values of the samples were determined for each variety. Maturity index of Memecik and Ayvalık olive cultivars were determined as 2.20 and 2.81, respectively. .

Fatty Acid Composition

FAs of Memecik and Ayvalık olive oils were evaulated as palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic, arachidic, gadoleic, behenic and lignoceric acids. Major fatty acids of the Memecik and Ayvalık olive oils subjected to different MV heating times were shown in Table 1. As can be seen in Table 1, ANOVA analysis showed that there were significant differences between the olive oil samples (p<0.05) in terms of fatty acid composition. Fatty acid percentages were within the range indicated by IOC (2015-b). At the beginning of the MV heating exposure, as the major fatty acid oleic acid percentage of Memecik and Ayvalık olive oil was 67.70 % and 68.81 % respectively. Due to the increase on oleic acid level of Ayvalık olive oil the linoleic and linolenic acid were decreased. This can be explained by the activity of oleate desaturase enzyme that transforms oleic acid to linoleic and catalyse the formation of double bonds (Gutierrez et al., 1999). Abd El-Moneim Mahmoud et al. (2009) reported that MV heating for 30 min affected the extra virgin and refined olive oils fatty acid composition. Farag et al. (1992) found an increase of saturated (16:0) and a decrease of unsaturated (18:1 and 18:2) fatty acid percentage after prolonged MV heating of vegetable oils. Farag et al. (1992) and Yoshida (1993) reported that vegetable oils with high degrees of unsaturated fatty acids were found mfore sensitive to the effect of MV energy. Caponio et al. (2002), Cossignani et al. (1998), Abd El-Moneim Mahmoud et al. (2009) evaluated that a reduction of the unsaturated fractions (MUFA (Monounsaturated Fatty Acid) and PUFA (Polyunsaturated Fatty Acid)) of olive oil occurred with MV treatment.

Table 1. FA (%) of olive oil samples subjected to different MV heating times

Time (min)	(C 16:0) Palmitic	(C 16:1) Palmitoleic	(C 18:0) Stearic	(C 18:1) Oleic	(C 18:2) Linoleic	(C 18:3) Linolenic	SFA	MUFA	PUFA
Memecik (M)									
M0	14.04±0.00^{f}	1.17±0.00^{a}	2.37±0.00^{c}	67.70±0.04^{d}	12.89±0.03^{b}	0.76±0.01^{c}	17.06±0.00^{e}	69.24±0.04^{d}	13.65±0.04^{b}
M3	14.02±0.03^{f}	1.17±0.00^{a}	2.38±0.01^{c}	67.69±0.02^{d}	12.90±0.01^{b}	0.78±0.01^{bc}	17.04±0.03^{e}	69.23±0.03^{d}	13.68±0.00^{b}
M5	14.47±0.06^{b}	1.16±0.01^{b}	2.35±0.01^{d}	67.26±0.08^{f}	12.91±0.03^{ab}	0.80±0.01^{ab}	17.45±0.06^{c}	68.79±0.08^{f}	13.71±0.02^{ab}
M10	14.11±0.00^{ef}	1.16±0.00^{ab}	2.34±0.00^{d}	67.47±0.00^{e}	13.05±0.01^{a}	0.80±0.00^{ab}	17.12±0.02^{de}	69.00±0.01^{e}	13.85±0.01^{a}
M15	14.20±0.01^{de}	1.17±0.01^{ab}	2.33±0.00^{d}	67.37±0.06^{ef}	13.05±0.01^{a}	0.81±0.01^{a}	17.19±0.01^{d}	68.91±0.05^{ef}	13.86±0.01^{a}
Ayvalık (A)									
A0	14.33±0.06^{c}	1.06±0.00^{c}	2.47±0.00^{b}	68.81±0.06^{c}	11.44±0.00^{a}	0.60±0.00^{d}	17.55±0.06^{c}	70.35±0.06^{c}	12.04±0.00^{c}
A3	14.46±0.01^{b}	1.06±0.01^{cd}	2.49±0.02^{b}	68.81±0.18^{c}	11.32±0.18^{c}	0.59±0.01^{d}	17.69±0.00^{b}	70.36±0.19^{c}	11.91±0.19^{c}
A5	13.85±0.05^{g}	1.05±0.01^{d}	2.51±0.00^{a}	69.60±0.09^{a}	11.14±0.03^{d}	0.55±0.01^{e}	17.12±0.04^{de}	71.15±0.08^{a}	11.69±0.04^{c}
A10	14.91±0.14^{a}	1.01±0.01^{f}	2.52±0.01^{a}	68.88±0.06^{c}	10.82±0.06^{f}	0.54±0.01^{e}	18.20±0.13^{a}	70.40±0.09^{c}	11.36±0.06^{e}
A15	14.20±0.01^{cd}	1.02±0.00^{e}	2.51±0.00^{a}	69.30±0.07^{b}	10.99±0.07^{e}	0.54±0.00^{e}	17.56±0.00^{c}	70.84±0.06^{b}	11.53±0.07^{de}

mean±SD, $^{a-g}$ Different letters in the same column concerning all samples significantly different values (p<0.05) M: Memecik; A: Ayvalık; SFA: Saturated Fatty Acid, MUFA: Monounsaturated Fatty Acid, PUFA: Polyunsaturated Fatty Acid

Table 2. Minor FA (%) of olive oil samples subjected to different MV heating times

Time (min)	(C14:0) Miristic	(C 17:0) Margaric	(C 17:1) Margoleic	(C 20:0) Arachidic	(C 20:1) Gadoleic	(C 22:0) Behenic	(C 24:0 Lignoseric
Memecik (M)							
M0	0.02±0.00	0.04±0.00	0.07±0.00	0.42±0.00	0.30±0.00	0.11±0.00	0.06±0.00
M3	0.02±0.00	0.04±0.00	0.07±0.00	0.42±0.00	0.31±0.00	0.12±0.01	0.05±0.01
M5	0.02±0.00	0.04±0.00	0.06±0.00	0.42±0.00	0.31±0.00	0.11±0.01	0.06±0.01
M10	0.02±0.00	0.04±0.00	0.06±0.00	0.42±0.00	0.31±0.00	0.12±0.01	0.07±0.01
M15	0.02±0.00	0.04±0.00	0.06±0.00	0.42±0.00	0.31±0.00	0.14±0.01	0.05±0.01
Ayvalık (A)							
A0	0.02±0.00	0.11±0.00	0.18±0.00	0.43±0.00	0.30±0.00	0.12±0.00	0.07±0.00
A3	0.02±0.00	0.12±0.01	0.19±0.01	0.43±0.00	0.31±0.01	0.12±0.00	0.06±0.01
A5	0.02±0.00	0.13±0.01	0.21±0.00	0.43±0.00	0.30±0.00	0.12±0.01	0.07±0.01
A10	0.02±0.00	0.13±0.00	0.21±0.00	0.43±0.00	0.31±0.02	0.12±0.00	0.07±0.01
A15	0.02±0.00	0.13±0.00	0.23±0.01	0.43±0.00	0.29±0.00	0.15±0.01	0.04±0.00

*mean±SD, M: Memecik; A: Ayvalık

Triacylglcerol (TAG) composition

Olive oil is composed of triacylglycerols (97-98%) and minor compounds (around 2 %). The FAs and triacylglycerol content of virgin olive oil differs considerably depending mainly on latitude, climate, variety and stage of maturity of olives (Sevim et al., 2013). 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 (Galeano Diaz et al., 2005). As seen on the Table 3, the percentage of 1,2,3-trioleylglycerol (OOO) was determined to be the highest. The percentage of OOO was followed by the 2,3-dioleyl-1-palmitoylglycerol (POO) and 2,3-dioleyl-1-linoleyllglycerol (LOO), respectively. The analysis of triacylglycerols allows the identification and the quantification of 19 triacylglycerols. the olive oils that was studied characterized by four primary TAG: LOO, PLO (palmityllinoleylolein), OOO and POO account for more than 85% of the total are of the peaks in profile. LLL (trilinolein), POP (palmityloleylpalmitin) and SOO (stearyldiolein) were also present in low percentages. TAG composition of Memecik and Ayvalık olive oils significantly affected from the MV heating time (p<0.05) (Table 3). Data presented in this study OOO percentage of both olive oils showed a drastically reduction until 3 min of treatment, after 5 min an increase was observed until 10 min heating time. Then a decrease was determined until 15 min for Memecik olive oil and an increase was observed for Ayvalık olive oil until 15 min MV heating time. Albi et al. (1997) reported that thermal degradations of TAG were more abundant than were oxidative ones, especially after MV heating, and this effect was more evident for highly unsaturated vegetable oils. Caponio et al. (2002) mentioned that MV treatment produced a significantly greater amount of polar compounds and of products of triglyceride oxidation and polymerization than traditional heating. The ECN42 difference of Memecik and Ayvalık olive oil did not exceed the limit of 0.2 % determined by European Commission for extra virgin olive oil.

Conclusion

This study was performed to determine the effect of MV heating with different exposure times (0, 3, 5, 10 and 15 min) on fatty acid and triacylglycerol compositions of Memecik and Ayvalık olive oils. FA analyses were performed with Gas Chromatography; TAG analyses were

performed with High Performance Liquid Chromatography systems. All parameters are significantly affected by the time of heating, some of them decreased some of them increased. At the study ANOVA analysis showed that there were significant differences between the olive oil samples (p<0.05) in terms of FAs. According to TAG analyses it was determined that Memecik and Ayvalık olive oils significantly affected from the MV heating time (p<0.05).

Table 3. Triacylglcerol composition (%) of olive oil samples subjected to different MV heating times

	Memecik (M) Time (min)					Ayvalık (A) Time (min)				
	M0	**M3**	**M5**	**M10**	**M15**	**A0**	**A3**	**A5**	**A10**	**A15**
LLL	0.21	0.23	0.23	0.24	0.24	0.12	0.15	0.15	0.15	0.15
OLL	3.47^{ab}	3.09^{bcd}	3.12^{bc}	3.65^{a}	3.41^{ab}	2.83^{cde}	2.56^{e}	2.62^{e}	2.67^{de}	2.62^{e}
LOO+PLnP	15.62^{a}	13.35^{c}	13.43^{c}	15.53^{a}	14.42^{abc}	15.20^{ab}	14.04^{bc}	13.92^{bc}	14.36^{abc}	14.6^{abc}
PLO +SLL	9.19^{c}	10.81^{a}	10.45^{ab}	9.975^{b}	10.13^{b}	8.63^{cd}	8.61^{cd}	8.35^{d}	8.40^{d}	8.29^{d}
OOO	31.38^{d}	24.73^{h}	25.42^{g}	28.76^{e}	26.98^{f}	33.56^{a}	31.21^{d}	31.25^{d}	31.98^{c}	32.64^{b}
SLO+POO	23.23^{e}	29.05^{a}	28.24^{a}	24.62^{cd}	26.67^{b}	23.82^{de}	26.52^{b}	26.34^{b}	25.53^{bc}	24.48^{cd}
POP	4.08^{e}	6.18^{a}	6.04^{a}	4.72^{c}	5.43^{b}	3.63^{f}	4.47^{cd}	4.61^{cd}	4.50^{cd}	4.28^{de}
SOO	3.74^{c}	3.19^{f}	3.20^{f}	3.46^{d}	3.32^{e}	4.12^{a}	4.01^{ab}	3.96^{b}	3.99^{b}	4.06^{ab}
ECN42	0.69^{c}	0.72^{b}	0.73^{b}	0.66^{d}	0.77^{a}	0.51^{e}	0.48^{f}	0.46^{f}	0.48^{f}	0.48^{f}
ECN44	6.73^{ab}	6.33^{b}	6.53^{ab}	7.11^{a}	6.82^{ab}	5.43^{c}	5.01^{c}	5.21^{c}	5.16^{c}	5.15^{c}
ECN46	27.54^{ab}	26.85^{abcd}	27.03^{abc}	28.12^{a}	27.34^{abc}	26.25^{bcde}	25.51^{de}	25.25^{e}	25.32^{e}	25.87^{cde}
ECN 48	59.22^{d}	60.25^{bcd}	60.03^{cd}	58.46^{d}	59.43^{cd}	61.56^{abc}	62.73^{a}	62.76^{a}	62.79^{a}	62.25^{ab}
ECN 50	4.87^{bc}	4.88^{b}	4.80^{bc}	4.77^{c}	4.77^{c}	5.30^{a}	5.32^{a}	5.34^{a}	5.29^{a}	5.29^{a}
ECN 42 Difference	0.12^{b}	0.11^{bc}	0.10^{bcd}	0.19^{a}	0.08^{de}	0.07^{e}	0.09^{cde}	0.07^{e}	0.01^{f}	0.03^{f}

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

[a-h] Different letters in the same line concerning all samples significantly different values (p<0.05)
M: Memecik; A: Ayvalık; LLL (trilinolein), OOO (triolein), LOO (linoleyldiolein), SLO+POO (palmityldiolein), SOO (stearyldiolein), POP (palmityloleylpalmitin), PLO+SLL (palmityllinoleylolein)

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