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Volatile Compounds, Phenolic Content, and Antioxidant Capacity in Sultan Hawthorn (*Crataegus azarolus* L.) Leaves

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ABSTRACT

Nowadays, there is considerable interest in plants such as hawthorn that have a rich source of secondary metabolites (volatile and phenolic compounds) in their leaves, with beneficial effects on health. This study investigated the Volatile Compounds (VCs), total phenolic content, and antioxidant activities of Sultan hawthorn leaves collected at three different times based on fruit maturity stages (immature, mature, and over-mature). Our main goal was to determine whether the volatile profile, total phenolic content, and antioxidant activity would change depending on the leaf collection time. A total of 78 VCs were identified in the leaves, 11 of which were for the first time. With the progress in fruit maturity, the levels of most VCs varied, the phenolic content and antioxidant activity increased, and acidity decreased. Benzaldehyde and α-farnesene were the principal VCs accounting for 61% of total VCs identified in leaf at the overmature stage. The principal component analysis successfully separated volatile compounds in hawthorn leaves along the fruit maturity stages. For the first time, the present study provided a general overview of the secondary metabolites in leaves from Sultan hawthorn cultivar along fruit maturity stages. The hawthorn leaf collected at the overmature fruit stage proved to have high potential in secondary metabolites and antioxidant capacity.

Keywords: Overmature fruit stage, Secondary metabolites.

INTRODUCTION

Hawthorn (*Crataegus spp*.) is a tree belonging to the *Rosaceae* family and has more than 200 species worldwide (Özderin and Fakir, 2015). The leaves and flowers of several *Crataegus* species is called with the common name of hawthorn leaf and flower. Herbal remedies, including hawthorn leaves and flower and/or their combination are generally available as herbal tea (EMA, 2021). In folk medicine, these preparations, both green (immature) and red (mature) fruits, are widely used to treat heart failure and high blood pressure as well as diarrhea, insomnia, asthma, and inflammation (Fong and Bauman, 2002; Lakache *et al.,* 2014; Ozderin *et al.,* 2016 Lund *et al.,* 2017). The German Commission E has approved a standardized extract of leaves and flowers to treat heart failure (Sticher and Meier, 1997). These health benefits ascribed to the hawthorn leaves, flowers, or fruits are thought to be related to their high content of secondary metabolites such as phenolic compounds, terpenes, aldehydes, and organic acids (Chang *et al.,* 2002; Liu *et al.,* 2011; Keser *at al.,* 2014; Alirezalu *et al.,* 2018). Hawthorn leaves are a perfect source of phenolic compounds compared to fruits and flowers (Keser *et al.,* 2014; Alirezalu *et al.,* 2018). Most plant phenolic compounds have a high antioxidant capacity and are essential in protecting cells against oxidative damage caused by free radicals (Pandey and Rizvi, 2009).

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Researchers stated that phenolics such as chlorogenic acid, vitexin, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, acetylvitexin 2′′-*O*-rhamnoside were remarkably high in hawthorn leaf compared to bark (Wloch *et al.,* 2013). Among them, quercetin has also been noted to have an important adjuvant role in slowing Covid-19 disease progression (Di Pierro *et al.,* 2021). Like phenolic compounds, volatile compounds are the secondary metabolites in plants. Volatile compounds in the essential oil of leaves and flowers of *Crataegus* species are previously studied (Robertson *et al.,* 1993; Lakache *et al.,* 2014; Ozderin *et al.,* 2016). Sultan hawthorn is the first standard cultivar grown in Turkey. However, no study is available on volatile compounds, total phenolics, and antioxidant capacity in Sultan hawthorn leaves collected at three different times, even though the metabolites of biochemical pathways in leaves change (Pavlovic *et al.,* 2019). Therefore, we aimed to investigate the alteration of volatile compounds, total phenolic content, and antioxidant activity of Sultan hawthorn leaves based on the fruit maturity stage.

MATERIALS AND METHODS

The hawthorn leaves were sampled on September 13 (CT1), September 20 (CT2), and October 2 (CT3) in 2017 season, corresponding to immature, mature, and overmature fruit maturity stages, respectively. At each sampling time, leaves (about 200 g) were collected from 3 trees randomly selected in the orchard, Hatay, Türkiye (36° 43′ 09″ N, 36° 13′ 80″ E, elevation 812 m).

Extraction and HS-SPME-GC-MS Analysis of Volatile Compounds of Hawthorn Leaf

The volatile compounds were extracted utilizing the Headspace-Solid Phase Micro-Extraction (HS-SPME) Technique and were chromatographed by Gas Chromatography-Mass Spectrometry (GC-MS). At each sampling time, nine samples (3 tree x 3 triplicate) were analyzed for volatile compounds. VC analysis was carried out according to the method described by Güler *et al.* (2017). Briefly, leaves (approximately 20 g) were grounded in a chilled mortar. Three grams of grounded leaf sample were separately transferred to a 20 mL HS vial (Agilent, Palo Alto, USA). Sodium chloride solution (3% w/v) was added to HS vial. The vials capped crimp-top PTFE-silicone septum (Agilent Palo Alto, USA) immediately frozen at –20ºC until analysis.

Before analysis, the frozen vials were held at 4°C for overnight. The HS samples were kept at 55 °C with continuous stirring for 45 min for extraction of VCs. The VCs were adsorbed to a SPME fiber (50/30 µm; Supelco, Bellefonte, US) coated with carboxen, divinylbenzene, polydimethylsiloxane (CAR/DVB/PDMS) at the same temperature for 45 min.

The VCs were chromatographed on a capillary column (HP-Innowax; 60 m×0.25 µm film thickness×0.25 mm i.d.) equipped with 6890 GC and 5973 N mass spectrometer (Agilent, Palo Alto, USA). The column was initially held at 50°C for 5 minutes, then, programmed by a ramp of 5°C min^{-1} up to 240°C. The column was held for 5 minutes at final temperature. The SPME fibre was conditioned at 250°C for 30 minutes before analysis. Between the sampling stages, SPME fiber were injected routinely until no impurities were monitored. The constant flow at 1.0 mL min^{-1} of Helium as carrier was applied. Mass spectrometer was operated at the scan mode in the m z^{-1} range from 33 to 330 with 70eV electronic ionization. The volatile compounds were identified by matching the recorded mass spectra with WILEY 7n.1 and NIST 02.L libraries. The VCs were identified by taking care of retention index (RI) of compounds with above 85% similarity. The RI of detected compounds was calculated from 1 µL n-alkane series (C8-C20; Supelco 04070 Sigma, St. Louis,

USA) separated with the same chromatographic conditions. The relative concentrations of VCs were calculated by the ratio of peak area of each compound to total area of all peaks.

Preparation of Hawthorn Leaf Infusion

Hawthorn leaves separated from petiole were washed in pure water and dried at room temperature. Then, leaves were ground in a mortar until a fine powder was obtained. Distilled water previously heated to the boiling point was used for infusion. As described by Zhang *et al.* (2018), the leaf powder was infused for 5 min using a 1:50 (w:v) powder:water ratio. After that, the infusion was filtered through Whatman No. 1 (pore size 11 µm) filter paper (Whatman International Ltd., Maidstone, UK). The infusion was cooled to room temperature and used for analysis.

Physicochemical and Functional Properties of Hawthorn Leaf Infusions

Total solids and ash content of infusion samples were determined gravimetrically according to the standard method of AOAC (2000). pH value and titratable acidity were determined using a pH meter (Orion, Thermo, Beverly, USA) and 0.1N NaOH, respectively. Titratable acidity was expressed as citric acid (g L^{-1}). The color values (L*, a*, b*) of tea samples were determined using a colorimeter (Hunter ColorFlex-EZ, Virginia, USA) after calibrating by using black and white ceramic plates. The measurement was carried out at D65 illuminant and 10^{o} observation angle. The L*, a*, and b* values represent the brightness/darkness (100/0), redness/greenness (+/-) and yellowness/blueness (+/-), respectively.

The infusion samples diluted with distilled water (1:5, v:v) were used to determine the Total Phenolic Content (TPC) and Antioxidant activity (AOC) according to the protocols described by Masatcioglu *et al.* (2013) and Re *et al.* (1999), respectively. A TPC diluted infusion sample (0.25 mL) was mixed with 3.25 mL of distilled water, then Folin–Ciocalteu reagent (0.25 mL), and stirred for 3 min. Saturated Na_2CO_3 solution (0.5 mL) was added to mixture to neutralization and diluted to 5 mL of distilled water. Then, the mixture was vortexed (ZX3, Velp Scientifica, Usmate Velate, Italy) for 10 s and held at room temperature for 1 h in dark. An UV-VIS 1700 model spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure absorbance at 725 nm. A Gallic acid standard curve (R^2= 0.9996) with 5-points was used to calculate the total phenolic content. The TPC results were as Gallic Acid Equivalents (GAE) in dry weight (mg g^{-1}).

The ABTS (A1888, Sigma, St. Louis, USA) radical scavenging capacity method was used to determine the antioxidant activity of the infusions. Briefly, ABTS (7.5 mM) and $K_2S_2O_8$ (2.45 mM) was reacted at room temperature for 16 hours in dark to obtain ABTS radical cation (ABTS•+). Then, ethanol (1:2 v:v) was added to ABTS•+ solution to give an 0.8±0.02 absorbance value at 734 nm. ABTS•+ solution (10 mL) mixed with tea sample (100 µL) was vortexed thoroughly for 1 min. After 6 min incubation at room temperature, absorbance (734 nm) was measured using ethanol as blank. Trolox (23881-3, Sigma, St. Louis, USA) ranged from 200 to 600 ppm was used for calibration curve (R^2=0.9998). The results were expressed as Trolox Equivalent (TE) in dry weight (µmol g^{-1}).

Data Analysis

One-way Analysis Of Variance (ANOVA) was applied to determine the differences between sampling times using a SPSS statistical program (Version 24.00, IBM, USA). Duncan's test was applied to assess significantly different means among

collected times (P< 0.05). Correlation analysis between the total phenolic content and the antioxidant activity of samples was performed with the bivariate (Pearson's) correlation test (P< 0.05). The JMP software (Version 13, SAS Ins., North Carolina, USA) was used to analysis of principal component.

RESULTS AND DISCUSSION

Volatile Compounds of Hawthorn Leaf

A total of 78 VCs including alcohols (11), hydrocarbons (21), ketones (11), esters (16), aldehydes (18), and a benzene compound (1), were identified in the headspaces of hawthorn leaves (Table 1), as in finding of Ozderin *et al.* (2016) for *Crataegus* taxa leaves. A total of 15 VCs determined above 1% at each collecting time, accounting for approximately 80% of the identified volatiles, are listed in Table 2. In all three collection times, the volatile compound profile of the hawthorn leaf samples was almost like each other and most of the VCs were detected in trace levels. Aldehydes and hydrocarbons were the principal volatile compounds with regard to their number and their percentage composition. Regardless of leaf collecting time, aldehydes represented approximately 63% of total VCs identified, and the percentage of aldehydes increased from CT1 to CT2 and decreased again from CT2 to CT3 (Figure 1).

Benzaldehyde, the simplest aromatic aldehyde in nature, was the most abundant aldehyde (Table 2), in line with the findings of Robertson *et al.* (1993) and Ozderin *et al.* (2016). The tendency to decrease in benzaldehyde towards from CT1 to CT3 is compatible with an increase in benzene methanol, a reduction product of benzaldehyde (Table 2). Benzaldehyde can be derived from *trans*-cinnamic acid produced by the degradation of amino acid phenylalanine in many plants (Riu-Aumat ell *et al.,* 2005; Güler *et al.,* 2017). It is both a flavoring agent with carcino-static properties and a repellent to insects (Morgan, 2018). CT1-leaf had the highest level (51%) of benzaldehyde, followed by the CT2 (42%), and CT3 (39%) leaves. The other principal aldehydes determined in leaves were *trans*,*trans*-2,4-heptadienal, *trans*-2-hexenal, and *trans*,*cis*-2,6-nonadienal, which originated from the autoxidation of linolenic acid (Hatanaka, 1993). Among them, *trans*,*trans*-2,4-heptadienal was the second most abundant VC in CT1-leaf. It was not influenced by collection time, indicating that hawthorn leaf is rich in n-3 polyunsaturated fatty acids.

Alcohol chemical group decreased from CT1 to CT3 (Figure 1), but were the third most abundant compounds accounting for approximately 10% of total volatiles. *Cis*-3-hexenol was the predominant alcohol, followed by benzene methanol, hexanol, and *trans*-2-hexenol. Six-Carbon (C6) aldehydes and alcohols known as 'green leaf aldehydes and alcohols' were dominant in hawthorn leaf, which is derived from 13-hydroperoxide of linoleic and linolenic acid through the lipoxygenase pathway (Hatanaka, 1993; Güler *et al.,* 2013).

Esters, together with ketones, were the fourth most abundant VCs, which accounted for approximately 5% of the total volatiles identified. Among esters, *cis*-3-hexenyl benzoate was at a relatively higher level, but its level significantly decreased from CT1 to CT3 (Table 2), decreasing unsaturated C6 alcohols.

Regardless of the time of leaf sampling, hydrocarbons were the second most abundant chemical group (Figure 1). α-Farnesene (sesquiterpene) was the dominant hydrocarbon, presenting 18.6% of total VCs identified in CT3-leaf (Table 2). It has a high economic value due to its use in the pharmaceutical and cosmetic industries and its effect on insect resistance in many plant species (Liu *et al.,* 2019; Wang *et al.,* 2019). α-Farnesene, one of the major compounds identified in the olive tree and *Platanus orientalis* leaves, has recently been reported as one of the natural products effective

Table 1. The volatile compounds identified in hawthorn leaves collected at different times.

Volatile Compounds	RI	Leaf collection times[φ] CT1	CT2	CT3	Volatile Compounds	RI	Leaf collection times[φ] CT1	CT2	CT3
Esters (16)					*Aldehydes (18)*				
Butyl 2-butenoate[≠1]	1570				Acetaldehyde[≠1]	<800			
Butyl hexanoate[≠1]	1425				Benzaldehyde[≠1,2]	1551			
Butyl octanoate[≠1]	1623				3-Phenoxy-benzaldehyde	2072			
Butyl benzoate[≠1]	1891				*trans*-2-Hexenal[≠1,2]	1227			
Ethyl hexadecanoate[≠1]	>2000				Hexanal[≠1,2]	1060			
Hexyl hexanoate[≠1]	1620				Nonanal[≠1,2]	1404			
Hexyl decanoate[≠1]	2024				*trans*-2-Heptenal[≠1]	1341			
Hexyl benzoate[≠1,2]	>2000				*trans*,*trans*-2-4-Heptadienal[≠1,2]	1514			
cis-3-Hexenyl acetate[≠1]	1330				Octanal[≠1,2]	1303			
cis-3-Hexenyl 2-methyl-propanoate[≠1]	1471				2,6,6-Trimethyl-1-cyclohexene-1carboxaldehyde	1646			
cis-3-Hexenyl hexanoate[≠1]	1668				*trans*,*cis*-2,6-Nonadienal[≠1,2]	1606			
cis-3-Hexenyl benzoate[≠1]	>2000				*trans*,*trans*-2,4-Nonadienal[≠1]	1725			
Isopentyl propanoate	1493				*trans*-2-Decenal[≠1]	1662			
Methyl tetradecanoate	2021				*trans*,*cis*-2,4-Decadienal[≠1]	1787			
Methyl hexadecanoate[≠1]	>2000				*trans*,*trans*-2,4-Decadienal[≠1]	1835			
Methyl salicylate	1810				3-Dodecenal[≠1]	1772			
Hydrocarbons (21)					Salicyl aldehyde[≠1]	1711			
Alkane-alkene (11)					Geranial[≠1]	1755			
Cyclododecane[≠1]	>2000				*Alcohols (11)*				
Tetradecane[≠1]	1400				Hexanol[≠1,2]	1361			
Pentadecane[≠1]	1500				*trans*-2-Hexenol[≠1,2]	1414			
Hexadecane[≠1]	1600				*cis*-3-Hexenol[≠1,2]	1393			
Heptadecane[≠1]	1700				Octanol[≠1]	1565			
Octadecane[≠1]	1800				Octen-3-ol[≠1,2]	1457			
Eicosane[≠1]	2000				Benzeneethanol[≠1,2]	1938			
Tricosane[≠1]	>2000				Benzenemethanol[≠2]	1901			
Decylene[≠1]	1976				*cis*-Farnesol*[≠1]	1949			
Tridecylene	1347				Nerolidol[≠1]	2052			
trans-4,8-Dimethyl-1,3,7-nonatriene(C11)	1315				Salicyl alcohol[≠1]	2045			
Terpenes (10)					Surfynol[≠1]	2096			
4-Cyclopropylnorcarane[§≠1]	1695				*Ketones (11)*				
α-Terpinene[§≠2]	1716				1-Octen-3-one[≠1]	1313			
L-Carvone[§≠1]	1765				2(5H)-Thiophenone [≠1]	>2000			
Naphthalene[§]	1777				2-Nonanone[≠1]	1928			
α-Copaene[§≠1,2]	1506				6-Methyl-3,5-heptadien-2-one	1612			
β-Gurjunene[§]	1592				6-Methyl-5-hepten-2-one[≠1]	1352			
α-Bergamotene[§≠2]	1734				Geranylacetone[≠1]	1872			
α-Farnesene[§≠1,2]	1759				Hexahydrofarnesyl acetone[≠1]	>2000			
B-Bourbonene[§≠2]	1535				o-Hydroxyacetophenone[≠1]	1838			
13-Epimanoyl oxide[§]	>2000				α-Ionone[≠1]	1877			
Compounds with benzene (1)					β-Ionone[≠1]	1968			
1,2-Dichloro-benzene[≠2]	1462				β-Damascenone[≠1]	1849			

[φ] CT1, CT2, and CT3 indicate hawthorn leaves collected at three different times corresponding to immature, mature, and overmature fruit maturity stages, respectively. RI: Retention Index of VCs calculated by using *n*-alkanes (C_8-C_{20}) series. Three-point color scales from white to black represent compounds identified from minimum (not detected) to maximum percentages (51%) in hawthorn leaves and midpoint was taken as 50%. [§] Phenolic compound; [§] Terpene compounds; [#] Compounds have been previously determined in *C. azarolus* fruit (Dursun *et al.,* 2021), leaf and flower volatile oil content of hawthorn taxa (Ozderin *et al.,* 2016).

Table 2. The mean relative percentage composition of the major VCs in hawthorn leaves.[a]

No	Volatile Compounds	Leaf collection times			P value
		CT1	CT2	CT3	
1	Benzaldehyde	39.2±2.2[b]	50.9±4.7[a]	42.0±2.8[b]	*
2	α-Farnesene	2.8±0.1[b]	2.3±0.8[b]	18.6±0.2[a]	***
3	*trans*,*trans*-2,4-Heptadienal	7.3±0.2	7.2±1.3	7.8±0.0	ns
4	*trans*-2-Hexenal	5.6±0.8	4.3±1.1	3.6±0.6	ns
5	*cis*-3-Hexenol	5.4±0.4[a]	4.5±1.6[a]	1.4±0.4[b]	**
6	Benzenemethanol	1.5±0.1	2.1±0.7	2.3±0.3	ns
7	6-Methyl-5-hepten-2-one	2.8±0.1[a]	1.4±0.5[b]	1.6±0.5[b]	**
8	Geranylacetone	2.1±0.6[a]	1.4±0.7[ab]	0.7±0.1[b]	*
9	Acetaldehyde	0.8±0.5[b]	1.8±0.4[a]	0.9±0.3[b]	*
10	*cis*-3-Hexenyl benzoate	1.4±0.0[a]	1.0±0.4[a]	0.5±0.0[b]	**
11	3-Dodecenal	1.5±0.3[a]	0.8±0.3[b]	0.4±0.0[b]	**
12	Hexanol	1.4±0.2[a]	0.8±0.4[b]	0.5±0.3[b]	*
13	*trans*-2-Hexenol	1.8±0.1	0.9±0.6	1.3±0.3	ns
14	*trans*-4,8-Dimethyl-1,3,7-nonatriene	1.5±0.1[a]	0.8±0.2[b]	1.3±0.3[a]	*
15	*trans*,*cis*-2,6-Nonadienal	0.5±0.3[b]	0.5±0.2[b]	1.3±0.2[a]	**

[a] The results were expressed as means±standart deviations (n=9). The VCs had a relative percent value higher than 1% were considered major volatile compounds. CT1, CT2 and CT3 indicate hawthorn leaves collected at three different times corresponding to immature, mature, and overmature fruit maturity stages, respectively. (a-c) The mean values in the same row showing different small letters were significantly different (* P< 0.05; ** P< 0.01; *** P< 0.001), ns: not significant, P> 0.05.

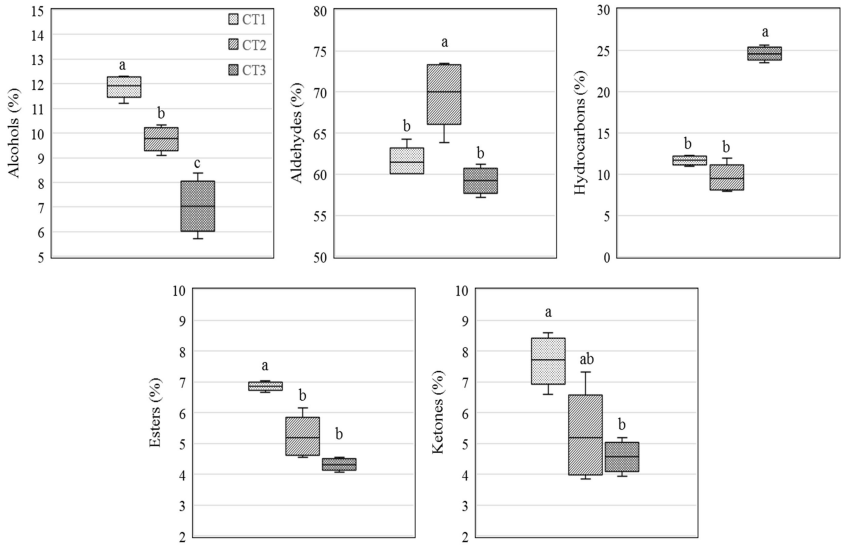

Figure 1. Box plots representing the percentage composition of the groups of VCs (except for compounds with benzene because of having a VC) in the leaves of Sultan hawthorn. CT1, CT2 and CT3 indicate hawthorn leaves collected at three different times corresponding to immature, mature and overmature fruit maturity stages, respectively. The center line of each box represents the mean, and the top and bottom of error bars represent the data's maximum and minimum, respectively (n= 9). [a-c] Mean values of hawthorn leaves collected at different times within each VC group were significantly different (P< 0.05).

against the COVID-19 virus (Güler *et al.,* 2017; Antonio *et al.,* 2020; Dursun *et al.,* 2021). Considering the potential health effects of α-farnesene, hawthorn leaves collected in CT3 may be more effective in medicinal usage. *Trans*-4,8-dimethyl-1,3,7-nonatriene, previously reported as a defensive compound against herbivores in bergamot essential oil (Turlings and Tumlinson, 1992), is substantially detected in Sultan hawthorn leaves.

Carotenoid-derived volatiles such as 6-methyl-5-hepten-2-one (sulcatone) and geranylacetone were also identified in hawthorn leaves. The first compound is derived from the catabolism of the chlorophyll phytol chain, and the latter from phytoene (Vogel *et al.,* 2008). Levels of sulcat one and geranylacet one changed significantly from CT1 to CT2.

According to discriminant analysis, PC1 (50.3%) and PC2 (33.3%) explained 83.6% of the total variance (Figure 2). PCA was successfully utilized in the discrimination of hawthorn species by Muradoğlu *et al.* (2021). In this study, we proved that the volatile compounds could also be used to discriminate and characterize hawthorn leaves based on the sampling times, indicating that PCA is a good indicator in such data analysis. CT1-leaf was distinguished from CT2 and CT3 leaves along PC1 having the highest percentages of hexanol, *trans*-2-hexenal, *trans*-2-hexenol, geranylacetone, *cis*-3-hexenol, 6-methyl-5-hepten-2-one, 3-dodecanal, and *cis*-4,8-dimethyl-1,3,7-nonatriene. CT2-leaf with the highest benzaldehyde and acetaldehyde was utterly separated from CT3 along PC2. CT3-leaf was characterized by the highest levels of α-farnesene, *trans*,*trans*-2,4-heptadienal,

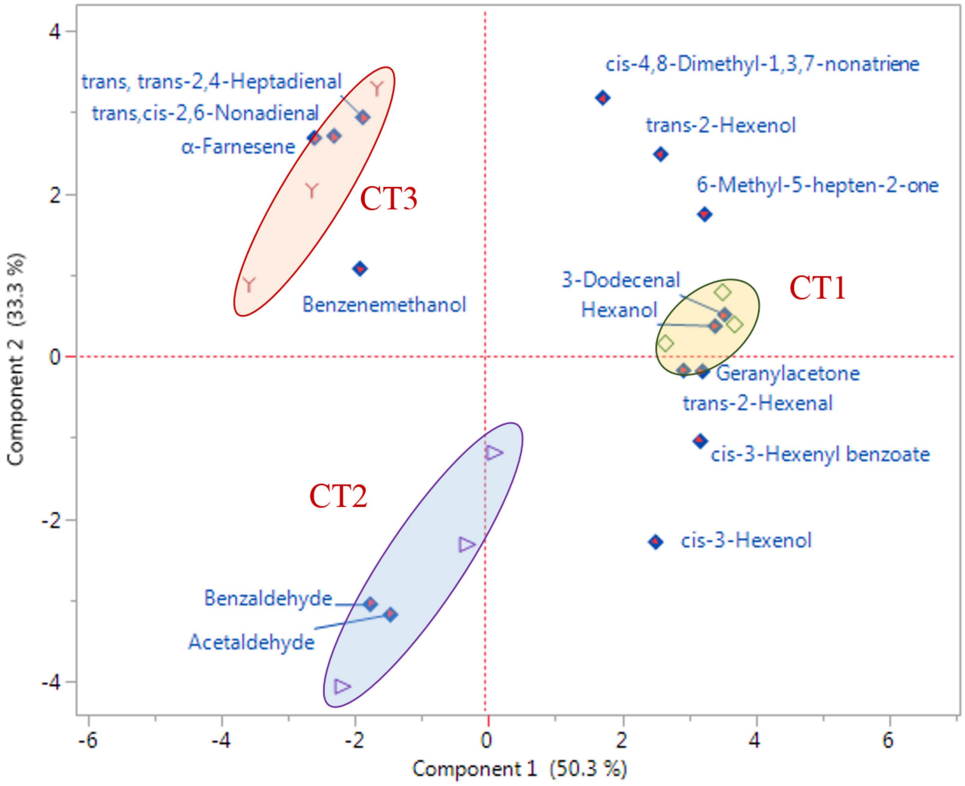

Figure 2. Results of principal component analysis of volatile compounds (numbered as specified in the Table 2) for hawthorn leaves. CT1, CT2 and CT3 indicate hawthorn leaves collected at three different times corresponding to immature, mature and overmature fruit maturity stages, respectively.

Table 3. Physicochemical and functional characteristics of infusions.[a]

Parameters	Leaf collection times[b]			P value
	CT1	CT2	CT3	
Total Solid (g L^{-1})	5.66±0.13	5.74±0.12	5.52±0.13	ns
Ash (g L^{-1})	0.63±0.07	0.58±0.13	0.58±0.13	ns
Titratable acidity (g L^{-1})	1.53±0.09^{a}	1.06±0.05^{b}	0.93±0.05^{c}	***
pH	7.04±0.01^{c}	7.17±0.01^{b}	7.34±0.01^{a}	**
L*	2.84±0.07^{a}	2.17±0.10^{b}	2.95±0.17^{a}	***
a*	-1.28±0.10^{a}	-1.06±0.16^{ab}	-0.92±0.04^{b}	*
b*	0.99±0.02^{b}	1.03±0.10^{b}	1.43±0.09^{a}	***
TPC (mg GAE g^{-1} DW)	29.91±0.11^{c}	33.25±0.32^{b}	34.57±0.18^{a}	***
AOA (µmol TE g^{-1} DW)	269.00±1.54^{c}	278.77±0.97^{b}	296.68±1.85^{a}	***

[a] The results were expressed as means±standard deviations (n= 9). CT1, CT2 and CT3 indicate hawthorn leaves collected at three different times corresponding to immature, mature, and overmature fruit maturity stages, respectively. DW: Dry Weight. [b] (a-c) The mean values in the same row showing different small letters were significantly different (* P< 0.05; ** P< 0.01; *** P< 0.001), ns: Not significant, P> 0.05.

benzenemethanol and *trans*,*cis*-2,6-nonadienal. A high negative eigenvector was obtained for benzaldehyde and acetaldehyde, which were the highest in CT2-leaf.

Physicochemical and Functional Properties of Hawthorn Leaf Infusion

This study is the first report on physicochemical and functional properties of infusions from leaves collected from 'Sultan' hawthorn trees at different times. The results of physicochemical analysis in infusion samples are shown in Table 3. The total solid and ash contents of leaf infusions were unchanged by the collection times, but the acidity was significantly varied. From CT1 to CT3, pH increased significantly (P< 0.01) and titratable acidity decreased. This may be related to utilizing the most acids in the process of respiration or an increase in alcoholic compounds in leaves because of their acid neutralizing capacity. A significant difference in color values of infusion samples was observed (Table 3). The L* (Lightness) and b* (yellowness) values were highest (2.95 and 1.43, respectively) at CT3 and the negative a* (greenness) value (1.28) was at CT1. Color values of leaf samples were also in line with the color of hawthorn fruit (Dursun *et al.,* 2021).

Total Phenolic Contents (TPCs) and Antioxidant activities (AOCs) of hawthorn leaf infusions are given in Table 3. The TPC increased significantly from CT1 to CT3, ranging from 29.91 to 34.57 mg GAE g^{-1} DW. The TPC values identified in leaf samples were within the ranges (12.41 to 82.74 mg GAE g^{-1} DW) obtained for leaves taken from 14 different hawthorn varieties (Alirezalu *et al.,* 2018). Sultan hawthorn leaf exhibited considerable antioxidant activity with values ranging from 269.00 to 296.68 µmol TE g^{-1} dry weight. The AOCs of leaf samples was also within ranges (75 to 379 µmol TE g^{-1}) obtained in 3 different *C. azarolus* var. *aronia* leaves (Özyürek *et al.,* 2012). The hawthorn leaf had higher TPC and antioxidant activity values in CT3 than in CT1 and CT2. Pavlovic *et al.* (2019) reported that hawthorn leaves had higher radical scavenging activities than hawthorn fruits. In addition, intake of polyphenol (1170 mg/day) is reported to be effective against cardiovascular diseases (Del Bo *et al.,* 2019). Thus, the consumption of 5 g Sultan hawthorn leaf powder from CT3 could provide nearly 50% of the required content associated with chronic disease.

CONCLUSIONS

This study determined the volatile compounds, phenolic content, antioxidant activity, and chemical composition in leaves

of Sultan hawthorn according to the fruit ripening stage. Total phenolic content and antioxidant activity increased significantly from CT1 to CT3 corresponding to immature to overmature fruit developmental stages. Based on discriminant analysis, volatile compounds classified Sultan hawthorn leaves according to the collection times. The findings from the present study have provided important information on the changes in leaf volatile compounds level, phenolic content, and antioxidant activity of Sultan hawthorn, the first standard cultivar in Turkey, according to fruit maturity stages. We envisage that the information will help decide the best time to harvest Sultan hawthorn leaves for use in folk medicine and food additives.

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ترکیبات فرار، محتوای فنلی و ظرفیت آنتی اکسیدانی در برگ های زالزالک سلطانی (.Crataegus azarolus L)

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چکیده

امروزه به گیاهانی مانند زالزالک (hawthorn) که منبع غنی از متابولیت‌های ثانویه (ترکیبات فرار و فنلی) در برگ‌های خود هستند و اثرات مفیدی بر سلامتی دارند، توجه زیادی می‌شود. در این پژوهش، ترکیبات فرار (VCs)، محتوای فنلی کل و فعالیت های آنتی اکسیدانی برگ زالزالک سلطانی که در سه زمان مختلف بر اساس مراحل بلوغ میوه (نابالغ، بالغ و بیش از حد بالغ) جمع آوری شده بود، بررسی شد.هدف اصلی ما تعیین این بود که آیا مشخصات موادفرار، محتوای فنلی کل و فعالیت آنتی اکسیدانی بسته به زمان نمونه‌برداری برگ تغییر می کند یا خیر. در مجموع ۷۸ VC در برگ ها شناسایی شد که ۱۱ مورد برای اولین بار بود. با پیشرفت در رسیدن وبلوغ میوه، مقدار بیشتر VCها تغییر کرد، محتوای فنلی و فعالیت آنتی اکسیدانی آن‌ها افزایش یافت و اسیدیته کم شد. نزآلدهید و α-فارنسن VCهای اصلی بودند که ۶۱% از کل VCهای شناسایی شده در برگ را در مرحله "بیش ازحد بالغ" تشکیل می دادند. تجزیه و تحلیل اجزای اصلی با موفقیت ترکیبات فرار در برگ زالزالک را در طول مراحل بلوغ میوه جداسازی کرد. این پژوهش برای اولین بار مروری کلی بر متابولیت های ثانویه برگ های کولتیوار زالزالک سلطانی در مراحل بلوغ میوه ارائه کرد. برگ زالزالک جمع‌آوری‌شده در مرحله میوه "بیش ازحد بالغ" ، پتانسیل بالایی در متابولیت‌های ثانویه و ظرفیت آنتی‌اکسیدانی دارد.