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Chemical characterization and storage stability of extra virgin olive oil extracted from Derik Halhalı cultivar

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ABSTRACT

Turkish olive cultivar known as “Halhalı” that is locally grown in Mardin (Derik) province, situated in the southeast Anatolia, was used for virgin olive oil (VOO) production. Halhalı olive was processed in the “Mobile Olive Oil Processing Unit” (TEM Oliomio 500-2GV, Italy) designed as the first mobile olive mill in Turkey. Some chemical and physical properties (colour, UV absorbance values, free fatty acid content, peroxide value, phenolic and tocopherol profiles) were determined and monitored during one year of storing in the dark at room temperature once in every three months. Results showed that up to the eighth month of storage, free fatty acid content, peroxide and UV-absorption values (K232 and K232 values) did not exceed the limits reported by International Olive Council (IOC) and olive oils were categorized as Extra Virgin Olive Oil (EVOO). Colour changed from green to yellow and UV absorbance values altered during storage. Total phenol and vitamin E (α-tocopherol) contents decreased by 18% and 16.38%, respectively. Luteolin and apigenin were the most abundant phenolics and their contents decreased up to 22% and 28% during storing, respectively.

Abbreviation: EVOO= Extra Virgin Olive Oil, IOC= International Olive Council, VOO= Virgin Olive Oil.

Introduction

Turkey is second in the number of olive trees (190 million) after Spain. Southeastern Anatolia located in upper Mesopotamia is the origin of olive trees, there are 91 registered olive cultivars in National Olive Collection in Olive Research Institute, Izmir. Derik Halhalı is one of the six olive cultivars (Derik Halhalı, Belluti, Hursuki, Mavi, Melkabazi and Zoncuk) cultivated in Derik district. The total number of the olive trees are 150,000 with the 8% of total olive trees, but 80% is Derik Halhalı. Halhalı fruits are medium sized with large seeds and oil content is between 21-27%. It has moderate yield with high periodicity and it is usually processed as table olive (Anonymous, 2016).

Olive oil differs among other edible oils due to high oleic acid, phenolics, vitamins, and other minor compounds. For this reason, it has unique nutritional value and sensory properties which do not exist in others. Virgin olive oil (VOO) is obtained from olive fruits by using only physical procedures, therefore it is ready to consume without refining processes. Thus, while other refined edible fats and oils might have trans fatty acids and heat contaminates such as 3-MCPD, 2-MCPD and glycidyl esters which are a risk factor in terms of food safety, olive oil does not contain these substances. Chemical composition varies in a broad range depending on cultivar, ripeness degree, ecologic conditions, growing region, processing techniques and storage. Free fatty acid content, peroxide value and oxidative rancidity increases, while total polyphenols, tocopherols and

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sterols decrease with increasing storage time. Up to 73% of total polyphenols decreased and this decrease was significantly higher in the samples with larger initial phenol contents (Mulinacci et al., 2013). Important losses of pigments (chlorophyll and carotenoids) contents of the commercial Arbequina virgin olive oil were reported after 12 months of storage (Morelló et al., 2004). Storage conditions is another important factor in olive oil quality. Storage of olive oil under nitrogen pressure in a dark place at room temperature (25-30 °C or lower) increases shelf life (Boskou et al., 2006). No change was observed in aromatic hydrocarbons of frozen samples during 12 months' storage (Mulinacci et al., 2013). Most of the α -tocopherol (79%) disappeared in four months, whereas <45% of the phenols were lost under diffused light during storage (Okogeri and Tasioula-Margari, 2002). EVOO with high antioxidant contents was still "excellent" after 240 days of storage at 40 °C (Lavelli, 2006). Psomiadou et al. (2000) suggested good handling is quite important for retaining high α -tocopherol levels of Greek VOO under the domestic condition for two years.

In this research, a mobile olive oil processing unit (MOOPU) was designed and used for olive oil production from "Halhali" cultivar. MOOPU was transferred into the orchard located in Mardin (Derik) region of Turkey in 2015-2016 harvest season. Therefore, it was possible to produce premium olive oil at optimum conditions within two hours after harvest. After filtration, olive oils were packaged in dark bottles with nitrogen gas in order to remove oxygen and stored one year at room temperature. Quality parameters were monitored during storage every three months.

Methods and Materials

Production of extra virgin olive oil (EVOO)

A "Mobile Olive Oil Processing Unit" (MOOPU) with state-of-the-art Oliomio equipment was designed in order to produce VOO. A special container was constructed and equipped with a knife crusher and a two-phase horizontal decanter (Oliomio D500, Italy). The mobile unit is an articulated lorry with a special semi-trailer measuring 2438 x 12,192 x 2896 mm which is divided into three separate sections. The first section is an olive accepting unit including: bunker, leaf removers, washer and crusher units of the system. The second section is processing unit including malaxer, decanter, filter and bag-in-box filling machine. The third section is support unit placed comprising a power plant and water supply tank. Processing unit was equipped by an air conditioner, isolation and filter ventilation systems and protected for temperature changes, dust and odour. MOOPU carried by a trailer truck to orchards in 2015-

2016 season. Olive fruits were harvested by hand picking in the early harvest period and processed to "cold press" VOO in the MOOPU in a few hours. Olive paste was prepared after crushing by a hammer mill and the paste was mixed in the malaxer at 27 °C for 15 min (Cold press). After decantation, VOO was filtered and filled in 250 mL amber glass bottles (headspace: 4 cm) by nitrogen gas. The bottles were stored at room temperature (18-24 °C) up to 12 months.

Chemical analysis

Chemical analysis including free fatty acid content, peroxide value and moisture content (MC) was performed according to the EEC 2568/91, AOCS Cd 8-53 methods, and ISO 662, respectively. Colour values (L , a , b values) were measured by spectrophotometer (Minolta, CM-3600d, Japan). L (lightness), b (yellowness), and a (redness) values were determined. UV-absorbance was measured according to the IOC method COI/T.20/Doc. No 19/Rev. 3. UV-absorbance was measured at 232, 266, 270 and 274 nm by using UV-spectrophotometer (Agilent 8453, USA). ΔK values were calculated with the following formula:

$$\Delta K = K_{270} - [(K_{266} + K_{274}) / 2]$$

Total phenolic content

The polar fraction was extracted and used for total phenolic and phenolic composition analyses. Olive oil sample (2.5 g) was weighed into a falcon tube. Hexane (6 mL) was added and shaken for 1 min. This solution was filtered through a solid phase extraction (SPE) cartridge (Superclean LC-Diol, USA) and collected in a glass tube. Then, hexane (6 mL) and 4 mL hexane:ethyl acetate (85:15, v/v) were passed through the SPE cartridge, respectively. The cartridge was washed with methanol:deionized water solution (1:1 v/v). The phenolic extract was evaporated (UniEquip Univapo 100 ECH, Canada). After the addition of 2 mL methanol:deionized water solution (1:1 v/v) the tubes were vortexed for 30 seconds. For the determination of total phenols Folin & Ciocalteu method was used and the results were expressed in terms of gallic acid equivalent (Romani et al., 2007; Inarejos-Garcia et al., 2009).

Phenolic composition

Ultra High-Performance Liquid Chromatography (UHPLC, Thermo Scientific Dionex Ultimate 3000, USA) and C18 column (4.6 mm inner diameter x 250 mm length and 5 μ m particle size, Acclaim 120, Thermo Scientific) were used for determination of phenolic profile. Prepared phenolic extract (1 mL) for total

phenolic content was passed through 0.45 μm microfilter (Merck, PVDF, Millipore Millex-HV, Germany) and poured into an amber vial. The column temperature was fixed at 30 °C and acetic acid:deionized water (1:1) (A), methanol (B), acetonitril (C) were used in a gradient flow program as the mobile phase. In the gradient program, eluents were 2.5% B, 2.5% C, and 95% A solution up to 60 min. Flow rate was 1 mL/min and diode array detector (DAD) was set in 280 nm, 320 nm and 335 nm. Apigenin, cafeic acid, gallic acid, luteolin, *m*-cumaric acid, *p*-coumaric acid, oleuropein, syringic acid, *trans*-furanic acid, vanilic acid, vanillin, tyrosol, 3-hydroxy tyrosol, 3,4-dihydroxy benzoic acid, 4-hydroxy benzoic acid, and 4-hydroxy phenyl acetic acid were purchased from Sigma-Aldrich Co. (Germany) and used as phenolic standards.

Tocopherol composition

Tocopherol composition was determined by using AOCS Official Method Ce 8-89, 1997. 2 g EVOO sample was weighed into a 25 mL volumetric flask. A small quantity of hexane was used for dissolving of oil, and then the flask was made up to volume. The solution was passed from syringe filter (0.45 μm) (PVDF, Millipore Millex-HV) into the HPLC vial. The samples (20 μL) injected to UHPLC. LiChrosorb SI 60-5 column (4.6 mm I.D \times 250 mm length and 5 μm particle size) was used for analysis. The column temperature was fixed at 30 °C during the process. The flow rate of analysis was 1 mL/min. Isopropanol: hexane (0.5:99.5, v/v) isocratic mix was used for the mobile phase, and chromatograms were obtained at 292 nm wavelength. Analysis time and injection volume were 30 min and 100 μL , respectively. Tocopherol standards used for determination of α , β , γ and Δ tocopherols.

Sensory Evaluation

Every month olive oil samples were transferred to Ayvalık Olive Oil Tasting Laboratory accredited by International Olive Council and TURKAK (Turkish Accreditation Agency). Method for the organoleptic assessment of virgin olive oil (COI/T.20/Doc. No. 15/Rev. 8, November 2015) was used. Eight trained tasting panels were able to assess the oils to determine the levels of positive attributes, such as fruitiness, bitterness and pungency. Negative attributes arising due to poor quality fruit, incorrect processing or storing, such as rancidity, musty and fusty, were determined by sensory panels. Descriptors were evaluated on a 0-10 intensity scale (a number between 0 and 10). Oils were served in coloured tasting glasses.

Statistical analysis

Statistical analysis was performed by SPSS 17 (SPSS Inc. Chicago, IL) statistical software and using One-way ANOVA method. All analyses were performed at least in duplicate and differences among all groups were determined by the Duncan test.

Results and discussion

Chemical analyses

Free acidity, peroxide, UV-absorbance and colour values of the olive oils produced in the Mobile Olive Oil Processing Unit (MOOPU) were shown in Table 1. Free fatty acid content, peroxide value results showed that Halhalı olive oil could be classified as extra virgin olive oil according to the International Olive Council standards up to the eighth month.

Free acidity and peroxide values of the samples differed significantly during a year storage period ($p>0.01$). Free fatty acid, which is an important criterion for classification of olive oils increased slightly in the second month and no change was observed until the end of storage time. During one-year storage free fatty acid values were under the IOC (International Olive Council standards) limit ($<0.8\%$) for extra virgin olive oil. Earlier studies showed that free acidity increased with storage depending on the packaging material, storage conditions and time (Méndez and Falqué, 2007; Baiano et al., 2014; Abdalla et al., 2014; Lavelli et al., 2006). Peroxide values (PV) which is the primary oxidation indicator showed an increasing trend up to the ninth month and after this month PV decreased. The minimum level of PV was observed in the twelfth month. Significant increases were reported on the PV of olive oil samples during short term (30 days) and long term (six years) of storage in different packaging materials at different conditions (Abdalla et al., 2014; Lavelli et al., 2006; Okogeri and Tasioula-Margari, 2002).

UV-absorbance values (K_{232} and K_{270}) which are advanced oxidation products changed during storage significantly ($p<0.01$). K_{232} values decreased up to the third month. The sharp increase was observed in the fourth month and it was stable up to the seventh month. An important increase was noticed in the eighth month and near to end of the storage, K_{232} value was decreased. It was the maximum in the eighth month. The highest and the lowest K_{270} values were in the second and first months, respectively (Table 1). Except for second, the third and fifth month of storage period K_{270} values were under IOC (International Olive Council standards) limitations. ΔK values were zero or below zero (results are not

shown). These results are in agreement in the related literature (Méndez and Falqué, 2007; Baiano et al., 2014; Lavelli et al., 2006; Okogeri and Tasioula-Margari, 2002). Baiano et al. (2014) reported that K_{232} value of Coratina olive oil increased up to the sixth year, then it decreased, at the end of final storage an increase was observed. Gutiérrez and Fernández (2002) showed that only two quality indices (K_{270} and sensory evaluation) of Picual and Hojiblanca olive oils decreased during storage at 2 °C in darkness and 30 °C in illumination. Quality deterioration resulted in downgraded olive oils, which were no longer extra virgin olive oils during storage and there was an excellent correlation between initial stability and the time to reach the limit of $K_{270} > 0.25$.

Colour analysis

Although colour is not regarded as an important quality feature for olive oil, it has a great influence on consumers' acceptance. Colour of virgin olive oils is depended on olive maturity and process conditions. Analysis of colour (L , a and b values) showed that colour of olive oil samples changed significantly during storage (Table 1). It has been attributed to the decomposition of colour pigments such as chlorophylls, pheophytins, xanthophylls and carotenes (Boskou, 2006). The lowest L values (lightness) was seen in the tenth month. The highest L value was observed at the end of storage time. Fluctuations were observed in a (redness) and b (yellowness) values during storage. The highest b value was obtained for the ninth month. After this month there was a decreasing trend in b value. The highest a value also observed in twelfth month.

Table 1. Oxidative stability parameters and colour values of Halhalı extra virgin olive oils during 12 months storage

STORAGE PERIOD (Month)	Free Fatty Acid Content (%)		Peroxide Value (meqO ₂ /kg Oil)	K_{232}	K_{270}	L value	a value	b value
	Acid Content (%)	Peroxide Value (meqO ₂ /kg Oil)						
0	0.2±0.00 ^b	11.58±0.118 ⁱ	1.6±0.00 ^e	0.07±0.00 ^k	33.61±0.007 ^a	1.64±0.014 ^c	9.60±0.127 ^a	
1	0.2±0.00 ^b	16.06±0.212 ^g	0.2±0.00 ^h	-0.24±0.00 ^m	33.11±0.714 ^a	1.54±0.085 ^c	9.59±0.113 ^a	
2	0.3±0.03 ^a	16.75±0.121 ^f	0.0±0.00 ⁱ	1.15±0.00 ^a	33.90±0.028 ^a	1.69±0.011 ^{bc}	10.01±0.127 ^a	
3	0.3±0.03 ^a	17.68±0.074 ^e	0.5±0.00 ^g	0.54±0.00 ^b	33.90±0.014 ^a	1.71±0.004 ^{bc}	10.00±0.042 ^a	
4	0.3±0.03 ^a	17.79±0.075 ^{de}	1.9±0.00 ^d	0.12±0.00 ^g	33.77±0.042 ^a	1.64±0.011 ^c	9.73±0.141 ^a	
5	0.3±0.01 ^a	18.00±0.001 ^d	1.0±0.00 ^f	0.33±0.00 ^c	33.78±0.021 ^a	1.69±0.011 ^{bc}	9.93±0.049 ^a	
6	0.3±0.00 ^a	18.94±0.003 ^c	1.9±0.00 ^d	0.13±0.00 ^f	33.91±0.445 ^a	1.90±0.064 ^a	10.49±0.608 ^a	
7	0.3±0.00 ^a	18.96±0.004 ^c	1.9±0.00 ^d	0.03±0.00 ^l	33.33±1.386 ^a	1.86±0.060 ^{ab}	10.07±1.655 ^a	
8	0.3±0.00 ^a	21.58±0.118 ^b	2.5±0.00 ^a	0.11±0.00 ^h	33.20±1.146 ^a	1.86±0.007 ^{ab}	10.91±0.269 ^a	
9	0.3±0.00 ^a	22.66±0.087 ^a	2.0±0.00 ^c	0.22±0.00 ^d	34.33±0.014 ^a	1.94±0.004 ^a	11.05±0.042 ^a	
10	0.3±0.00 ^a	18.94±0.028 ^c	2.1±0.00 ^b	0.14±0.00 ^e	27.03±2.666 ^b	0.81±0.018 ^d	12.15±0.361 ^b	
11	0.3±0.00 ^a	15.58±0.118 ^h	2.0±0.00 ^c	0.10±0.00 ⁱ	34.08±0.926 ^a	1.99±0.007 ^a	10.60±1.385 ^a	
12	0.3±0.00 ^a	11.32±0.015 ⁱ	1.9±0.00 ^d	0.07±0.00 ^j	34.24±0.481 ^a	2.00±0.053 ^a	10.79±0.686 ^a	

*Different superscript letters in the same column indicate significant difference between mean values ($p < 0.01$).

Table 2. Tocopherol Content of Halhalı (Mardin) monocultivar EVOOs during 12 months' storage (ppm).

STORAGE PERIOD (Month)	α -Tocopherol	β -Tocopherol	γ -Tocopherol
0	347.67±7.210 ^a	1.79±0.142 ^a	0.19±0.000 ^a
3	329.41±2.320 ^b	1.17±0.014 ^b	0.17±0.006 ^b
6	324.24±0.980 ^b	1.13±0.009 ^b	0.16±0.001 ^b
12	290.72±0.160 ^c	0.32±0.004 ^c	ND

*Different superscript letters in the same column indicate significant difference between mean values ($p < 0.01$). nd: not detected

Tocopherol profile

Tocopherol (α , β , γ) profile of Halhalı (Mardin) olive oils was determined every three months during 12 months of storage time (Table 2). The results showed that tocopherols contents (α , β , γ) decreased with increasing storage time as expected. The lowest tocopherols contents were obtained after 12 months of storing. It means that 16.38% of α -tocopherol, 82.12% of β -tocopherol and 100% of γ -tocopherol contents were decomposed during storage. Decreasing in α -tocopherol was lower than other tocopherol isomers. These results were in agreement with earlier reports (Psomiadou et al., 2000; Baiano et al., 2014; Okogeri and Tasioula-Margari, 2002; Rastrelli et al., 2002).

Total polyphenol

Total polyphenol contents of the Halhalı olive oil ranged from 288.55 ± 0.947 to 236.64 ± 0.635 ppm during storage expressed as gallic acid (Table 3). The highest total polyphenol values were determined at fresh oils and its amount decreased with time, but the decreases were not dramatic as well as tocopherols. Although after a year storage, 18% of total polyphenols were decomposed, higher total polyphenol content was detected than that of earlier findings reported for Halhalı olive and olive oil (Arslan and Schreiner, 2012). It has been reported that the total phenolics content of

Halhalı olive and olive oils harvested in Hatay region of Turkey were 178-231 ppm and 97 ppm, respectively. After a short term or long term storage significant decreases in total polyphenol were reported for monocultivar and commercial olive oils by Morelló et al. (2004), Abdalla et al. (2014) and Baiano et al. (2014). In addition to health benefits, phenolic compounds, acting as natural antioxidants, increase the resistance of the oil to storage and heating.

Phenolic profiles

The phenolic composition was determined every six months. 3,4-dihydroxy benzoic acid, 4-hydroxy benzoic acid, *trans*-ferulic acid, *m*-coumaric acid, luteolin, and apigenin were identified and quantified in the polar fraction of in Halhalı olive oil during storage time (Table 4). Luteolin (3',4',5,7-tetrahydroxyflavone) was the most abundant polyphenol among others. The initial content of luteolin was 256.60 ± 0.421 ppm and decreased to 199.31 ± 1.056 ppm at the end of storage. Apigenin was the second phenolic with the content of 7.18 ± 0.738 ppm. Its content decreased to 5.18 ± 0.158 ppm at the end of the storage period. These results confirmed that storage caused significant changes on the phenolic profile. Yorulmaz et al. (2009) reported that luteolin was the most abundant phenolic compound following *trans*-cinnamic acid and luteolin-7-glucoside.

Table 3. Changes in Total phenols of EVOOs during 12 months of storage (ppm)

Storage Period (Month)	EVOOs' Total Phenols
0	288.55 ± 0.947^a
3	278.12 ± 0.851^b
6	269.57 ± 0.695^c
9	250.32 ± 0.261^d
12	236.64 ± 0.635^e

*Different superscript letters in the same column indicate significant difference between mean values ($p < 0.01$).

Table 4. Changes in phenolic compounds of Halhalı (Mardin) EVOOs during 12 months of storage time (ppm)

Phenolic Compounds	Month		
	0	6	12
3,4-dihydroxy benzoic acid	3.22 ± 0.190^a	2.41 ± 0.033^b	1.93 ± 0.056^c
4-hydroxy benzoic acid	3.63 ± 0.094^a	3.11 ± 0.020^b	2.60 ± 0.074^c
<i>trans</i> -ferulic acid	0.67 ± 0.010^a	0.63 ± 0.005^a	0.56 ± 0.017^b
<i>m</i> -coumaric acid	0.45 ± 0.005^a	0.42 ± 0.000^a	0.36 ± 0.020^b
luteolin	256.60 ± 0.421^a	214.36 ± 0.735^b	199.31 ± 1.056^c
apigenin	7.18 ± 0.738^a	6.95 ± 0.071^b	5.18 ± 0.158^c

*Different superscript letters in the same row indicate significant difference between mean values ($p < 0.05$).
nd: not detected

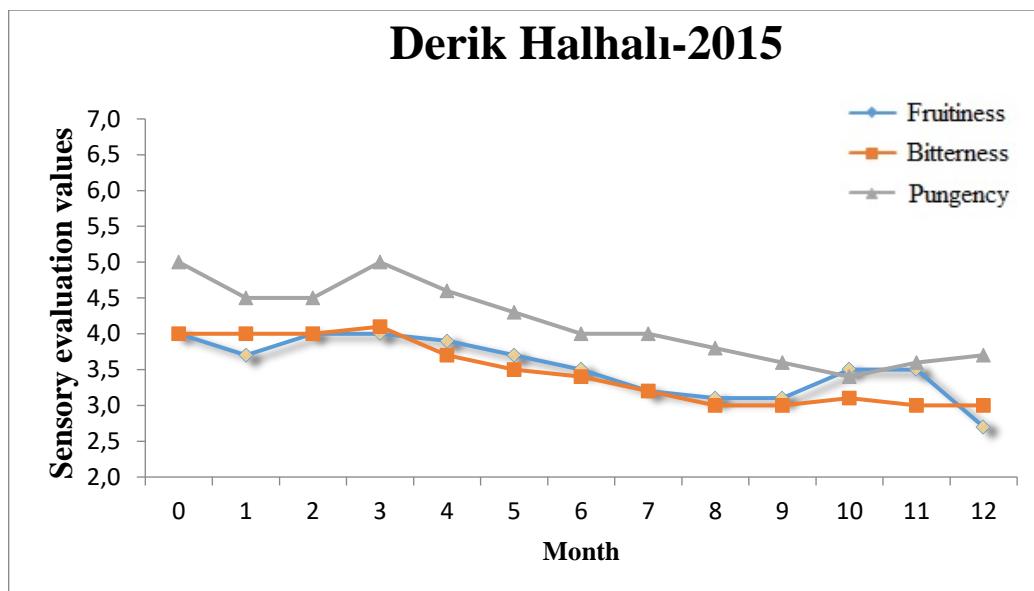


Fig. 1. Sensory evaluation of Halhalı EVOO during 12 months' storage

Morello et al. (2004) suggested that although storage did not appear to have any effect on vanilic acid or vanillin, which were present at low concentration, there was a significant decrease in the concentration of the rest of the quantified phenolic compounds. That reduction was more marked in the secoiridoid derivatives such as 3,4-DHPEA-EDA, p-HPEA-EDA and 3,4-DHPEA-EA indicating more active participation in the oxidative processes as they were more easily oxidized. Among the most representative phenolic compounds in olive oil, lignans seem to be the most stable during oil storage. Mulinacci et al. (2013) and Gómez-Alonso et al. (2007) showed an increase in tyrosol and hydroxytyrosol contents over time due to hydrolytic processes of the secoiridoidic derivatives. Gómez-Alonso et al. (2007) stated that the main phenols were the dialdehydic form of elenolic acid linked to tyrosol (p-HPEA-EDA), oleuropein aglycon, and the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA). Baiano et al. (2014) reported that there were increasing and decreasing trends in phenolic compounds (3,4-DHPEA, p-HPEA, vanillin, p-coumaric acid, 3,4-DHPEA-AC, 3,4-DHPEA-EDA, p-HPEA-AC, p-HPEA-EDA, 1-acetoxipinoresinol + *trans*-cinnamic acid, p-HPEA-EA) content.

Sensory evaluation

Halhalı olive oils had a very strong and green flavor with a peppery finish without any defect. It has a balance in terms of bitterness and fruitiness receiving a score between 3.0-4.0 and left a very fresh after taste in the mouth (Fig. 1). The pungency was 5.0 and it had fallen

to 3.5 scores for 12 months. This can be attributed to the decrease of phenolic compounds.

Conclusion

Halhalı is the major domestic olive cultivar in Mardin region of Turkey. Although the limited number of research on Halhalı olive oils composition in the literature, the authors are not aware of a study about the effects of storage on Halhalı olive oils. This is the first report on chemical composition and sensory properties of Halhalı olive oil produced in the mobile olive oil processing unit and the effects of storage. Free fatty acidity values were very low and it was stable during the storage. It showed that from tree to bottle the olive oil was produced in proper conditions. Although peroxide values were slightly exceeded the IOC limits (>20 meq O₂/kg oil) for eighth and ninth months of storage, UV-absorption values (K₂₃₂ and K₂₇₂) were in agreement with the IOC standard. It has a unique taste with no defect, fruitiness, bitterness and pungency in a balance and peppery finishing. The amount of total phenols extra virgin olive oils normally ranges between 50 and 1000 mg/kg, depending on cultivar, fruit's ripeness, processing and storage. It can be said that Derik Halhalı olive oil has higher total phenol content, which is uncommon in commercial ordinary Turkish olive oils. Luteolin and apigenin (flavonoids) which were major phenolics in Halhalı olive oils have multiple biological effects such as anti-inflammation, anti-allergy and anticancer, luteolin functions as either an antioxidant or a pro-oxidant biochemically. The level of these phenols in this cultivar is much higher in comparison the Spanish and Greek olive oils.

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