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EFFECTS OF PERIODS AND ALTITUDES ON THE PHENOLIC COMPOUNDS AND OIL CONTENTS OF OLIVES, cv. AYVALIK**Serhat USANMAZ¹, Ferda ÖZTÜRKLER², Murat HELVACI¹, Turgut ALAS¹, İbrahim KAHRAMANOĞLU¹, M. Atilla AŞKIN^{1*}**¹European University of Lefke, Faculty of Agricultural Sciences and Technologies, Lefke, Northern Cyprus, via Mersin 10 Turkey²Süleyman Demirel University, Faculty of Agriculture, Isparta, Turkey

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Abstract

This study aimed to investigate the influence of periods and altitudes on the phenolic compounds of olive fruits and leaves; and oil contents of olives cv. Ayvalık located in Balıkesir province in Turkey. Surveys conducted in 4 different periods (July 2009, October 2009, January 2010 and April 2010) on 3 different altitudes (0 m, 300 m and 600 m from sea level). Fruit and leaf samples were collected with 3 replications for each periods & altitudes interaction. Phenolic compounds of both leaf and fruit samples were then investigated. Furthermore, acid compounds of fruits, fruit moisture contents (%), leaf moisture contents (%), pulp contents (%), fruit weight (kg/1000 fruits) and oil contents (%) were also tested for each samples. According to the results obtained, among the surveyed altitudes, highest fruit moisture content was measured at 300 m with 43.54%, whereas among the survey periods, the highest moisture content was found in October with 49.34%. Not surprisingly, among the tested period & altitude interactions, October 300 m was found to have highest fruit moisture content with 50.53% and followed by October 600 m with 49.37%. Highest leaf moisture content was measured from 0 and 300 m altitudes with 48.43% and 48.21%, respectively, where the April period was found to have highest leaf moisture content among the periods with 48.09%. Pulp content (%) results of present study are in accordance with the fruit moisture content (%) results. Fruit weight (kg/1000 fruits) was found to be highest in October and followed by July and January with 3.30 kg, 2.93 kg and 2.71 kg, respectively. Oil contents (%) have found to have clear differences among periods with lowest 1.52% in July and 28.89% in January. Altitude was also found to influence oil content where it was found that highest oil content was measured at highest altitude. Among the tested acid, Oleic acid was found to be highest and is followed by Palmitic acid, Linoleic acid, Stearic acid and Palmitoleic acid. Periods and altitudes are found to influence acid contents. Highest Oleic acid content was measured at 300 m altitude where the highest contents of other acids were measured at 600 m altitudes. Similarly, with the acid contents, phenolic compounds in leaf and fruit samples were found to be influenced by periods and altitudes. Highest Oleuropein content ($\mu\text{g g}^{-1}$) in fruits measured in July at 0 m altitude with 825.50 where the lowest was measured at October 0 m 54.10. July period and 0 m altitude were found to have highest Oleuropein contents. On the other hand, no statistical difference was determined among the Oleuropein contents ($\mu\text{g g}^{-1}$) in leaves among the tested altitudes. Furthermore, January period was found to have highest Oleuropein contents among the tested periods which has the lowest in fruits. Results may suggest that, as fruits ripen, Oleuropein accumulation in fruits decrease whereas it increases in leaves.

Key words: fruit moisture, pulp contents, Oleuropein, Oleic acid, oil contents.**INTRODUCTION**

Olive tree (*Olea europaea* L.) is a member of Oleaceae family which is reported to be originated in Mesopotamia (including Southeast Anatolia). Today, olive and olive oil productions are important elements of Mediterranean cuisine (Delgado-Pertinhez et al., 2000; Owen et al., 2000). Olive fruit has been traditionally known to be beneficial for human health. Olives are rarely used in their natural form because of severe bitterness. However, they are either consumed as oil or table olives. Health benefits of olive oil have been associated with its well-balanced fatty acid composition (mainly oleic acid) and with the presence of vitamins and natural antioxidants (Medeiros, 2001). Olive oil is the primary source of fat in the Mediterranean diet which is associated with a low mortality for cardiovascular disease. But, Covas (2007) reported that the benefits of olive oil consumption are beyond a mere reduction of the LDL

cholesterol. According to Covas (2007), olive oil rich diets reduce the insulin requirements and decrease plasma concentration of glucose and insulin in type 2 diabetic patients. Olive and olive oil continues to have an important place in today's diet. In recent years, the consumption of olive oil and table olives has steadily increased in all over the world due to its beneficial effects on the protection against cardiovascular and neurodegenerative diseases, and its potential role in reducing the potential risk of several cancers (Pérez-Jiménez et al., 2007; Menendez et al., 2006).

According to FAO, 10.650.068 ha worldwide are olives orchards in 2016, with Spain having the highest harvested area with 2.573.473 ha (FAOSTAT, 2018). Total harvested area was 3.446.687 ha in 1965 which means about 3 fold increase in 50 years. Turkey had a total of 845.542 da olive production are in 2016. Total production was 19.267.493 tonnes in 2013 which equals a yield of about 1.8 tonnes per ha. Olive tree is drought tolerant because of its specific morphological mechanisms like extensive root system and stomata undersides of the leaves (Orgaz and Fereres, 1997). Despite these characteristics, lack of soil water affects fruit trees and yields too. Olive is native to the Mediterranean and well adapted to the seasonally dry, semiarid climate (Meikle 1977). The chemical composition and quality of olive oil are highly influenced by geographical production area (Temine et al, 2006; Örmeci and Aşkın, 2017), production systems (Patumi, 1999) and variety and oil extraction process (Baconi et al., 2007; Okatan et al., 2017). Since the beginning of 21st century, consumer's awareness on the active phenolic compounds is increasing (Bayçın et al., 2007). Due to that, not only the olive fruits but also the olive leaves had strong attention of the scientists with mainly the sectors of medicine, cosmetics and food (Meirinhos et al., 2005). Most of the phenolic compounds of olives were reported to be beneficial against some diseases and have anti-fungal activity (Gülcü and Demirci, 2008; Türköz et al., 2008). Around 100 phenolic compounds were determined where Oleuropein was reported to be most valuable of them (Benavente-Garcia et al., 2000; Savournin et al., 2001). Other important phenolic compounds of olives are Luteolin, Apigenin, Clorogenic acid, p-cumaric acid, Hydroxytyrosol, Gallic acid, Ferulic acid, Benzoic acid and Rutin trihydrate (Gülcü and Demirci, 2008). These phenolic compounds were reported to vary according to the cultural practices, production region, harvest time and altitude (Boskou, 1996; Mousa and Gerasopoulos, 1996; Skevin et al., 2003). In the light of these information, present study aimed to investigate the influence of periods and altitudes on the phenolic compounds of olive fruits and leaves; and oil contents of olives cv. Ayvalık located in Balıkesir province in Turkey.

MATERIALS AND METHODS

Present study conducted in Balıkesir region in Turkey. Surveys conducted in 4 different periods (July 2009, October 2009, January 2010 and April 2010) on 3 different altitudes (0 m, 300 m and 600 m from sea level). Periods for fruits corresponds to the fruit ripening periods as July: green, October: semi-black and January: black. Fruit and leaf samples were collected with 3 replications for each periods & altitudes interaction. Phenolic compounds of both leaf and fruit samples were then investigated. Furthermore, acid compounds of fruits, fruit moisture contents (%), leaf moisture contents (%), pulp contents (%), fruit weight (kg/1000 fruits) and oil contents (%) were also tested for each samples. Samples were stored in locked nylon bags under -18 C until analysis.

Moisture content of both fruits and leaves were determined suddenly after harvest according to TSE Incubator technique reported by Anonim (2001). Olives were crushed in mortar. Thus, 5 g was sampled and weighted. Next, samples were dried under incubator at 105±1 C and weighted again to determine moisture content. Oil contents were determined according to the AOCS (1993) by using Soxhlet Extraction machine (Büchi Universal Extraction System B-811, Germany). Determination of the oil acid composition of the samples from different period and altitude was carried with GC/MS according to the method of Marquard (1987). Finally, phenolic contents of olive fruit and leaf samples were performed with HPLC according to Capanio et al. (1999).

Raw data from the experiments were subjected to Analysis of Variance and mean separations were done with Duncan's multiple range test by using SPSS 22.0.

RESULTS AND DISCUSSIONS

Fruit moisture contents of the olives were found to be highest in October at 300 m altitude with 50.53% moisture. It was observed that fruit moisture content is increasing from July to October and then it was declined (Table 1). No correlation has been observed between fruit moisture and altitude where fruit moisture increased when altitude increased until 300 m and then it decreased. On the contrary to fruit moisture contents, leaf moisture showed some changes. Leaf moisture content found to decrease as fruits ripen and after harvest too. Highest leaf moisture content was observed in April. Furthermore, altitude was also found to have significant effect on the leaf moisture content where it decreased as altitude increases.

Pulp contents of the olives were also found to be different for periods and altitudes. It was determined that pulp content is increasing from July to October and then it decreases until January. The peak period was found to be October. Similar phenomena found for altitude, where it decreases until 300 m altitude and then it decreases. Similar results were found for fruit weight. The highest fruit weight (kg/1000 units) was measured in October with 3.30 kg and is followed by July and January with 2.93 kg and 2.71 kg, respectively. On the other hand, results showed that, oil content of the fruits is increasing as the fruits mature. The highest oil content was measured in January with 24.89% and is followed by October and July with 14.76% and 1.52%, respectively. It was also found that altitude has a slight effect on oil content and it increases as the altitude increase.

Table 1. Changes in some characteristics of fruit and leaf samples according to periods and altitudes

Periods / Altitudes	Fruit Moisture Content (%)	Leaf Moisture Content (%)	Pulp Content (%)	Fruit weight (kg /1000 units)	Oil Content (%)
July - 0 m	37.97 b	45.87 cd	71.87 c	2.61 e	1.27 e
July - 300 m	40.37 b	48.57 b	76.87 b	2.62 e	1.45 e
July - 600 m	48.10 a	44.87 cd	79.33 ab	3.55 b	1.83 e
October - 0 m	48.13 a	49.27 b	79.67 ab	2.89 d	13.83 d
October - 300 m	50.53 a	46.50 c	80.53 a	3.05 d	14.87 cd
October - 600 m	49.37 a	44.17 d	81.60 a	3.96 a	15.57 c
January - 0 m	36.83 b	49.57 b	72.13 c	3.13 cd	24.17 b
January - 300 m	39.73 b	45.37 cd	79.13 ab	3.34 bc	24.37 b
January - 600 m	25.23 c	46.27 c	67.37 d	1.65 f	26.13 a
April - 0 m		49.03 b			
April - 300 m		52.40 a			
April - 600 m		42.83 e			
July	42.14 b	46.43 b	76.02 b	2.93 b	1.52 c
October	49.34 a	46.64 b	80.60 a	3.30 a	14.76 b
January	33.93 c	47.07 b	72.88 c	2.71 c	24.89 a
April		48.09 a			
Altitude 0 m	40.98 b	48.43 a	74.56 b	2.88 a	13.09 b
Altitude 300 m	43.54 a	48.21 a	78.84 a	3.00 ab	13.56 b
Altitude 600 m	40.90 b	44.53 b	76.10 b	3.06 a	14.51 a

In present study, oleic (C18:1), palmitic (C16:0) and linoleic (C18:2) acids were found as major fatty acids in olive oil samples (Table 2.).

Table 2. Fatty acid composition (%) of olive oils extracted from olives harvested in different periods from different altitudes

Periods / Altitudes	Palmitic acid % (C16:0)	Palmitoleic acid % (C16:1)	Stearic acid % (C18:0)	Oleic acid % (C18:1)	Linoleic acid % (C18:2)
July - 0 m	21.17 ab	0.93 bc	2.68 b	66.37 a	9.08 f
July - 300 m	22.54 a	1.09 b	2.15 d	64.66 b	9.57 e
July - 600 m	21.15 ab	1.30 a	2.50 bc	64.83 b	10.23 d
October - 0 m	22.51 a	1.41 a	2.25 cd	60.99 d	12.84 b
October - 300 m	20.34 b	0.94 bc	2.84 b	65.26 ab	10.64 d
October - 600 m	20.57 b	1.34 a	2.49 bc	63.08 c	12.52 b
January - 0 m	20.25 b	0.84 c	3.70 a	60.89 d	14.09 a
January - 300 m	18.63 c	0.94 bc	3.77 a	64.81 b	11.87 c
January - 600 m	22.78 a	1.01 bc	3.52 a	58.33 e	14.00 a
July	21.62 a	1.10 b	2.44 b	65.28 a	9.63 c
October	21.14 ab	1.23 a	2.53 b	63.11 b	12.00 b
January	20.55 b	0.93 c	3.66 a	61.34 c	13.32 a
Altitude 0 m	21.31 ab	1.06 b	2.88 a	62.75 b	12.00 a
Altitude 300 m	20.50 b	0.99 b	2.92 a	64.91 a	10.69 b
Altitude 600 m	21.50 a	1.21 a	2.84 a	62.08 b	12.25 a

These results are in accordance with the findings of Allalout et al. (2009) and Toker et al. (2015). Other important acids were found to be as palmitoleic (C16:1) and stearic (C18:0) acids. Fatty acids have important effects on oxidative stability and are also important due to their nutritional value (Boskou, 1996). It was found that both harvesting period and altitude has a significant effect on the fatty acid composition of the olive fruits. Highest oleic acid content was measured in July at 0 m altitude. Among the harvesting periods, July was found to have highest (65.28%) and is followed by October (63.11) and January (61.34). These results showed that as fruits ripen, oleic acid content is decreasing. Altitude was also found to significantly influence oleic acid content and samples from 300 m altitude was measured to have highest oleic acid content. The second highest fatty acid content of the samples, the palmitic acid, was also found to be similarly affected by harvesting period, but the effect of altitude was reverse. Highest palmitic acid contents were measured at 0 m and 600 m altitude and the lowest from 300 m altitude.

The evaluation of total phenols in olive oil samples from different ripening period and altitude are given in Table 3. Total phenol contents showed different responses to changes in ripening period and altitude. Similarly Jolayemi et al. (2016) reported that phenolic compounds of the olive oils were significantly affected by harvest time. For example gallic acid and vanillic acid contents of the fruits increased as fruits ripen, while on the other hand hydroxytyrosol, sinapinic acid, rutin trihydrate and oleuropein decreased. Tyrosol, p-coumaric acid, quercetin dehydrate and luteolin contents of the fruits increased during ripening, but after October, they started to decrease. Higher amount of oleuropein derivatives (sum of tyrosol and hydroxytyrosol) in oils were determined in early harvested fruits in September. These results are in accordance with the findings of Jolayemi et al. (2016). Luteolin was also significantly affected by all factors and found to be lowest in October and increased in January, in accordance with the findings of Boselli et al. (2009). The effect of altitudes on the phenolic profiles of the olive oils is differing than the ripening period. From 0 m to 300 m altitude, almost all phenolic compounds showed slight decrease, and it decreased at the samples collected from 600 m.

Total phenols in olive leaf samples from different ripening period and altitude are given in Table 4. Total phenol contents in leaves showed different responses to changes in ripening period and altitude as in olive oils. Contrary to the olive oils, oleuropein in leaves showed increase as time passes and fruits ripen. The highest oleuropein content was determined in January as $586.92 \mu\text{g g}^{-1}$. However, similarly to the contents in olive oil samples, oleuropein content in leaves was measured lowest at 300 m altitude and higher at 0 m and 600 m altitudes. Sinapinic acid content in leaves also showed similar responses to the harvesting period while it increased during time and then decreased after January (full fruit maturity). However, the response of sinapinic acid content in leaves is reverse to the response in olive oils. The effects of altitude on the phenolic profiles of the olive leaf samples were also found meaningful for some phenolic compounds. Gallic acid, tyrosol and benzoic acid showed increase while altitude increase and sinapinic acid and rutin trihydrate showed decrease as altitude increase.

Table 3. Phenolic profiles (mg/kg) of Ayvalik olive oils at three harvest times (July, October and January) and three altitudes (0 m, 300 m, 600 m)

Periods / Altitudes	Gallic acid	Hydroxytyrosol	Tyrosol	Vanillic acid	p-coumaric acid	Ferulic acid	Sinapinic acid	Benzoic acid	Rutin trihydrate
July - 0 m	2.40 a	27.60 b	10.20 b		4.50 a	7.10	11.10 a		11.27 a
July - 300 m	1.07 cd	40.30 b	3.17 c-e	3.50 b	1.80 b				12.27 a
July - 600 m	0.70 de	114.10 a	10.57 b		2.07 b				14.60 a
October - 0 m	0.60 de	70.80 ab	1.87 de	3.47 b	0.57 d				4.17 b
October - 300 m	0.30 e	34.00 b	1.50 e		1.60 bc		2.37 c	3.37 b	3.27 b
October - 600 m	0.17 e	76.67 ab	6.40 bc		0.87 cd		7.17 b	7.37 a	10.50 a
January - 0 m	2.07 ab	64.20 ab	9.30 b	13.80 a	4.07 a		7.07 b	3.47 b	4.87 b
January - 300 m	1.60 bc	27.27 b	7.47 bc	7.67 b	2.27 b		2.00 c	2.30 b	4.77 b
January - 600 m	2.70 a	38.47 b	15.37 a	15.60 a	2.40 b		1.57 a		3.20 b
July	1.39 b	60.67 a	7.98 b	3.50 b	2.79 a	7.10	11.10 a		12.71 a
October	0.36 c	60.49 a	3.26 c	3.47 b	1.01 b		4.77 b	5.37	5.98 b
January	2.12 a	43.31 a	10.71a	12.36 a	2.91 a		3.54 b	2.88	4.28 b
Altitude 0 m	1.69 a	54.20 ab	7.12 b	8.63 b	3.04 a	7.10	9.08 a	3.47 b	6.77 a
Altitude 300 m	0.99 b	33.86 b	4.04 c	5.58 b	1.89 b		2.18 c	2.83 b	6.77 a
Altitude 600 m	1.19 b	76.41 a	10.78 a	15.60 a	1.78 b		4.37 b	7.37 a	9.43 a

Table 4. Phenolic profiles ($\mu\text{g g}^{-1}$) of Ayvalik olive tree leaves at three harvest times (July, October and January) and three altitudes (0

Periods / Altitudes	Gallic acid	Hydroxytyrosol	Tyrosol	Vanillic acid	P-coumaric acid	Ferulic acid	Sinapinic acid	Benzoic acid	R
July - 0 m	1.17 c	79.87 a	4.30 e	8.70	3.00		11.10 b	13.87 e	7
July - 300 m	1.97 b	8.97 c	14.37ab					27.90 a	9
July - 600 m	3.00 a	19.07bc	15.57 a				2.17 c	28.67 a	7
October - 0 m		24.87 b	10.70 d				17.57a	23.30 bc	9
October - 300 m		17.50 bc	11.30 cd				5.87 c	19.87 cd	4
October - 600 m		19.37 bc	11.90 cd				4.50 c	21.50 cd	2
January - 0 m		25.10 b	10.10 d				16.27 ab	18.27 d	3
January - 300 m		18.37 bc	12.20 cd				15.17 ab	23.70 bc	5
January - 600 m		26.17 b	13.10 a-c				4.80 c	26.40 ab	5
April - 0 m		18.97 bc					3.17 c	11.07 e	2
April - 300 m		14.67 bc	14.37 ab		9.70	4.60	2.00 c		4
April - 600 m	1.67 bc	12.10 c	12.47 a-c				1.40 c	28.20 a	2
July	1.93	35.97 a	11.41 b	8.70	3.00		6.63 b	23.48 a	7
October		20.58 bc	11.30 b				9.31 ab	21.56 ab	5
January		23.21 b	11.80 ab				12.08 a	22.79 a	5
April	1.67	15.24 c	13.42 a		9.70	4.60	2.19 c	19.63 b	3
Altitude 0 m	1.17 b	37.20 a	8.37 b	8.70	3.00		12.03 a	16.63 c	5
Altitude 300 m	1.97 a	14.88 b	13.06a		9.70	4.60	7.68 b	23.82 b	5
Altitude 600 m	2.20 a	19.18 b	13.26 a				3.22 c	26.19 a	4

CONCLUSIONS

Results showed that both ripening period and altitude has a significant influence on the acid contents and phenol profiles of olive oils and leaves of Ayvalık variety. Highest leaf moisture content was measured from 0 and 300 m altitudes with 48.43% and 48.21%, respectively, where the April period was found to have highest leaf moisture content among the periods with 48.09%. Fruit weight (kg/1000 fruits) was found to be highest in October and followed by July and January with 3.30 kg, 2.93 kg and 2.71 kg, respectively. Oil contents (%) have found to have clear differences among periods with lowest 1.52% in July and 28.89% in January. This result showed that oil content is significantly increasing as fruits ripen. Among the tested acid, Oleic acid was found to be highest and is followed by Palmitic acid, Linoleic acid, Stearic acid and Palmitoleic acid. Phenolic compounds in leaf and fruit samples were also found to be influenced by periods and altitudes. Highest oleuropein content ($\mu\text{g g}^{-1}$) in fruits measured in July at 0 m altitude with 825.50 where the lowest was measured at October 0 m as 54.10 $\mu\text{g g}^{-1}$. July period and 0 m altitude were found to have highest oleuropein contents. Results may suggest that, as fruits ripen, oleuropein accumulation in fruits decrease whereas it increases in leaves.

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