Int. J. Sec. Metabolite, Vol. 4: 3 (2017) pp. 195-204

IJSM International Journal of Secondary Metabolite

Special Issue 1: Research Article

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

Identification of Volatile Compounds (VCs) in the Leaves Collected from 'Gemlik', 'Halhalı' and 'Sarı Hasebi' Olive Tree Varieties

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Received: 05 May 2017 – Revised: 19 June 2017 - Accepted: 21 August 2017

Abstract: There is a considerably interest on some fruits and leaves extracts such as olive leaf, due to their beneficial health effects. Olive leaf has been consumed as tea for many years. However, the studies on volatile compounds (VCs) of leaves are scarce. Therefore, this study was aimed to evaluate of VCs in the leaves collected from 'Gemlik', 'Halhalı' and 'Sarı Hasebi' olive trees varieties grown in Hatay province. The VCs were analyzed by gas chromatography-mass spectrometry (GC-MS) using solid phase micro-extraction (SPME). The 97 out of 127 VCs identified were found common in all the olive leaves. Terpenes, aldehydes, alcohols and ketones were identified in the olive leaves as major VCs groups, which accounted for about 36-60%, 20-28%, 6-14% and 4-8% of total VCs identified in leaves, respectively. The relative proportions of these chemical groups showed considerably differences among olive leaves. α-Cubebene was found as major VC followed by *trans*-caryophyllene, α-farnesene, *trans*-2-hexenal, benzeneethanol, nonanal, *trans*,*trans*-2,4-heptadienal, cycloisosativene, *trans*-4,8-dimethyl-1,3,7-nonatriene, 2,4-heptadienal, α-humulene, α-muurolene and benzaldehyde. These compounds accounted for 56-75% of total VCs identified in the olive leaves. While 'Halhalı' olive leaf had highest ($p<0.01$) levels of α-cubebene (31.79%), cycloisosativene (7.69%) and α-muurolene (4.05%), 'Sarı Hasebi' had *trans*-caryophyllene (23.16%), *trans*-4,8-dimethyl-1,3,7-nonatriene (4.65%), α-humulene (3.64%) and 'Gemlik' had benzeneethanol (6.93%), nonanal (5.07%), and benzaldehyde (2.17%) at the highest levels. This study has showed that olive leaves from each variety are a good terpene source that makes them important in terms of beneficial effects on health.

Keywords: Olive tree leaves, Volatile compounds, Gemlik, Terpenes

1. INTRODUCTION

Turkey is the fourth biggest olive producing country after Spain, Italy and Greece with 1 768 000 tons in an 826.092 ha area [1]. These Mediterranean countries are producing approximately 65% of the world's total olive production since olive tree belongs to a member of Oleaceae family that is well adapted to Mediterranean basin. Olive is important in context of religion since it has also been praised as a blessed tree and fruit in the Holy Quran. Olive is not used as a natural fruit because of its extremely bitter taste but is rather consumed either as olive oil or as table olive. Olive leaves are known as olive by-products, which are remained during oil extraction, fruit harvesting and olive tree pruning. They are burned, grounded or thrown in away as a by-product that is resulted in environmental pollution or wasting a resource [2].

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ISSN: 2148-6905 online /© 2017 DOI: 10.21448/ijsm.370128

According to Herraro et al. [3], olive leaves represent approximately 10% of the weight of olives collected for oil extraction. But, recently olive leaves have been used in folk medicine in Mediterranean regions for arthritic pains, decreasing blood pressure, antimicrobial and antiviral effects, supporting immune system, relax and benefits to the cardiovascular system [4-5-6]. These beneficial effects could be related to phenolic or volatile compounds. According to Talhaoui et al. [2], due to phenolic compounds olive leaf has a matrix rich in antioxidants and also shows anti-inflammatory property. Kubo et al. [7] have reported that olive leaves are the main sources of glycosides, oleuropein and ligstrosides. The degradation of these compounds by a β-glucosidase enzyme resulted in the releasing of volatile compounds such as aldehydes. They may show antimicrobial property. However, detailed studies on the volatile compounds of olive leaves are scarce [8]. Therefore, the specific objects of the present study were; to identify the volatile compounds of olive leaves from different varieties such as 'Gemlik', 'Halhalı' and 'Sarı Hasebi and also if the olive varieties can be grouped based on the volatile compounds identified in the olive leaves.

2. MATERIAL and METHODS

The leaf samples of three olive tree varieties ('Gemlik', 'Halhalı' and 'Sarı Hasebi') were collected from Hatay province located in the Eastern Mediterranean Region of Turkey. 'Gemlik' trees were 10 years old and, 'Halhalı' and 'Sarı Hasebi' were 45 years old. No pesticide was used for pest control in olives. The leaves were randomly collected from each olive tree variety in December month by hand. They were dried in an oven at 30-35 °C for 3 days. Thereafter, VCs analyses of leaves from each variety were carried out according to the procedure described by Guler [9] with minor modification. For VCs analyses, samples were prepared in triplicate from each cultivar. For this purpose, 3 g of dried leaves was separately transferred to a 20 mL head-space vial (Agilent, Palo Alto, CA, USA), adding 3 mL NaCl solution (3% w/v). The vials were sealed using crimp-top caps with TFE-silicone headspace septa (Agilent). The VCs were adsorbed on a fibre consisting of divinylbenzene/ carboxen/polydimetylsilioxan (Supelco, Bellefonte, PA, USA) using Solid Phase Micro-extraction (SPME) technique. For extraction of VCs, the vials were kept at 60°C for 45 min in a water bath then SPME fiber was inserted to the headspace vial and held for 30 min at constant temperature. Desorption of VCs from fiber was performed in the injection port at 250°C for 5 min. The VCs were separated on a HP-Innowax capillary column (60 m x 0.25 mm id x 0.25 μm film thickness; Agilent, USA). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature program was initially held at 50°C for 5 min and then programmed from 50°C by a ramp of 5°C min^{-1} up to 230°C, which was held for 5 min. Identification of VCs was carried out by a computer-matching of their mass spectral data with those of known compounds from the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) library, version 02.L. Based on the peak resolution, their areas were estimated from the integrations performed on selected target ions. Relative percent qualitative recovery per compound was expressed as the target ion response divided by the total target response from the integrated suite of compounds per sample.

All data was subjected to ANOVA and means were compared using Duncan's multiple range test (SPSS Version 17.0; SPSS Inc., Chicago, IL, USA) to determine statistically differences at $p<0.05$ level. All the data on VCs obtained from olive leaves were used for discriminant function analysis based on Eigenvalues.

3. RESULTS and DISCUSSIONS

To our knowledge, this study is the first report on VCs identified in the leaves of the different olive varieties. Thus, it would be very difficult to compare them now. As shown in Table 1., a total of 127 VCs were identified in experimental olive leaves. VCs were grouped according to their chemical classes and the profile was composed of 27 terpenes (21 sesquiterpenes and 6 monoterpenes), 22 aldehydes, 17 ketones, 15 alcohols, 14 phenyls and phenols, 11 esters, 8 alkenes, 7 alkanes, 3 furans and 3 the others. ‘Gemlik’ and ‘Sarı Hasebi’ leaves had a similar VC number whereas ‘Halhalı’ olive leaf had the less VC number. Twenty-six VCs are listed in Table 2, which constituted the majority of volatile compounds accounting for 75%, 69% and 56% of total VCs identified in ‘Halhalı’, ‘Sarı Hasebi’ and ‘Gemlik’ olive leaves, respectively. ‘Gemlik’ variety was considerably different from ‘Sarı Hasebi’ and ‘Halhalı’, in terms of the percent proportions of volatile compounds identified in the leaves. As shown in Tables 1 and 2., and in Fig. 1, terpenes, especially sesquiterpenes, were the major chemical group found in all the olive leaves, in terms of their number and their percentage composition. A similar tendency was obtained for *Ficus carica* leaves which were rich in potential health-promoting phytochemicals [10].

While the main terpene and also VC identified in the headspace of ‘Gemlik’ and ‘Sarı Hasebi’ olive leaves was *trans*-caryophyllene, it was α-cubebene in ‘Halhalı’ leaf. *Trans*-caryophyllene (TC) is an important constituent of the essential oils derived from several species of medicinal plants such as *Cordia verbenacea.* The recent studies have indicated that TC had multiple pharmacological effects including anti-inflammation, anti-apoptosis and neuroprotection [11]. When compared with ‘Halhalı’ and ‘Gemlik’ olive leaves, ‘Sarı Hasebi’ leaf is one of important tools for the management and/or treatment of especially inflammatory diseases due to its considerably high *trans*-caryophyllene and also α-humulene (α-caryophyllene) percentages [11].

Tablo 1. The volatile compounds identified in olive leaves

Volatile Compounds (127)	RT	RI	Gemlik	Halhalı	Sarı Hasebi
Terpenes (27)					
Monoterpenes (6)					
γ-Terpinene	16.55	1398	+	+	+
Styrene	16.98	1423	+	+	+
trans-4,8-Dimethyl-1,3,7-nonatriene	18.43	1511	+	+	+
cis-α-Bisabolene	28.92	>2100	+	+	-
Camphene	29.09	>2100	+	+	+
Allo ocimene	33.68	>2100	+	+	+
Sesquiterpenes (21)					
α-Ylangene	22.98	1873	+	+	+
Cycloisosativene	23.75	1943	+	+	+
α-Cubebene	23.99	1965	+	+	+
β-Bourbonene	24.56	2022	+	-	+
β-Gurjunene	26.18	>2100	+	+	+
β-Cubebene	26.66	>2100	-	+	+
trans-Caryophyllene	26.86	>2100	+	+	+
α-Gurjunene	27.25	>2100	+	+	+
Caryophyllene	28.49	>2100	+	-	+
α-Humulene	28.65	>2100	+	+	+
α-Amorphene	28.99	>2100	+	+	+
Zingiberene	29.57	>2100	+	+	+
α-Copaene	29.72	>2100	+	-	+
α-Muurolene	29.80	>2100	+	+	+
α-Farnesene	30.14	>2100	+	+	+
Δ-Cadinene	30.56	>2100	+	+	+
α-Amorphene	30.68	>2100	+	-	+
trans-β-Farnesene	31.01	>2100	+	+	+
γ-Muurolene	31.41	>2100	+	-	+
Epi-ligulyl oxide	31.50	>2100	+	+	+
cis-Calamenene	32.33	>2100	+	+	+

Table 1. (Continued)

Aldehydes (22)					
cis-2-Butenal	8.95	1083	+	+	+
Hexanal	10.43	1138	+	+	+
trans-Citral	13.71	1262	+	+	+
Heptenal	14.39	1291	+	+	+
trans-2-Hexenal	15.65	1353	+	+	+
Octanal	18.00	1484	+	+	+
trans-2-Heptenal	19.16	1561	+	+	+
Nonanal	21.18	1800	+	+	+
trans,*trans*-2,4-Hexadienal	21.64	1754	+	+	+
2-Octenal	22.30	1811	+	+	+
trans,*trans*-2,4-Heptadienal	23.33	1905	+	+	+
2,4-Heptadienal	24.15	1979	+	+	+
Benzaldehyde	25.09	2072	+	+	+
trans,*trans*-2,6-Nonadienal	26.51	>2100	+	+	+
2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde	27.49	>2100	+	+	+
trans-2-Decanal	27.88	>2100	+	+	+
Safranal	28.12	>2100	+	+	+
trans,*trans*-2,4-Nonadienal	29.35	>2100	+	+	+
2-Undecenal	30.42	>2100	+	+	+
trans,*cis*-2,4-Decadienal	30.75	>2100	+	+	+
Tridecanal	31.76	>2100	+	-	+
4-Methoxy-benzenaldehyde	36.62	>2100	+	+	+
Ketones (17)					
6-Methyl-5-hepten-2-one	19.51	1586	+	+	+
11H-Dibenzo[b,e][1,4]diazepin-11-one, 5,10-dihydro-5-[3-(methylamino)propyl]-	23.44	1915	+	+	+
3,5-Octadien-2-one	24.58	2024	+	+	+
4,8-Dimethyl-nona-3,8-dien-2-one	25.65	>2100	+	-	-
3,5-Octadiene-2-one	26.13	>2100	+	-	-
6-Methyl-3,5-heptadien-2-one	26.70	>2100	+	-	-
Phenyl methyl ketone	28.31	>2100	+	+	+
Glycocyanidine	29.26	>2100	+	+	+
β-Damascenone	32.09	>2100	+	+	-
trans-Geranylacetone	32.62	>2100	+	+	+
3-Phenyl-2-nutanone	33.02	>2100	+	+	+
β-Ionone	34.62	>2100	+	+	+
2,3-Epoxy-β-ionone	35.77	>2100	+	+	+
cis-Cinerolone	36.84	>2100	+	+	+
6,10,14-Trimethyl-2-pentadecanone	37.99	>2100	+	+	+
3-Ethyl-4-methyl-1H-pyrrole-2,5-dione	41.02	>2100	+	+	+
5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	43.03	>2100	+	+	+
Alcohols (15)					
Hexan-1-ol	19.79	1605	+	+	+
cis-3-Hexen-1-ol	20.80	1684	+	+	+
1-Octen-3-ol	22.60	1838	+	-	+
cis-1,5-Octadien-3-ol	23.62	1931	+	+	+
Octan-1-ol	25.46	>2100	+	+	+
2,6-Dimethyl-cyclohexanol	27.01	>2100	+	+	+
1-Nonanol	28.01	>2100	+	+	+
β-Citronellol	30.48	>2100	+	+	-
α-Methyl-benzenemethanol	31.81	>2100	+	+	+
Benzenemethanol	33.24	>2100	+	+	+
Benzeneethanol	34.02	>2100	+	+	+
cis-Farnesol	34.22	>2100	+	+	+
Nerolidol	36.27	>2100	+	+	+
(1S*,6S*,7S*)-Tricyclo[5.3.2.0(1,6)]dodecan-7-ol	38.96	>2100	+	+	+
D11-Dodecene-1-ol	39.31	>2100	+	-	+

Table 1. (Continued)

Phenyls and Phenols (14)					
1,2,3,4-Tetramethylbenzene	17.32	1443	+	+	-
Safranal	22.78	1855	+	+	+
trans-Anethole	32.23	>2100	+	+	+
2-Methoxy-phenol	32.95	>2100	+	+	+
2-Methyl-naphthalene	33.83	>2100	+	+	+
2-Methoxy-4-methyl-phenol	34.96	>2100	+	+	+
Phenol	35.90	>2100	+	+	+
2-Propenyl-benzene	36.71	>2100	+	+	+
2,6-Dimethyl-phenol	37.32	>2100	+	-	-
4-Methyl-phenol	37.40	>2100	+	-	-
m-Cresol	37.55	>2100	+	-	+
2-Methoxy-4-(2-propenyl)-phenol	39.12	>2100	+	-	+
Tymol	39.78	>2100	+	+	+
Biphenylene	40.05	>2100	+	+	+
Esters (11)					
Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	13.43	1250	+	+	+
Heptanoic acid, methyl ester	17.83	1474	+	-	+
Hexanoic acid, methyl ester	21.00	1699	+	-	+
Nonanoic acid, methyl ester	23.85	1952	+	-	+
Tiglic acid, *cis*-3-hexenyl ester	28.39	>2100	+	+	+
Acetic acid, 2-phenylethyl ester	30.03	>2100	+	+	+
Benzoic acid, 2-hydroxy-, methyl ester	31.27	>2100	+	+	+
Acetic acid, 2-phenylethyl ester	31.96	>2100	+	+	+
Benzoic acid, *cis*-3-hexenyl ester	38.35	>2100	+	+	+
Hexadecanoic acid, methyl ester	39.67	>2100	+	+	+
Phthalic acid, ethyl ester	42.94	>2100	+	+	+
Alkenes (8)					
7-Methyl-3,4-octadiene	20.17	1635	+	+	+
3,7-Dimethyl-1-octene	20.29	1644	+	+	+
trans-3,5-Dimethyl-1,6-octadiene	21.45	1738	+	+	-
2,5-Dimethyl-2,4-hexadiene	24.53	2019	+	-	+
4,8-Dimethyl-1,7-nonadiene	25.88	>2100	+	-	+
1,2,4,4-Tetramethyl-cyclopentene	27.63	>2100	+	+	+
cis-2,6-Dimethyl-2,6-octadiene	28.17	>2100	+	+	+
1,4-Diethyl-1,4-dimethyl-2,5-cyclohexadiene	33.34	>2100	+	+	+
Alkanes (7)					
9-Methyl-nonadecane	22.16	1798	+	+	+
5-Methyl-dodecane	25.00	2064	+	-	+
8-Hexyl-pentadecane	25.57	>2100	+	-	+
Hexadecane	26.38	>2100	+	+	+
Heptadecane	28.79	>2100	+	+	+
cis-1,2-Divinylcyclohexane	30.23	>2100	+	+	+
Cyclododecane	34.77	>2100	+	+	+
Furans (3)					
2-(2-Propenyl)-furan	27.38	>2100	+	+	+
β-Agarofuran	29.18	>2100	+	+	+
Dihydro-β-agarofuran	32.45	>2100	+	+	+
Others (3) (Unkown-Alkyne-Acid)					
Unknown	18.94	1546	+	+	+
1,4-Dimethoxy-2-butyne	27.73	>2100	+	+	+
trans-3-Hexenoic acid	34.71	>2100	+	-	+

RI: retention index based on the identified VCs retention time (RT) and calculated from a linear equation between each pair of straight alkanes (C5-C25).; + and -: compounds are identified and unidentified in leaves, respectively.

α-Cubebene identified in 'Halhalı' olive leaf as the major VC is a dibenzocyclooctadiene lignin and it has a potential antioxidant property [12]. It has previously been identified in *Schisandra chinensis* herb as the most plentiful VC [13]. This plant is a well-known medicinal herb that ameliorates cardiovascular symptoms. Benzenethanol, cycloisosativene and α-farnesene was identified in Gemlik, 'Halhalı' and 'Sarı Hasebi' olive tree leaves as the second most abundant VCs, respectively. Benzenethanol (Phenylethyl alcohol) was the main component of Rose oils obtained from rose blossom, which is used in ophthalmic drug products as preservative and in food and also cosmetic industries as a flavoring substance [14].

Table 2. The relative percentages of the major volatile compounds (VCs) identified in 'Gemlik', 'Halhalı' and 'Sarı Hasebi' olive leaves.

Major Volatile Compounds (26)	Gemlik	Halhalı	Sarı Hasebi	Analyses of Variance
α-Cubebene	5.54±0,65[b]	31.79±1,74[a]	3.56±0,28[b]	$F(148,5) = 423.4$, $p< .001$
trans-Caryophyllene	11.17±1,19[b]	3.85±0,12[c]	23.16±1,08[a]	$F(148,5) = 219.9$, $p< .001$
α-Farnesene	6.69±0,74	5.55±1,22	9.52±1,35	$F(9,55) = 6.50$, $p> .05$
trans-2-Hexenal	5.18±0,47	6.86±0,59	7.30±1,33	$F(9,55) = 3.15$, $p> .05$
Benzeneethanol	6.93±0,66[a]	3.09±0,13b	3.98±0,78[b]	$F(9,55) = 22.6$, $p< .05$
Nonanal	5.07±0,20[a]	3.05±0,54[b]	4.03±0,12[ab]	$F(9,55) = 17.5$, $p< .05$
trans,*trans*-2,4-Heptadienal	4.28±0,06	3.60±0,33	3.84±0,24	$F(9,55) = 4.05$, $p> .05$
Cycloisosativene	1.20±0,08[b]	7.69±0,17[a]	0.88±0,02[b]	$F(148,5) = 2432.6$, $p< .001$
trans-4,8-Dimethyl-1,3,7-nonatriene	2.65±0,22[ab]	1.79±0,07[b]	4.65±1,07[a]	$F(9,55) = 10.72$, $p< .05$
2,4-Heptadienal	3.10±0,05	2.07±0,40	2.56±0,18	$F(9,55) = 8.22$, $p> .05$
α-Humulene	1.85±0,05[b]	0.64±0,01[c]	3.64±0,05[a]	$F(148,5) = 2778.5$, $p< .001$
α-Muurolene	0.43±0,01[b]	4.05±0,45[a]	0.31±0,03[b]	$F(30,82) = 135.8$, $p< .01$
Benzaldehyde	2.17±0,06[a]	1.25±0,04[b]	1.18±0,14[b]	$F(30,82) = 74.56$, $p< .01$
Benzenemethanol	2.07±0,20[a]	0.90±0,03[b]	1.13±0,20[b]	$F(9,55) = 28.03$, $p< .05$
11H- Dibenzo[b,e][1,4] diazepin-11 one, 5,10-dihydro-5-[3-(methyl amino)propyl]-	1.32±0,62	1.39±0,25	1.29±0,45	$F(9,55) = 0.023$, $p> .05$
Benzoic acid, 2-hydroxy-, methyl ester	1.40±0,05[a]	0.68±0,18[b]	1.91±0,26[a]	$F(9,55) = 22.66$, $p< .05$
6-Methyl-5-hepten-2-one	1.62±0,07	1.10±0,04	1.10±0,31	$F(9,55) = 5.05$, $p> .05$
3,5-Octadien-2-one	1.49±0,03[a]	0.44±0,05[b]	1.24±0,17[a]	$F(30,82) = 50.59$, $p< .01$
γ-Terpinene	0.94±0,19	0.85±0,33	1.36±0,83	$F(9,55) = 0.54$, $p> .05$
Unknown	0.70±0,29[b]	1.79±0,20[a]	0.64±0,14[b]	$F(9,55) = 17.48$, $p< .05$
trans-3,5-Dimethyl-1,6-octadiene	1.76±0,23[a]	1.32±0,15[a]	nd	$F(30,82) = 66.20$, $p< .01$
Styrene	1.40±0,46	0.41±0,05	1.03±0,33	$F(9,55) = 4.71$, $p> .05$
3,7-Dimethyl-1-octene	0.76±0,07[b]	1.38±0,02[a]	0.44±0,00[c]	$F(148,5) = 263.5$, $p< .001$
2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde,	1.00±0,03[a]	0.29±0,12[b]	0.49±0,04[b]	$F(30,82) = 48.39$, $p< .01$
trans-2-Decanal	0.99±0,15[a]	0.26±0,07[b]	0.42±0,11[b]	$F(9,55) = 23.52$, $p< .05$
β-Cubebene	nd	1.16±0,12[a]	0.50±0,02[b]	$F(148,5) = 153.4$, $p< .001$

Mean values (n = 3) followed by different letters in the same row indicate significant differences ($p< 0.05$) for the olive leaves. nd: not detected.

Cycloisosativene is one of the major compounds found in *Euphorbia macrorrhiza* which is one of medicinal plants used in the treatment for skin diseases, gonorrhea, migraine, intestinal parasites and warts [15]. Cycloisosativene identified in 'Halhalı' leaf as the second most abundant VC was significantly higher than that of the other olive varieties. While *trans*-2-hexenal was the third most abundant VC in 'Sarı Hasebi' and 'Halhalı' olive leaves, α-farnesene was in 'Gemlik' olive leaf. Both *trans*-β- and α-farnesene occur in a wide range of plant and animal species. *Trans*-β-farnesene molecule was identified to be effective kairomone for the predators. The aphids releasing *trans*-β-farnesene alone were found to be attractive for *Adalia bipuncata* L. [16]. Additionally, β-farnesene has a bitter flavor note, which is high in some hop varieties with bitter flavor [17].

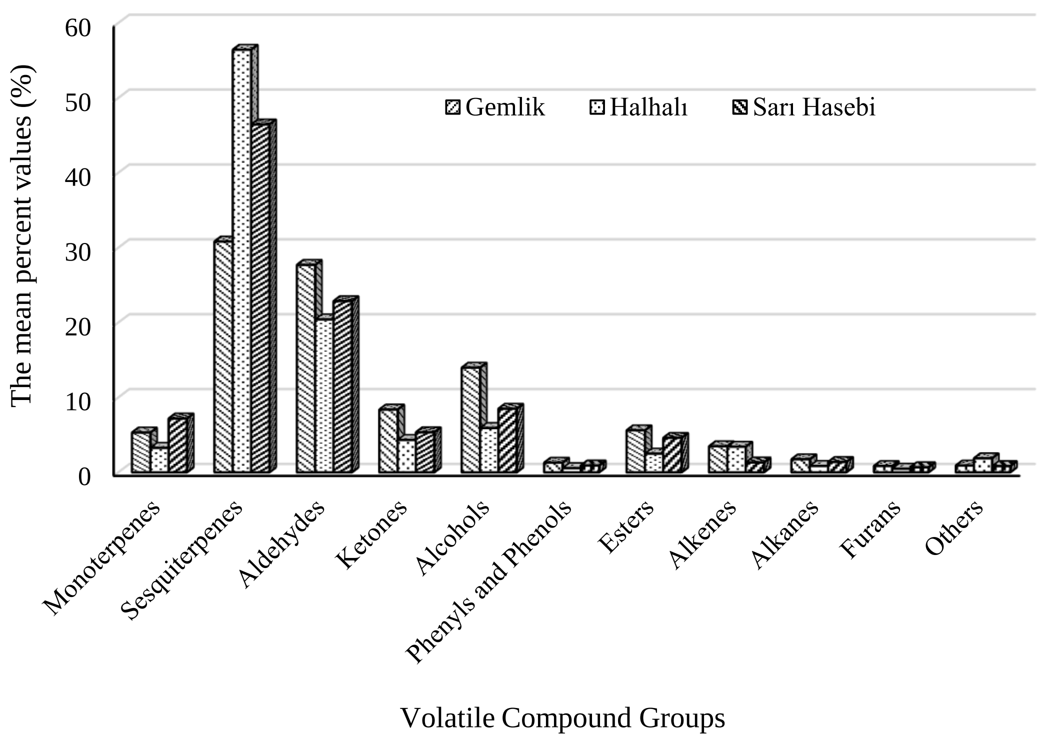

Figure 1. The mean percent values of volatile compound groups of olive leaves according to chemical families.

Another major compound, *trans*-2-hexenal, has an activity against a large number of microorganisms [8]. α-Muurolene is considerably high in 'Halhalı' leaf, compared with the other varieties. It was previously found to be in essential oil of *Calendula officinalis* flowers [18]. On the other hand, 'Halhalı' leaf had the lower percentages of *trans*-4,8-dimethyl-1,3,7-nonatriene and α-humulene than those in 'Gemlik' and 'Sarı Hasebi' leaves. These VCs have been considerably high, especially in 'Sarı Hasebi' leaf. *Trans*-4,8-dimethyl-1,3,7-nonatriene has been previously identified in Bergamot essential oil and maize leaves, which is a defense chemical compound against to herbivores [19]. Nonanal was identified in all the leaves but it was considerably higher in 'Gemlik' leaf than the other varieties. This saturated aldehyde is derived from the oxidation of linoleic acid by 9-lipoxygenase. This finding indicates that 'Gemlik' olive may have had the higher linoleic acid content, followed by 'Sarı Hasebi' and 'Halhalı'. Actually, the fatty acid composition of olive fruit may be estimated by the determining of the profiles of VCs in olive leaves. It is even possible to make a comparison between olive varieties.

VCs including β-bourbonene, caryophyllene, α-cubebene, α-amorphene, γ-muurolene, tridecanal, 1-octen-3-ol, D11-dodecene-1-ol, m-cresol, 2-methoxy-4-(2-propenyl)-phenol, heptanoic acid methyl ester, hexanoic acid methyl ester, nonanoic acid methyl ester, 2,5-dimethyl-2,4-hexadiene, 4,8-dimethyl-1,7-nonadiene, 5-methyl-dodecane, 8-hexyl-pentadecane, trans-3-hexenoic acid were routinely identified in 'Gemlik' and 'Sarı Hasebi' olive leaves whereas they were not found in 'Halhalı' olive leaf. The remaining 29 VCs were sporadically identified in all the leaves.

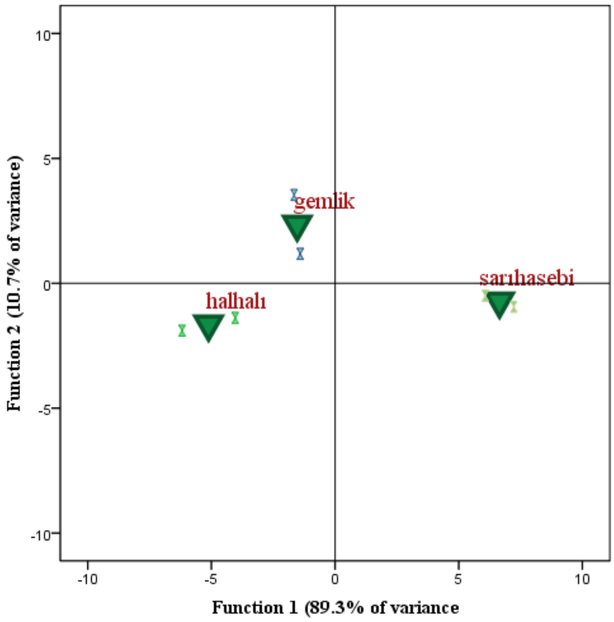

Figure 2. Discriminant analysis of the percentage composition of volatile compounds (VCs) in ''Gemlik'', ''Halhalı'' and ''Sarı Hasebi'' olive leaves.

Discriminant analysis was applied to data on VCs obtained from the leaves of three olive varieties analyzed. According to discriminant analysis based on Eigenvalues, VCs identified in the olive leaves could be used to discriminate and classify the olive tree varieties (Fig. 2). As shown in Fig. 2., the olive varieties were completely different from each other. Table 3 shows that the olives in each discriminant function, in proportion to the magnitude of their variation value (bold numeric), are independent from the olives in the other discriminant function.

Table 3. Results of discriminant function on the volatile compounds of olive varieties.

Factors	**Discriminant function**	
	1	**2**
Gemlik	-1.534	**2.357**
Halhalı	**-5.117**	-1.639
Sarı Hasebi	**6.651**	-0.718
Eigenvalues	48.5109	5.8399
Explained variance%	89.3%	10.7%

Olive varieties in the same discriminant function are related to each other, according to positive and negative variation. According to discriminant function, 'Halhalı' olive leaf showed a negative variation with 'Sarı Hasebi', and also both was related with each other. 'Halhalı' olive leaf had the highest α-cubebene, cycloisosativene and α-muurolene, and also the lowest *trans*-caryophyllene and α-humulene percentages. However, in terms of VCs mentioned, 'Sarı Hasebi' olive leaf has a tendency opposite to that of 'Halhalı' leaf.

The leaf of ‘Gemlik’ olive variety was clearly different from the other two varieties (Fig. 2. and Table 3.) since it had the highest benzenethanol, benzenealdehyde, nonanal and benzenemethanol percentages. Therefore, the olive variety groups of ‘Gemlik’, ‘Halhalı’ and ‘Sarı Hasebi’ can be grouped based on the volatile compounds identified in their leaves. Additionally, volatile compounds in olive leaves may be a tool for the assessment of olive origin as varietal markers. However, future studies should increase the number of varieties for accurate generalization.

4. CONCLUSION

This study confirmed that the profile and proportions of VCs identified in the leaves collected from olive trees changed depending on the variety. In terms of the proportion of volatile compounds identified in headspace of olive leaves, ‘Gemlik’ variety was considerably different from ‘Sarı Hasebi’ and ‘Halhalı’ varieties. According to discriminant analysis, VCs such as α-cubebene, cycloisosativene, α-muurolene, *trans*-caryophyllene, α-humulene, benzenethanol, benzenealdehyde, nonanal and benzenemethanol can be used to discriminate and classify the olive varieties. In general, the major volatile compounds identified in the leaves of three olive tree varieties were similar to those in medical plants. Thus, olive tree leaves may be used in folk medicine or in food supplements as a raw material. In addition, some VCs identified in olive leaves such as nonanal may give an idea of the fatty acid composition of olive fruit. An important result of this work is that olive tree varieties may be discriminated or classified by volatile compound contents of olive leaves and also a SPME method can be used as a quality assessment system.

Conflict of Interests

Authors declare that there is no conflict of interests.

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