Turkish Journal of Agriculture - Food Science and Technology, 9(12): 2265-2271, 2021
DOI: https://doi.org/10.24925/turjaf.v9i12.2265-2271.4610

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Phytochemicals and Aroma Compounds Content of Crabapple (*Malus tribolata* C.K. Schneid.) Genotypes in Kahramanmaraş Province

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ARTICLE INFO

Research Article

Received : 06/08/2021
Accepted : 12/09/2021

Keywords:
Wild apple
Phenolic
Antioxidant
Volatile compounds
Apple aroma

ABSTRACT

Crabapple (*Malus tribolata* C.K. Schneid.), which one of the rare wild fruit trees in Turkey. This study was carried out to determine the aroma compounds, phenolic compound, antioxidant capacity and some fruit properties of fruits obtained from 2 different crabapple genotypes. The amount of phenolic substance was done by Folin-Ciocalteu method; antioxidant analysis was done using DPPH technique. With respect to antioxidant capacities, the highest value (70.12%) was determined in 46 EL 01 genotype. We found that 46 EL 01 genotype had the highest total phenolic content (839.13 mg/100 g). Determination of volatile compounds that play a major role in fruit quality using the HS-SPME/GC/MS technique, total of 37 aroma compound, namely 7 alcohols, 2 terpenes, 5 aldehydes, 17 esters, 1 ketone, 3 acids and 2 other compounds, were found in two different crabapple genotypes. Total aroma compounds in 46 EL 01 and 46 EL 02 genotypes were calculated as 101.78 µg/L and 102.26 µg/L, respectively. As a result, it has been determined that crabapple, which is a wild fruit, has high phenolic and antioxidant contents and also has many aroma compounds.

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Introduction

Crabapple (*Malus tribolata* C.K. Schneid.), which is also known as wild apple, is a species of apple with different synonyms which are widely grown in Asia, Europe and North America (Li et al., 2016). It is one of the little known forest trees in our country, and some scientific studies have been carried out on the species in recent years. It is often used in various types of wine, vinegar and fruit juices rather than direct consumption in different parts of the world due to its small and sour fruits (Aladedunye and Matthaus, 2014). In addition to its critical position in the biological diversity, crabapple is also one of the most important sources for food processing and nutritional elements (Jiang et al., 2014; Wang et al., 2015). Cardiovascular diseases and cancer are among the leading causes of death in many industrially developed countries. The relationship between these diseases and nutrition has been a popular research topic in recent years (Lu and Foo, 2000; Boyer and Liu, 2004; Sevindik et al., 2021).

Phenolic compounds are natural plant secondary metabolites which affect inner and outer quality parameters such as appearance, aroma and health protection (Chen et al., 2014; Mohammed et al., 2019). In addition, they also bear value due to their high levels of antioxidant activity. It was also revealed that in addition to antioxidant activities of phenolic compounds in fruits, they were also effective in terms of antimicrobial activity (Lattanzio, 2003; Irkin et al., 2008; Pehlivan et al., 2021).

Apple is an easily accessible phenolic source as a fruit (Escarpa and Gonzalez, 1998). Phenolic compounds are powerful antioxidants which can effectively protect the human body against diseases caused by oxidative destruction (Weng and Yen, 2012).

Fruit aroma can be considered as an importance indicator of fruit quality. While some aroma compounds are almost common in nearly all types of fruit, other aromas may be peculiar to a single or a few types of fruit (El Hadi et al., 2013). In recent years, consumers have started to demand more high quality and aromatic fruits, thus encouraging producers to grow aromatic fruits and fruit breeders to focus on breeding in various types of aromatic fruits (Kafkas, 2004; Ozcan et al., 2020). In today's world, scientific community pays particular attention to aroma compounds in fruits (Feng et al., 2006; Li et al., 2008a, Boyaci and Yilmaz, 2020). In this respect, several studies have been recently conducted on apple, which is one of the richest fruit species in terms of aroma compounds, which revealed more than 350 different aroma compounds, including esters, alcohols and aldehydes (Fellman et al., 2003; Nie et al., 2006, Beyhan et al., 2017).

Various studies on crabapple demonstrated that the fruit was rich in physiological activities as far as its aroma, phenolic compounds and antioxidant capacities were concerned (Yoshizawa et al., 2004; Aladedunye and Matthaus, 2014; Li et al., 2016; Sharma and Nath, 2016). Phenolic compounds are also in high demand in food industries and cosmetics because of the health-promoting benefits of these compounds, but there is little information about such wild edible fruit varieties that are locally available and underused. However, the number of studies on the compounds and functions of crabapple is fairly limited in the regions where it is grown (Li et al., 2016; Mohammed et al., 2020). Similarly, although it is grown under different synonyms in different regions of Turkey, no studies have been so far carried out on phytochemical properties and aroma compounds of crabapple. Therefore, the present study aims to determine fruit properties, phytochemical properties and aroma compounds of crabapple (*Malus tribolata* C.K. Schneid.)

Materials and Methods

The materials in the present study consisted of fruits obtained from two different crabapple (*Malus tribolata* C.K. Schneid.) genotypes which were naturally grown under similar ecological conditions in central Kahramanmaraş province of Turkey.

Color and fruit weight

Fruit and fruit peel colours belonging to crabapple genotypes on the predicted harvest date were determined using Lovibond (TR 300; Amebury, Germany) in order to classify colour values as $L*$ (brightness), $a*$ (red) and $b*$ (yellow). Fruits belonging to each crabapple genotypes were weighed on a precision scale with a sensitivity range of 0.01 in five different replicate groups containing five fruits in order to calculate average values.

Phytochemical Properties

Twenty crabapples samples in each genotype were squeezed, and the obtained fruit juices were centrifuged for five minutes at 2000 rpm. The total amount of soluble solids content (TSSC), and pH level in the obtained fruit juices were measured using a digital refractometer (Krüss, A.KRUSS optronic, Germany) and a pH scale (HI 2211 pH/ORP meter, HANNA Instruments). For titrable acidity (%), 6 g fruit juice was titrated using an automatic titrator (Automatic Potentiometric Titrator, AT-510; KEM Kyoto Elect., Tokyo, Japan) at 0.1 N NaOH until it reached pH 8.2. Titrable acidity was expressed in malic acid in percentage.

Total Phenolic Content

Total phenolic content in crabapple genotypes extracts were measured using Folin-Ciocalteu method developed by (Singleton et al., 1999) 0.5 g of each sample (unpeeled) was weighed in 50 mL capped test tubes and homogenized using 2500 µL Methanol for 30 seconds. The homogenized sample was centrifuged at 2000 rpm for five minutes. Filtrates obtained from the upper phase of each extract (50 µL, three replicates) were put in a screwed and capped test tube, and 250 µL Folin Ciocalteu reagent and 750 µL sodium carbonate (20 %) were added. These tubes were vortexed and kept at room temperature in a dark environment for two hours. Later, the mixture absorbance at 760 nm was measured using UV-VIS spectrophotometer. Finally, total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per 100 g apple extract.

Antioxidant Content

Antioxidant content was used by modifying the method developed by (Hatano et al., 1989). 0.5 g of each sample was weighed in a 50 mL capped test tube and homogenized using 2500 µl Methanol for 30 seconds. The homogenized sample was centrifuged at 2000 rpm for five minutes. Filtrates obtained from the upper phase of each extract (50 µL, three replicates) were put in a screwed and capped test tubes and mixed with DPPH solution diluted with methanol (1950 µL) (0.2 mM in methanol). These tubes were vortexed and keep at room temperature in a dark environment for half an hour. Following the color formation (from dark purple to light yellow), the mixture absorbance at 517 nm was measured using UV-VIS spectrophotometer. The control solution was prepared using a mixture of methanol and DPPH radical reagent solution. Scavenging activity was calculated using the following formula:

DPPH (%) = [(A517 control A517 sample)/ A517 control] ×100

Analysis of volatile aroma compounds

Fruit juice samples obtained from ripe fruits was placed in a glass tube. The extraction of volatile aroma substances with the HS-SPME/GC/MS (Headspace Solid Phase Micro Extraction/Gas Chromatograpghy Mass Spectrometry) technique, the adsorption of the aroma substances in the headspace by the syringe was achieved. Volatile aromas adsorbed by the syringe were desorbed into the injector part of the GC-MS using the polar column. Samples were analyzed for 70 minutes using Innowax (30m x 0.250 mm, 25 mikron) column in HS-GC/MS (Perkin Elmer) using polar column. Identification processes are; By using Wiley and NIST Library Scanning Software, the peaks determined in the GC were compared with reference compounds or mass spectra in the computer memory (Urek, 2016).

Statistical analysis

Triplicate phytochemical properties analyses were performed and a completely randomized design using analysis of one-way analysis of variance (ANOVA).

Results and Discussion

Colour and fruit weight

Fruit properties of crabapple genotypes are given in Table 1. The fruit weights of 46 EL 01 and 46 EL 02 genotypes were measured as 17.31 g and 12.53 g, respectively. Fruit shell colours were found as *L*: 73.21, *a*:-3.16, *b*: 44.36 for 46 EL 01 genotype and as *L*: 73.64, *a*:-3.11, *b*: 46.02 for 46 EL 02 genotype. Fruit flesh colors were found as *L*: 83.23, *a*:-1.14, *b:* 24.22 for 46 EL 01 genotype and as *L*: 82.17, *a*:-1.78, *b*: 25.91 for 46 EL 02. Similar to the findings of the present study, in a study on crabapple grown in Kahramanmaraş province of Turkey, (Ak, 2019) calculated fruit weight ranging between 9.10 and 12.50 g and found fruit shell colour and flesh colour as *L*: 63.5, *a*: -3.0, *b*: 31.1 and *L*: 69.2, *a*: -3.3, *b*: 36.7, respectively.

In a two-year study on crabapple, (Tashev and Petkova, 2006) observed that the weights of crabapple fruit were 6.37 g and 8.14 g in Bulgaria and Greece, respectively, in 2002, while the same weights were 9.66 g and 4.38 g in 2003. Similarly, in a study on naturally grown crabapple in Turkey, (Yılmaz and Ok, 2012) reported that average fruit weight was 8.63 g and that fruit size differed depending on the genotypes and harvest year.

Phytochemical properties

Some phytochemical properties regarding the amount of soluble solids content (TSSC), pH, titrable acidity (TA), total phenolic content and amount of antioxidants in different crabapple genotypes are given in Table 2. The average amounts of soluble solids content (TSSC), in 46 EL 01 and 46 EL 02 genotypes were calculated as 13.76% and 13.26%, respectively. In addition, pH levels in 46 EL 01 and 46 EL 02 genotypes were calculated as 4.16 and 4.53, respectively. Finally, the amounts of titrable acidity in 46 EL 01 and 46 EL 02 genotypes were calculated as 0.66% and 0.23%, respectively. On the other hand, (Ak, 2019) reported in different studies focusing on crabapple fruits in different that the amount of TSSC, pH level and the amount of titrable acidity were 26.73%, 3.30% and 2.77%, respectively. In this respect, (Eberhardt et al., 2000) stated that acidity in crabapples differed depending on the genotypes and regional conditions, ranging between 0.1 and 6 g in 100 g.

Total phenolic contents in 46 EL 01 and 46 EL 02 genotypes were found as 839.13 mg GAE/100g and 789.78 mg GAE/100g, respectively. Average antioxidant capacity in 46 EL 01 and 46 EL 02 genotypes were calculated as 70.12% and 50.32%, respectively. (Li et al., 2014) reported in a study on 10 different crabapple fruits that total phenolic content ranged between 02.83−1265.94 mg GAE/100g and the average content was 566.58 mg GAE/100 g, while (Cinar et al., 2020) stated in a study on different crabapple fruits that total phenolic content and was between 0.3-22 (GAE mg g^{-1}), while antioxidant activity was calculated as 33 %, respectively. (Yoshizawa et al., 2004) analyzed DPPH radical scavenging activity in fruit juices obtained from 42 different crabapple fruits and reported that crabapple displayed a very powerful antioxidant activity, which makes it a promising source of antioxidant. In addition, (Mertoglu and Evrenosoglu, 2019) calculated total phenolic content in different cultivars such as Jerseymac, Summer Red, Vista Bella, Williams Pride, Granny Smith, Fuji, Golden Delicious and Pink Lady as 494.81 mg.GAE L^{-1}, 158.67 mg.GAE L^{-1}, 225.36 mg.GAE L^{-1}, 230.42 mg.GAE L^{-1}, 164.35 mg.GAE L^{-1}, 212.6 mg.GAE L^{-1}, 221.14 mg.GAE L^{-1} and 167.22 mg.GAE L^{-1}, respectively. Various studies demonstrated that total phenolic content and antioxidant activity in crabapple fruits were higher compared to other apple cultivars (Chen et al., 2007; Valavanidis et al., 2009) which overlaps with the findings in the present study. To add this, different phenolic compounds in different *Malus* types can be attributed to differences in genetic variation, environmental conditions and geographical locations (McRae et al., 1990; Awad et al., 2000). The above-mentioned discussion indicates that total phenolic content varies significantly in different plant species. Therefore, it can be inferred from the findings of the present study that crabapple fruit can be used as a rich source of phenolic compound and antioxidant.

Volatile Aroma Compounds

A total of 37 aroma compounds, namely 7 alcohols, 2 terpenes, 5 aldehydes, 17 esters, 1 ketone, 3 acids and 2 other compounds, belonging to crabapple genotypes the HS-SPME/GC/MS (Headspace Solid Phase Micro Extraction / Gas Chromatograpghy Mass Spectrometry) technique is determined and given in Table 3. Total aroma compounds in 46 EL 01 and 46 EL 02 genotypes were calculated as 101.78 µg/L and 102.26 µg/L, respectively. The highest values in both genotypes in terms of aroma compounds given in Table 3 were observed in caproleic acid with 29.87 µg/L and 28.74 µg/L, respectively. It was followed by terpenes (α-Farnesene with 20.33 µg/L and 21.23 µg/L) and alcohol compounds (Citronellol <L-> with 10.19 µg/L and 11.09 µg/L).

Table 1. Fruit properties of crabapple genotypes

Genotypes	Fruit shell colour			Fruit flesh colour			Fruit weight
	L	*a*	*b*	*L*	*a*	*b*	(g)
46 EL 01	73.21	-3.16	44.36	83.23	-1.14	24.22	17.31
46 EL 02	73.64	-3.11	46.02	82.17	-1.78	25.91	12.53

Table 2. The phytochemical properties of crabapple genotypes

Genotypes	TSSC (%)	pH (%)	TA (%)	Total Phenolic Content (mg GAE/100g)	Antioxidant (DPPH %)
46 EL 01	13.76±0.09	4.16±0.22	0.66±0.11	839.13±2.34	70.12±2.20
46 EL 02	13.26±0.05	4.53±0.15	0.23±0.06	789.78±2.89	50.32±1.60

Table 3. Volatile aroma compound in different crabapple genotypes (µg/L)

Aroma Compounds	R.T.[1]	46 EL 01	46 EL 02
Alcohols			
2-methyl- 1-Propanol	6.609	0.92	0.63
2-methyl- 1-Butanol	9.694	1.90	1.86
Carbospol	13.885	0.23	0.53
Citronellol <L->	26.297	10.19	11.09
Dec-9-en-1-ol	27.025	0.88	0.36
Pinane-2-thiol	28.793	1.33	1.35
2-(dodecyloxy)- Ethanol	34.557	0.27	0.23
Total		15.72	16.05
Terpenes			
α-Farnesene	24.185	20.33	21.23
α-Santalene	31.879	4.73	6.73
Total		25.06	27.96
Aldehydes			
<cis-4-> Decenal	24.826	0.45	0.39
<cis-> Undec-8-enal	30.218	1.33	1.22
Undecylene aldehyde	31.162	0.76	0.59
<2,4-trans, trans-> Undecadienal	31.966	0.43	0.37
Dodec-2(E)-enal	33.183	1.16	1.10
Total		4.13	3.67
Esters			
Acetic acid, butyl ester	6.268	0.29	0.23
Hexanoic acid, ethyl ester	10.548	1.05	1.11
Acetic acid, hexyl ester	11.645	0.44	0.42
Octanoic acid, ethyl ester	16.163	2.59	2.75
Hexanoic acid, 2-methylbutyl ester	16.785	0.54	0.33
2-methylpropyl octanoate	19.263	0.77	0.57
Tiglate <propyl->	19.327	0.53	0.25
Decanoic acid, ethyl ester	21.464	1.26	1.35
Octanoic acid, 3-methylbutyl ester	21.942	1.09	1.03
ethyl E-4-decenoate	22.166	1.06	1.00
Tiglate <isoamyl->	24.962	0.43	0.58
Non-2(E)-enoic acid <methyl-> ester	27.258	3.95	4.15
Non-2(E)-enoic acid <methyl-> ester	27.454	0.65	0.53
Cyclohexaneethyl acetate	26.695	0.56	0.5
Non-2-ynoate <methyl->	28.194	1.34	1.16
Geranyl formate	28.436	0.57	0.65
Nonyl acetate	28.628	0.33	0.22
Total		17.45	16.83
Ketone			
dihydro- 2(3H)-Furanone	21.300	0.78	0.58
Total		0.78	0.58
Acids			
Citronellic acid	29.619	4.33	4.08
Caproleic acid	30.998	29.87	28.74
Nonanoic acid	33.087	0.45	0.60
Total		34.65	33.42
Other components			
Acetylvaleryl	13.816	0.54	0.45
Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	27.923	3.45	3.30
Total		3.99	3.75

[1]Retention Time (min).

2-methyl-1-Propanol, 2-methyl-Butanol, Carbospol, Citronellol <L->, Dec-9-en-1-ol alcohol compounds were found in both genotypes. It was observed that the highest alcohol compound in both genotypes were in Citronellol <L-> with 10.19 µg/L and 11.09 µg/L.

α-Farnesene and α-Santalene, which are terpene compounds, were also found in both genotypes, and it was observed that the former was higher, with 20.33 µg/L and 21.23 µg/L, in both genotypes compared to the latter.

Wild apples usually are richer in terpene content compared to other cultivars (Chen et al., 2007; Wang et al., 2014). In a study on aroma compounds in 10 different wild apples, (Zhang et al., 2017) found that volatile terpene content was higher in *Malus prunifolia* (Willd.) Borkh.

compared to *Malus baccata* (L.) Borkh. and the most abundant terpene was α-farnesene in all compounds. Similarly, in the present study, α-farnesene compound followed alcohols as the second highest aromatic compound in crabapple genotypes.

Aldehyde compounds, namely <cis-4-> Decenal, <cis-> Undec-8-enal, Undecylene aldehyde, <2.4-trans, trans-> Undecadienal and Dodec-2(E)-enal, were found in both genotypes, while the amount of <cis-> Undec-8-enal compound was higher compared to other compounds, with 1.33 µg/L and 1.22 µg/L, in both genotypes. Aldehydes are more dominant in unripe apples (Paillard et al., 1990; Mattheis et al., 1991).

However, as a result of fruit ripening, the amount of aldehydes decreases, whereas alcohol and ester concentrations increase (Kakiuch et al., 1986). Acetic acid butyl ester, Hexanoic acid, ethyl ester, Acetic acid, hexyl ester, Octanoic acid, ethyl ester, Hexanoic acid, 2-methylbutyl ester, 2-methylpropyl octanoate, Tiglate <propyl->, Decanoic acid, ethyl ester, Octanoic acid, 3-methylbutyl ester, ethyl E-4-decenoate, Tiglate <isoamyl->, Non-2(E)-enoic acid <methyl-> ester, Non-2(E)-enoic acid <methyl-> ester, Cyclohexaneethyl acetate, Non-2-ynoate <methyl->, Geranyl formate and Nonyl acetate, all of which are crucial ester compounds in apple aroma, were also found in both genotypes. Among these ester compounds, octanoic acid and ethyl ester compounds were calculated as the highest compounds in both genotypes with 2.59 µg/L and 2.75 µg/L. (Fellman et al., 2003) stated that apple aroma was a combination of various aroma compounds because three chemical groups, i.e. ester, aldehyde and alcohols, played a vital role in apple aroma, ester compound being the foremost element. As for ketone compounds, only dihydro-2(3H)-Furanone was found in both genotypes with 0.78 µg/L and 0.58 µg/L. Citronellic acid, Caproleic acid and Nonanoic acid were found in the crabapple genotypes in the present study. Caproleic acid was found in higher concentrations compared to other acid compounds in both genotypes with 29.87 µg/L and 28.74 µg/L, respectively.

Other compounds in aroma compounds were Acetylvaleryl and Phenol, 2.6-bis (1,1-dimethylethyl)-4-methyl- in both genotypes. It was observed that Phenol, 2.6-bis (1,1-dimethylethyl)-4-methyl- was found in a higher amount compared to the other compound, with 3.45 µg/L and 3.3 µg/L, in both genotypes, respectively. Aroma affects fruit quality to a great extent, which makes it a decisive factor in apple growing (Valavanidis et al., 2009). Several studies have been carried out on apple aroma in recent years, and more than 350 aroma compounds such as esters, alcohols and aldehydes were revealed in apples (Yahia, 1994; Nie et al., 2006). It was also reported that the compounds in question often varied depending on various factors such as cultivar, ripeness, climatic conditions during the harvest, post-harvest applications and storage conditions (Dixon and Hewett 2000; Nie et al., 2006; Ozcan and Sutyemez, 2019). Compares the aroma of unripe with ripened apples and points out that the main volatile compounds of apple were esters and alcohols (Wang et al., 2007). In the study, in which he determined 136 aroma components belonging to 12 different categories in three apple varieties (Zaofengtian, Vista Bella, Liaofu), 1-hexanol, 2-hexenal, acetic acid butyl ester, acetic acid hexyl ester etc. identified as the main compounds. While previous research on aroma components has generally focused on cultivated apples, studies conducted by different researchers on the aroma components of crabapples are limited (Li et al., 2008a; Li et al., 2008b; Zhao et al., 2014). Our results and other results show both similarities and dissimilarities in terms of major aroma compounds which seems to be very crabapple genotypes dependent. The situation, environmental conditions and growing measures also have some influence on the form of the aroma compounds of fruits.

Conclusion

In this study, 2 different crabapple genotypes grown in Kahramanmaraş under completely natural conditions were evaluated in terms of antioxidant, phenolic compounds and aroma compounds. In both crabapple genotypes, a total of 37 aroma contents were determined, including 7 alcohols, 2 terpenes, 5 aldehydes, 17 esters, 1 ketone, 3 acids, 2 other compounds, and the aroma compound amount was the highest, respectively. In both genotypes, acids were identified as terpenes and alcohol compounds. According to the findings obtained, it was determined by this study that two different crabapple genotypes are very rich in phenolic compounds, have strong antioxidant capacities and are rich in aroma components. In this respect, it has been determined that crabapple is a natural phytochemical source in terms of nutrition and health.

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