Emirates Journal of Food and Agriculture. 2023. 35(1): 78-85
doi: xxxxx
http://www.ejfa.me/

RESEARCH ARTICLE

Evaluation of physicochemical characteristics, antioxidant activity and phenolic profile of *Crataegus* species in Malatya, Turkey

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ABSTRACT

This study aims to evaluate the pomological and physicochemical characteristics, antioxidant activity and phenolic profile of the three cultivars of hawthorn. A total of nine phenolic compounds were identified in *C. orientalis* subsp. *orientalis* fruits by using RP-HPLC-DAD system. These comprised of phenolic acids (i.e., gallic, chlorogenic, caffeic, syringic and p-coumaric acids) and flavonoids (i.e., catechin, epicatechin, rutin and procyanidin-B2). The major phenolic compounds in the hawthorn samples were procyanidin-B2, rutin and chlorogenic acid. The total phenolic contents of samples ranged from 2.86 to 13.81 mg gallic acid equivalent/g dry weight (DW). β-Carotene contents and antioxidant activities (DPPH and ABTS assays) of fruits were found to be high levels. In conclusion, the hawthorn cultivars used in the study differ in composition as they are subspecies of each other. It was concluded that the phenolic compounds of hawthorn are the primary substances responsible for antioxidant activity, and the fruit has shown that it has an important place with these properties. It is thought that this study will lead to the use of hawthorn in the food industry in the future, thus adding value to this fruit and enabling it to be used in new products.

Keywords: Antioxidant capacity, Bioactive compounds, *Crataegus* spp., Hawthorn, Phenolic compounds

INTRODUCTION

Fruit and vegetable consumption delays the aging in human and reduces the risks of some diseases such as cancer, rheumatoid arthritis, lung diseases, cataracts, Parkinson's or Alzheimer's diseases (Szajdek and Borowska, 2008), and is also used for the treatment of cardiovascular disorders, hypertension and atherosclerosis. The current protective effect is attributed to phytochemicals such as phenolic compounds, vitamins (C and E), carotenoids, which have antioxidant properties in the structures of fruits and vegetables (Nabavi et al., 2015).

Phenolic compounds are secondary metabolites with high antioxidant activity and formed due to the plant's defense mechanism against environmental stress conditions (UV radiation, pathogens, etc.). The antioxidant activity is realized by the scavenging of reactive oxygen species in tissues. These compounds act by inhibiting the activity of metals and enzymes that catalyze oxidation reactions (Szajdek and Borowska, 2008).

Hawthorn (*Crataegus* spp.) is a fruit that grows naturally in Turkey, belonging to the *Rosaceae maloidea* sub-family (Dönmez, 2004). It is mainly grown in northern regions of East Asia, Europe, and North America (González-Jiménez et al., 2018). Hawthorn which generally has thorns grows mainly on limestone soils, wooded and sunny areas up to 1500 m above sea level. This species does not need a lot of water when growing (Nazhand et al., 2020). The genus *Crataegus* represents about 280 species, including hybridized species (Kumar et al., 2012; Edwards et al., 2012). Approximately 21 species have been identified in Turkey, and it has been reported that there are more taxa in the ongoing studies, and these taxa are determined as "Hawthorn" (Dönmez, 2004).

Crataegus spp. plant is rich in phenolic compounds and these phenolic compounds in hawthorn differ between

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Received: 08 June 2022; **Accepted:** 19 October 2022

different parts (fruit, leaf and flower) of the plant. Different parts of *Crataegus* species have antioxidant, antiviral, anti-inflammatory, antimicrobial, gastroprotective, antihyperlipidemic, hypoglycemic, hepatoprotective and immunostimulant activities (Kumar et al., 2012). It is reported that fruits, leaves and flowers of hawthorn species are used to treat heart diseases due to their antispasmodic, cardiotonic, hypotensive and antiatherosclerotic activities in European countries (Edwards et al., 2012). Swaminathan et al. (2010), reported a cardioprotective and antiradical effect of phenolic extracts from Crataegus oxycantha fruits.

González-Jiménez et al. (2018), study investigating the polyphenol profile and antioxidant content of fruit extracts reported that hawthorn has excellent potential as a natural source of antioxidant phenolic compounds and can be used as a nutraceutical and functional food.

It has been reported that flavonoids and oligomeric proanthocyanidins (OPCs), which constitute the major components, are responsible for the pharmacological activities of hawthorn (Yao, 2008). Catechin and (-)-epicatechin, procyanidin-B2 are the main flavan-3-ols detected in hawthorn fruit, while quercetin, chlorogenic acid, gallic acid, caffeic acid, naringenin and cratenacin are other phenolics of the fruit (Nabavi et al., 2015; Coklar et al., 2018). There is an increasing interest in these natural antioxidant sources, which are rich in phenolic compounds. Hawthorn grows in almost every region of Turkey, and the lack of determination of the characteristics of this fruit may cause the extinction of very valuable hawthorn species and varieties. In this regard, the current study focused on evaluating some pomological characteristics, some physicochemical properties (pH, total acidity, total solid content, soluble solids content and maturity index), total phenolic contents, antioxidant capacities (DPPH and ABTS) and phenolic compound contents of 3 different *C. orientalis* fruits grown in Malatya province. In addition, β-Carotene, vitamin A and sugar contents of fruits were investigated in HPLC.

MATERIAL AND METHODS

Fruit sample and chemicals

In the research, three different fruits belonging to the hawthorn plant belonging to the non-cultured *C. orientalis* L. species in the natural flora of Malatya province were used (H1: Yellow-small, H2: Yellow-big, H3: Orange, Fig. 1). *C. orientalis* subsp. *orientalis* fruits were collected in November 2019 from Hekimhan (38° 48' 59.49" N, 37° 55' 50.8" E) county of Malatya in Turkey and taxonomically identified by Prof. Dr. Ali Aslan DÖNMEZ, a senior taxonomist from the Department of Biology, Hacettepe University, Ankara, Turkey. The colors of hawthorn fruits vary from yellow to orange. Fruits were cut into halves and seeds were removed and stored in polyethylene bags at −20 °C until extraction.

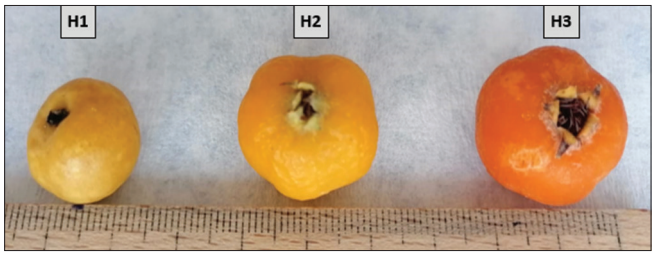

Fig 1. H1: Yellow-small hawthorn, H2: Yellow-big hawthorn, H3: Orange hawthorn.

All chemicals were used to analytical-grade. Folin-Ciocalteau phenol reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl), phenolic acid standards (rutin, catechin, caffeic, chlorogenic, procyanidin-B2, epicatechin, syringic, p-coumaric and gallic acid), sugar standards (fructose, glucose and maltose), organic acid standard (citric acid), vitamin A standard (β-carotene), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), potassium chloride and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, USA). The other chemicals were obtained from Merck (Darmstadt, Germany).

Pomological features

Pomological features, including sizes and weight measurements, of 3 hawthorn cultivars were determined using a digital micrometer and an analytical balance, respectively.

Extraction of phenolic compounds

Phenolic compounds were extracted according to the method described by Karabulut et al. (2018) with slight modifications. Extraction procedure is demonstrated in flow diagram (Fig. 2). Briefly, frozen hawthorn puree was thawed at room temperature. A 10 g of sample was homogenized with 35 mL of solvent mixture (1:80:19, v/v/v, ratio HCl: methanol:water) and then sonicated using ultrasonic bath. The mixture was centrifuged (SL16R Centrifuge Series, Thermo Fisher Scientific, Massachusetts, USA) at 4900 × g for 6 min at 4 °C. Supernatants were collected and used for total phenolic content, DPPH, ABTS assays, and individual phenolic compounds after filtration through a 0.45 μm nylon filter (Lubitech, Songjiang, China). The extract was kept at −18 °C until analysis.

Physicochemical analyses

Total acidity was determined by alkali titration method and results were expressed as g citric acid per 100 g of fresh

weight (FW). pH of hawthorn samples was measured by a pH meter (Orion Star A111, Thermo Scientific, USA) using standard methodology (AOAC, 2005). Total solid content of the samples were determined using a vacuum oven (70°C, 0.07–0.08 MPa) until constant weight and results were given by gravimetric calculation (İsmail and Gökçe Kocabay 2021). °Brix was determined by using a digital refractometer PTR 300 model (Index Instruments., Cambridgeshire, UK) at 20 °C and results were expressed in °Bx. The maturation index was calculated as the ratio of °Bx to total acidity reported in Valero and others (2006). Surface color which expressed as L*, a*, b*, Chroma, and hue angle (h°), was measured using a chromameter (Konica Minolta, model CR-5, Osaka, Japan) with a 3 mm petri dish with illuminant D-65 and adjusted observer of 10° angle.

Total and individual phenolic composition

The total phenolic content in the hawthorn fruits was determined using Folin-Ciocalteu's method as described in Kocabey et al. (2016). The absorbance was read at 725 nm using a UV- spectrophotometer (Shimadzu model UV-1700, Shimadzu Corporation, Kyoto, Japan). The results were expressed as mg of gallic acid equivalents (GAEs) per 1 g of DW. Individual phenolic composition of hawthorn fruit extracts was determined by RP-HPLC (Shimadzu Corp., Model LC 20AD prominence, Kyoto, Japan) as described in Karabulut et al. (2018).

Antioxidant activity

DPPH assay: DPPH radical-scavenging activity of hawthorn fruit extracts was evaluated according to a modified version of the method described by Brand-Williams et al. (2005). Aliquots of the hawthorn extract (100 µL) were mixed with 1.9 mL of DPPH (1 mg/mL) in methanol and absorbance was measured at 517 nm after 60 min at 30 °C using UV-Vis spectrophotometer. A calibration curve was acquired from measuring the absorbance of known concentrations of trolox. The results were indicated as mmol trolox equivalent (TE) per 1 kg of DW. Each value is the mean ± SD of triplicate measurements.

ABTS assay: ABTS assay in hawthorn fruit extracts was evaluated according to a modified version of the method described by Re et al. (1999). A calibration curve was obtained from measuring the absorbance of known concentrations of trolox. The results were stated as mmol TE/kg of DW. Each value is the mean ± SD of triplicate measurements.

β-Carotene and vitamin A tests

The analysis procedure was applied as Sadler et al. (1990) described with slight modifications. 1 mL of the total extract containing β-Carotene was taken into the HPLC vial, the hexane in the extract was evaporated under a nitrogen stream at ambient temperature. The residue was resolved in 1 mL tetrahydrofuran: methanol (1:9, v/v) containing 0.1% BHT and filtered through a 0.45 µm PTFE filter (Lubitech, Songjiang, China). A 20 µL of the extract was then injected into the HPLC (250 × 4.6 mm, 5 mm; GL Sciences Inc., Tokyo, Japan). The mobile phase consisted of methanol and acetone (70:30, v/v) and elution was achieved at an isocratic flow rate of 1.0 mL/min at 450 nm (for β-Carotene) and 325 nm (for vitamin A). A calibration curve was prepared with known concentrations of β-Carotene and vitamin A standards and quantifications were calculated using the calibration curves. The concentrations were expressed as mg/kg DW.

Organic acid and sugars

Determination of organic acid and sugars was achieved according to the method described by Demir et al. (2014) with slight modifications. Briefly, 5 g of hawthorn puree was diluted with 25 mL of MilliQ water (Millipore, Bedford, MA, USA) and centrifuged at 4900 × g for 20 min at 4 °C. The extract was filtered through a 0.45 µm nylon filter (Lubitech, Songjiang, China) and the filtrate was used for HPLC analysis of sugars (glucose, fructose and maltose) and organic acid (citric acid) as described in Sturm et al. (2003). Quantification was carried out using a calibration curve prepared with five-point concentrations of corresponding standards. The concentrations were expressed as mg/kg DW.

Statistical analysis

The obtained data were analyzed using one-way analysis of variance (ANOVA) by statistical software (SPSS for Windows, version 25.0) to analyze the significance of individual variations among the control and experimental groups. Duncan's multiple comparison test was used to compare means at a level of $P<0.05$.

RESULTS AND DISCUSSION

Pomological features and physicochemical analyses

Some pomological characteristics of hawthorn fruits collected from different regions are given in Table 1. There

Table 1: Some pomological characteristics of 3 different cultivars of hawthorn

Hawthorn varieties	Fruit weight (g)	Fruit seed (mm)	Fruit width (mm)	Fruit length (mm)	Index (width/length)
H1	2.12 ± 0.10^{a}	2.00 ± 0.00	15.74 ± 0.39^{a}	13.32 ± 0.22^{a}	1.18 ± 0.02^{b}
H2	4.30 ± 0.16^{b}	3.00 ± 0.00	20.03 ± 0.63^{b}	18.32 ± 0.32^{b}	1.09 ± 0.04^{a}
H3	5.75 ± 0.14^{c}	5.00 ± 0.00	21.66 ± 0.16^{c}	19.58 ± 0.66^{c}	1.11 ± 0.04^{a}

Values followed by same superscripts in a column do not differ significantly ($P<0.05$)
(H1: Yellow-small hawthorn; H2: Yellow-big hawthorn; H3: Orange hawthorn)

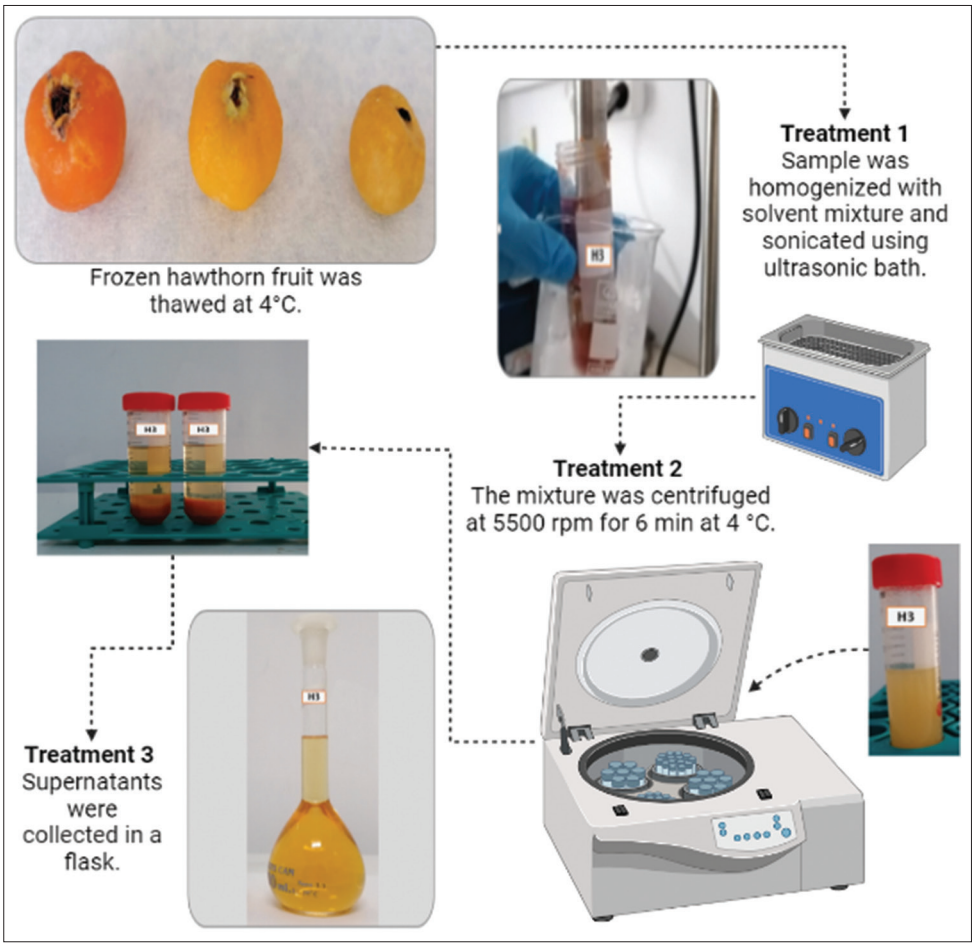

Fig 2. Flow diagram of the extraction.

were some differences in some pomological characteristics. H3 has the highest fruit weight (5.75±0.14 g), while H1 has the least (2.12±0.10 g). Seed hawthorn cultivars (H1, H2 and H3) were determined as 2.00±0.00, 3.00±0.00 and 5.00±0.00 mm, respectively. Ercisli et al. (2015) reported that the fruit weight of the hawthorn species they selected from Malatya varied between 0.76-4.27 g and the soluble solids content varied between 6.71-15.83°Bx. The difference in fruit weights and dimensions of germplasm under the same geographical conditions may originated from the genotypic effects. Higher fruit weight and a higher flesh ratio are the most crucial desirable fruit characteristics in hawthorn breeding programs. It has been reported that fruit length and width of hawthorn genotypes in Turkey ranged between 8.43±0.12 and 17.58±0.23 mm and between 6.56±0.93 and 20.71±1.22 mm, respectively (Gurlen et al., 2020).

Table 2 shows some physicochemical properties of 3 different cultivars of hawthorn. In the study, total acidity values (as citric acid) in H1, H2 and H3 hawthorn samples were determined as 1.24%, 0.96% and 1.28%, respectively. While the total acidity value was found close to each other

Table 2: Some physicochemical properties of 3 different cultivars of hawthorn

Physicochemical properties	Hawthorn varieties		
	H1	H2	H3
pH	4.17±0.01^{b}	4.17±0.01^{b}	4.12±0.01^{a}
Total acidity (% citric acid)	1.24±0.00^{b}	0.96±0.01^{a}	1.28±0.04^{b}
Total solid content (%)	29.86±0.03^{c}	22.58±0.09^{a}	24.09±0.07^{b}
Soluble solids content (°Brix)	12.99±0.00^{b}	11.98±0.00^{a}	16.99±0.00^{c}
Maturity index	10.48±0.02^{a}	12.48±0.10^{b}	13.30±0.46^{c}

Values followed by same superscripts in a row do not differ significantly (P<0.05), (H1: Yellow-small hawthorn; H2: Yellow-big hawthorn; H3: Orange hawthorn)

in the H1 and H3 samples, a lower value was determined in the H2 sample (P<0.05). Gundogdu et al. (2014) determined the total acidity as 1.78% for *C. orientalis* var. *orientalis* and expressed citric acid as the dominant organic acid for some *Crataegus* species. Citric acid is known to be the predominant organic acid in most *Crataegus* species, followed by malic and succinic acid (Gurlen et al., 2020; Gundogdu et al., 2014). In addition, in this study, citric acid contents from lowest to highest were determined as

H3 (10.97±0.05 mg/kg DW), H1 (13.12±0.35 mg/kg DW) and H2 (16.54±0.33 mg/kg DW) varieties (Table 7). In different studies, the citric acid content for *C. orientalis* was expressed as 2.532 g/100 g FW (Gundogdu et al., 2014) and an average of 1.56 g/100 g FW (Muradoğlu et al., 2019). No significant difference was observed between the pH values of hawthorn ($P>0.05$). It is seen that the H1 variety has the highest total dry matter content (29.86±0.03%), while the H3 variety has the highest soluble solids content (16.99°Bx). The maturity index (SSC/TA) of hawthorn cultivars was determined as 13.30±0.46 (H3), 12.48±0.10 (H2) and 10.48±0.02 (H1). Yanar et al. (2011) reported that the total soluble solids content and pH value of hawthorn genotypes varied between 11.66% and 24.00% and 3.12 and 4.09, respectively, in some studies conducted in a different regions of Turkey. In the study performed by Alirezalu et al. (2020) for 15 different species belonging to the genus *Crataegus*, pH (3.03-4.35), titration acidity (0.75-1.17%), total dry matter (5.27-19.43%) and the soluble solids contents (14.99-23.43%) ranged a wide interval. The physicochemical characteristics of fruits are important indicators of their quality and maturation.

Color directly affects the appearance and the consumer acceptability of the fruits and the color result are shown in Table 3. The effect of cultivar on the color values was significant and the color in the fruits ranged from yellow to orange. In this study, L* values from lowest to highest were determined as H3, H1 and H2 varieties. The a* values represented reddish color and showed a higher value in orange-colored hawthorn fruits (H3) with 24.07±0.57. The b* values were higher in yellow-colored H1 (36.41±0.49) and H2 (34.14±0.44) cultivars. The h° values of H1, H2 and H3 in the hawthorn cultivars were 64.94±0.55, 66.31±0.31 and 54.11±0.89, respectively and the h° values increase as the color getting darker.

Total phenolic contents, phenolic profile and antioxidants capacity

Total phenolic content values of hawthorn fruits (H1, H2 and H3) are shown in Table 4. The total phenolic contents detected in the samples of H1, H2 and H3 were 13.81±0.93, 2.86±0.24 and 13.43±0.89 mg GAE/g DW, respectively. The phenolic content of plant fruits is influenced by genotype, habitat conditions and ripeness. Flavonoids, one of the phenolic compounds, have been reported as the most critical and major compounds responsible for the important pharmacological activities of *Crataegus* species. The total and individual phenolic compounds are the primary agents responsible for the antioxidant activity of hawthorn fruits. It is seen that different results have been obtained in studies examining the antioxidant activity and total phenolic content of the fruits of varying hawthorn species. Čopra-Janićijević et al. (2018) determined that the total phenolic content in extract for *C. microphylla* fruits ranged between 2.47 and 8.63 mg GAE/g DW, which are in agreement with our present results. Coklar et al. (2018) determined the total phenolic content in fresh hawthorn fruit belonging to *C. orientalis* species as 13.36 mg GAE/g DW. Tahirović and Bašić (2014) determined the total phenolic content of the fruit between 2.02 and 4.60 mg GAE/g FW in the extraction they carried out for *C. monogyna* L. using different ratios of water and different solvents (water, ethanol, methanol). It is observed that the phenolic yield generally increases in extractions where organic solvents are mixed with water in certain proportions (Spigno et al., 2007).

Identification of phenolic compounds was carried out by comparing with the retention times of authentic standards. A total of nine phenolic compounds were determined in HPLC chromatograms at 280, 320 and 360 nm wavelengths in hawthorn fruits. Five major phenolic compounds, including procyanidin B2, rutin, syringic acid, epicatechin,

Table 3: Color values for 3 different cultivars of hawthorn

Hawthorn varieties	Color parameters				
	L*	a*	b*	Chroma (C*)	Hue angle (h°)
H1	44.37±0.54^{b}	17.02±0.21^{b}	36.41±0.49^{b}	40.19±0.37^{b}	64.94±0.55^{b}
H2	49.82±0.62^{c}	14.72±0.44^{a}	34.14±0.44^{a}	37.27±0.38^{a}	66.31±0.31^{c}
H3	39.28±0.26^{a}	24.07±0.57^{c}	33.60±0.81^{a}	41.63±0.59^{c}	54.11±0.89^{a}

Values followed by same superscripts in a column do not differ significantly ($P<0.05$)
(H1: Yellow-small hawthorn; H2: Yellow-big hawthorn; H3: Orange hawthorn)

Table 4: Antioxidant capacities and total phenolic content of 3 different cultivars of hawthorn

Hawthorn varieties	Total phenolic content (mg GAE/g DW)	ABTS (mmol TE/kg DW)	DPPH (mmol TE/kg DW)
H1	13.81±0.93^{b}	57.45±0.93^{a}	95.26±0.36^{b}
H2	2.86±0.24^{a}	62.78±0.59^{b}	72.64±0.86^{a}
H3	13.43±0.89^{b}	62.01±0.67^{b}	114.57±0.23^{c}

Values followed by same superscripts in a column do not differ significantly ($P<0.05$)
(H1: Yellow-small hawthorn; H2: Yellow-big hawthorn; H3: Orange hawthorn)

AQ1 **Table 5: Phenolic compounds (mg/kg DW) of 3 different cultivars of hawthorn**

Wavelength	Phenolic compounds	Hawthorn varieties		
		H1	H2	H3
280 nm	Gallic acid	4.78±0.74^{c}	3.46±0.01^{b}	1.41±0.01^{a}
	(+)-Catechin	146.42±0.69^{c}	30.62±0.50^{a}	82.14±0.55^{b}
	Procyanidin-B2	294.91±0.13^{b}	63.17±0.54^{a}	314.94±0.44^{c}
	(-)-Epicatechin	46.51±0.88^{b}	20.58±0.37^{a}	88.11±0.88^{c}
	Syringic acid	4.77±0.14^{a}	195.98±0.53^{c}	79.73±0.97^{b}
320 nm	Chlorogenic acid	206.02±0.30^{c}	40.23±0.56^{a}	115.61±0.40^{b}
	Caffeic acid	0.80±0.03^{c}	0.23±0.00^{b}	0.12±0.01^{a}
	p-Coumaric acid	48.47±0.73^{c}	6.35±0.86^{a}	38.96±0.70^{b}
360 nm	Rutin	282.65±0.25^{c}	23.29±0.64^{a}	216.66±0.43^{b}

Means±SD within a row without a common lowercase superscript differ significantly ($P \leq 0.05$).
(H1: Yellow-small hawthorn; H2: Yellow-big hawthorn; H3: Orange hawthorn)

Table 6: β-Carotene and vitamin A content of 3 different cultivars of hawthorn

Hawthorn varieties	ß-Carotene (mg/kg DW)	Vitamin A (mg/kg DW)
H1	70.14±0.87^{a}	35.76±0.86^{a}
H2	159.22±0.60^{b}	50.34±0.82^{c}
H3	638.27±0.94^{c}	42.92±0.11^{b}

Values followed by same superscripts in a column do not differ significantly ($P<0.05$)
(H1: Yellow-small hawthorn; H2: Yellow-big hawthorn; H3: Orange hawthorn)

and chlorogenic acid, were quantified in extracts of fruits of three varieties of *C. orientalis*. The most common phenolic compounds detected in hawthorn fruits (H1, H2 and H3) were procyanidin-B2 (294.91±0.13 mg/kg DW), rutin (282.65±0.25 mg/kg DW); syringic acid (195.98±0.53 mg/kg DW), procyanidin-B2 (63.17±0.54 mg/kg DW), procyanidin-B2 (314.94±0.44 mg/kg DW), rutin (216.66±0.43 mg/kg DW), respectively.

Since phenolic compounds, especially procyanidins and flavonoids, are the major bioactive compounds in *Crataegus* fruits, the presence of these compounds is compatible with antioxidant activity (González-Jiménez et al., 2018). In our study, it was determined that procyanidin-B2, a dimer of epicatechin and catechin, is the most important phenolic compound of *C. orientalis*. Çoklar and Akbulut (2016), who studied the phenolic contents of fruits from the *C. orientalis* species, defined rutin as one of the essential phenolic components. In the study of Muradoğlu et al. (2019) rutin, catechin and caffeic acid are the main phenolic compounds found in the fruits of four different species of *Crataegus*. It was determined that *C. monogyna* subsp. *monogyna* Jocq was the species with the highest rutin content among all species, and the rutin content was 61.6 mg/100 g FW on average. Čulum et al. (2018) determined the contents of rutin, gallic acid and chlorogenic acid in different hawthorn species were in the range of 0.03-13.49 mg/g DW, 0.001-0.082 mg/g DW, 0.19-8.70 mg/g DW, respectively, which are in agreement with our present results.

Antioxidant activity (DPPH and ABTS) values of hawthorn fruits (H1, H2 and H3) are shown in Table 4. ABTS antioxidant activities of the samples of H1, H2 and H3 were 57.45±0.93, 62.78±0.59 and 62.01±0.67 mmol TE/kg DW, respectively, while DPPH antioxidant activity values were 95.26±0.36, 72.64±0.86 and 114.57±0.23 mmol TE/kg DW, respectively. The ABTS^{+} method is generally applied to extract hydrophilic and lipophilic antioxidative substances from the food matrix (Re et al., 1999). Coklar et al. (2018) determined the antioxidant activity in fresh hawthorn fruit belonging to *C. orientalis* species as 35.78 mmol TE/kg DW and 57.74 mmol TE/kg DW for DPPH and ABTS, respectively, which lower than our results. In one study, examining the antioxidant activity of 18 hawthorn species grown in Turkey, it was noted that the antioxidant activity was in the range of 2.91-57.61 μmol TE/g FW (Ercisli et al. 2015). Çoklar et al. (2016) investigated the antioxidant activity of hawthorn (*C. orientalis*) fruit and the effects of different solvents on the extraction of phenolic compounds. They have determined that the antioxidant activity values were in the range of 0.66 to 4.06 mmol TE/100 g DW.

The antioxidant activity studies on *Crataegus* species have exhibited a higher level of antioxidant potential due to their polyphenolic compounds such as flavonoids and procyanidins. It is thought that factors such as growing conditions, natural habitat, genotype, growth stage, extraction procedure, solvent content used in the extraction and total phenolic contents are important role on the antioxidant activity with regardless of methodology (ABTS, DPPH or any others).

β-Carotene, vitamin A and sugar contents

β-Carotene (providing precursors for vitamin A synthesis) are the most common lipid-soluble antioxidants in fruits and vegetables. β-Carotene turns into vitamin A in intestinal epithelial cells and palmitate. Vitamin A has important functions in increasing the body's resistance to infections, vision functions, bone growth, skin development,

Table 7: Sugar and organic acid contents of 3 different cultivars of hawthorn

Hawthorn varieties	Glucose (mg/kg DW)	Fructose (mg/kg DW)	Maltose (mg/kg DW)	Citric acid (mg/kg DW)
H1	161.73±0.37[a]	75.76±0.08[a]	13.38±0.27[b]	13.12±0.35[b]
H2	201.30±0.51[b]	179.41±0.04[c]	9.80±0.01[a]	16.54±0.33[c]
H3	204.00±0.33[c]	178.15±0.19[b]	9.72±0.65[a]	10.97±0.05[a]

Values followed by same superscripts in a column do not differ significantly (P<0.05)
(H1: Yellow-small hawthorn; H2: Yellow-big hawthorn; H3: Orange hawthorn)

cell division, reproduction and also strengthening the immune system. β-Carotene and vitamin A contents of H1, H2 and H3 hawthorn cultivars are shown in Table 6. β-Carotene content of H3 (638.27±0.94 mg/kg DW) sample was determined to be higher than H1 and H2 samples (P>0.05). However, the vitamin A content was not higher in H3 sample, H2 hawthorn contained (50.34±0.82 mg/kg DW) higher vitamin A than other samples. The orange color of the H3 sample correlated with the β-Carotene content as seen in Table 6. Ibrahim et al. (2017) determined that the content of vitamin A and β-Carotene in *C. laevigata* ranged from 0.76 to 1.14 mg/kg and 2.88 to 3.87 mg/kg, respectively.

Fructose and glucose were identified as the principal sugars in *C. orientalis* and their levels varied importantly depending on hawthorn samples (Table 7). Glucose content was higher in all hawthorn samples compared to other sugars. The glucose, fructose and maltose contents (mg/kg DW) of the H1, H2 and H3 hawthorn samples were determined as 161.73±0.37, 75.76±0.08 and 13.38±0.27; 201.30±0.51, 179.41±0.04 and 9.80±0.01; 204.00±0.33, 178.15±0.19 and 9.72±0.65, respectively. Muradoglu et al. (2019) determined the glucose, fructose and maltose content for *C. orientalis* as 9.89 mg/100 g FW, 17.16 mg/100 g FW and 0.036 mg/100 g FW, respectively. The results presented here are agreement with those reported by previous studies for *Crataegus* species.

CONCLUSIONS

This study determined physicochemical characteristics, antioxidant activity, and total and individual phenolic contents of 3 different hawthorn samples belonging to the same variety. While the amount of solid soluble content of the H3 sample is higher than the other samples, it is the H1 sample with the highest total dry matter content. The phenolics in its content affect the antioxidant activity of hawthorn fruit. While the total phenolic contents of H1 and H3 samples are similar, the H2 sample has a lower total phenolic content. The most abundant phenolic compound in H1 and H3 samples was procyanidin-B2. The most abundant phenolic compounds in the H2 sample are syringic acid procyanidin-B2 and chlorogenic acid respectively. When the β-Carotene contents of the samples were examined, it was concluded that the orange-colored H3 sample had the highest β-Carotene content. The most abundant sugar in hawthorn samples is glucose, fructose and maltose, respectively. It is thought that the different colored hawthorn samples used in the study are different subspecies from each other and it affects the hawthorn compositions.

The authors are grateful for the financial support of the Scientific Research Council of Inonu University (BAP Project Number FDK-2019-1994).

Author contributions

Fatma Sezer Öztürk: Research design, conducted research work, and data analysis; İncilay Gökbulut: Research design, data analysis, guidance, and correction of the manuscript. Ali Adnan Hayaloğlu: Guidance, data analysis, and manuscript correction.

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Author Queries???

AQ5: Kindly cite table 5 in the text part