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Research Article
(Araştırma Makalesi)

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Determination of chemical composition of some blackthorn genotypes (*Prunus spinosa* L.)

Bazı çakal eriği (*Prunus spinosa* L.) genotiplerinin kimyasal bileşiminin belirlenmesi

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ABSTRACT

Objective: This study aimed to analyze the chemical and functional properties of fruits from five selected *Prunus spinosa* L. genotypes grown in Seydişehir district of Konya province, and determine the differences among the genotypes.

Material and Methods: In the research, fruit properties such as fruit weight, pericarp color, flesh color were determined, while total soluble solids (TSS), pH, titratable acid (TA), total phenol content, antioxidant level and volatile organic compounds were analyzed. PCA analysis was employed to distinguish variations among the genotypes.

Results: Five different blackthorn genotypes were examined for fruit properties, revealing variations. Fruit weight ranged from 1.31 g to 2.67 g. TSS content was determined to be between 11.9% and 13.2%. pH values varied from 3.35 to 4.22, while TA ranged from 0.83% to 1.30%. Total phenol content ranged from 356.92 mg GAE/100 g to 387.56 mg GAE/100 g. Total antioxidant levels were determined to be between 65.13% and 77.06%. Thirty different compounds were detected in the analysis of volatile organic compounds, including seven different acids.

Conclusion: Significant diversity among the blackthorn genotypes has been identified. Statistical analyses have shown that B01T genotype has the highest total phenol content, while B05T genotype has the highest antioxidant level. The presence of various volatile organic compounds in blackthorn indicates the diversity of its chemical profile. The findings support the notion that blackthorn has a beneficial potential for health and is a valuable candidate for the development of health-focused food products.

ÖZ

Amaç: Bu çalışmada, Konya iline bağlı Seydişehir ilçesinde yetişen seçilmiş beş *Prunus spinosa* L. genotipinin meyvelerindeki kimyasal ve fonksiyonel özellikleri analiz edilerek genotipler arasındaki farklılıklar belirlenmiştir.

Materyal ve Yöntem: Araştırmada, meyve ağırlığı, perikarp rengi, et rengi gibi meyve özellikleri belirlenmiş, suda çözünebilir kuru madde (SÇKM), pH, titre edilebilir asitlik, toplam fenol içeriği, antioksidan seviyesi ve uçucu organik bileşikler analiz edilmiştir. PCA analizi genotipler arasındaki varyasyonları ayırt etmek için kullanılmıştır.

Araştırma Bulguları: Beş farklı çakal eriği genotipinin meyve özellikleri incelenmiş ve varyasyonları ortaya konmuştur. Meyve ağırlığı 1.31 g-2.67 g arasında değişmiştir. SÇKM %11.9-%13.2 arasında tespit edilmiştir. pH değerleri 3.35-4.22 arasında, titre edilebilir asitlik ise %0.83-%1.30 arasında belirlenmiştir. Toplam fenol içeriği 356.92 mg GAE/100 g ile 387.56 mg GAE/100 g arasında değişmiştir. Toplam antioksidan miktarları %65.13 ile %77.06 arasında belirlenmiştir. Uçucu organik bileşik analizinde otuz farklı bileşik tespit edilmiş, bunlar arasında yedi farklı asit belirlenmiştir.

Sonuç: Çakal eriği genotipleri arasında önemli çeşitlilik tespit edilmiştir. İstatistiksel analizler, B01T genotipinin en yüksek toplam fenol içeriğine, B05T genotipinin ise en yüksek antioksidan seviyesine sahip olduğunu göstermiştir. Çakal eriğinde çeşitli uçucu organik bileşiklerin bulunması, kimyasal profilinin çeşitliliğini göstermektedir. Bulgular, çakal eriğinin sağlık için faydalı bir potansiyele sahip olduğunu ve sağlık odaklı gıda ürünlerinin geliştirilmesinde değerli bir aday olduğunu desteklemiştir.

INTRODUCTION

Blackthorn (*Prunus spinosa* L.) in temperate climate regions of Asia (Meschini et al., 2017; Owczareka et al., 2017), Europe, West Asia, and Northwest Africa (Elez-Garofulić et al., 2018), can be grown naturally, especially in the Central, North, West and South regions of Türkiye (Marakoğlu et al., 2005). *Prunus spinosa*; 'blackthorn' or 'sloe', which is in the *Rosaceae* family, including fruit species such as almond, cherry, and peach, is called 'Çakal eriği' or 'Güvem' in Türkiye (Karakas et al., 2019). It has been known as one of the ancestors of *Prunus domestica* L., has a chromosome number of 2n=32 (Aparajita et al., 2002). It blooms white blossoms in March and April. The trees are shrub-like, thorny, densely branched, 1-4 m tall, and deciduous. The leaves are elliptical, 2-3 cm in length, hairy at first and after glabrous. Its flowers consist of 5 petals and 5 sepals and are in hermaphrodite form and pollinated by insects and self-fertilized. After fertilization, the fruits begin to develop and usually ripen very late which may take until late autumn. The fruits of *P. spinosa* are bluish-black in color and very acidic and tannic at the beginning of maturity (Kırca, 2022).

The fruit, flower, bark, and root of *P. spinosa* , which has been consumed since ancient times, are used for medicinal purposes. The blackthorn is also used in the food industry for the production of jams and various beverages (liquor, wine, juice, compote and tea) (Veličković et al., 2014). Sikora et al. (2013) reported that the freeze-drying process of blackthorns did not significantly affect the nutrient and antioxidant content. This allows this species to be frozen and consumed out of season.

Raw blackthorn fruits consist of water (≅68%), sugars, acids, astringency substances like tannins and polyphenols, proteins, cellulose, anthocyanins, minerals (K, P, Fe, Cu, Na), provitamin A, vitamins (B1, B2, C, PP) (Leterme et al., 2006; Ruiz-Rodríguez et al., 2014). It is also rich in phenolics and antioxidants (Sikora et al., 2013; Ruiz-Rodríguez et al., 2014; Veličković et al., 2014). The fruits of *P. spinosa* contain many bioactive compounds such as tocopherols (α-tocopherol, β-tocopherol, -tocopherol, and δ-tocopherol), L-ascorbic acid, β-carotene, anthocyanins (cyanidin-3-routine, peonidine-3-routine and cyanidin-3-glycoside) and flavonoids (routine, quercetin, and hyperoside) (Fraternale et al., 2009; Ruiz-Rodríguez et al., 2014; Ayla et al., 2017).

Blackthorn is used in phytotherapy. The flowers, bark and root of blackthorn have been traditionally used in public medicine for their diuretic and laxative properties, due to their ability to remove excess sodium ions, harmful products of metabolism, reduce the permeability of blood vessels, and counteract inflammation of the urinary tract (Elez-Garofulić et al., 2018). Recently, blackthorn has attracted attention both as an industrial food plant due to its rich source of phenolic compounds and as a pharmacological nutraceutical medicinal plant (Kumarasamy et al., 2004, 2007; Pinacho et al., 2015; Yuksel, 2015; Mikulic-Petkovsek et al., 2016; Meschini et al., 2017). It is an antispasmodic and anti-inflammatory agent in the treatment of various coughing diseases, hypertension, regulation of menstruation, diabetes and gastrointestinal disorders. It has an antiseptic effect (due to the presence of tannins) and shows activity against inflammation of the mucosal layer of the digestive tract (Borkowski et al., 1994; Kültür, 2008). *P. spinosa* is used to treat skin problems and soothe stomach cramps (Browics, 1972). Blackthorn is an excellent source of polyphenolic compounds that can significantly reduce the negative effects of free radicals found in fresh fruit extracts in the organism. Therefore, they have an important role in the prevention of neurodegenerative diseases, cardiovascular diseases, and cancer (Burits & Bučar, 2000).

The existing study was carried out on blackthorn genotypes naturally grown in the Seydişehir district of Konya, Türkiye. In the study, the phenolic components, antioxidant capacity, and volatile organic compounds of the blackthorn genotypes were determined and the differences between the genotypes were revealed.

MATERIALS and METHODS

Plant materials and sample preparation

The study utilized fruits sourced from five distinct genotypes of blackthorn (*P. spinosa*), all naturally grown under comparable ecology in Seydişehir, located within the Konya province of Türkiye. The visuals of the fruits of the blackthorn genotypes are presented in Figure 1. The location of the region where the study was conducted is presented in Figure 2. The coordinates and altitude information determined by the genotypes are presented in Table 1.

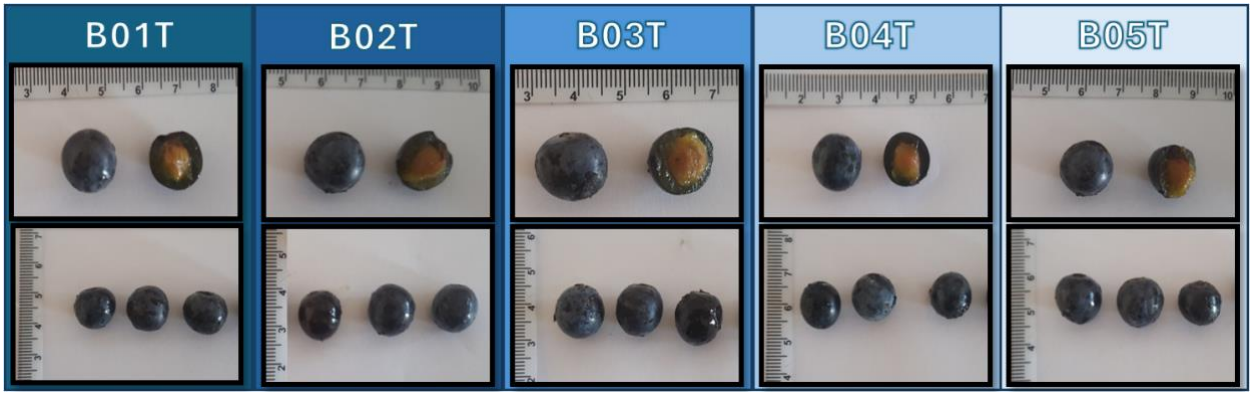

Figure 1. Visuals of the fruits of the blackthorn genotypes.

Şekil 1. *Çakal eriği genotiplerinin meyvelerine ait görseller.*

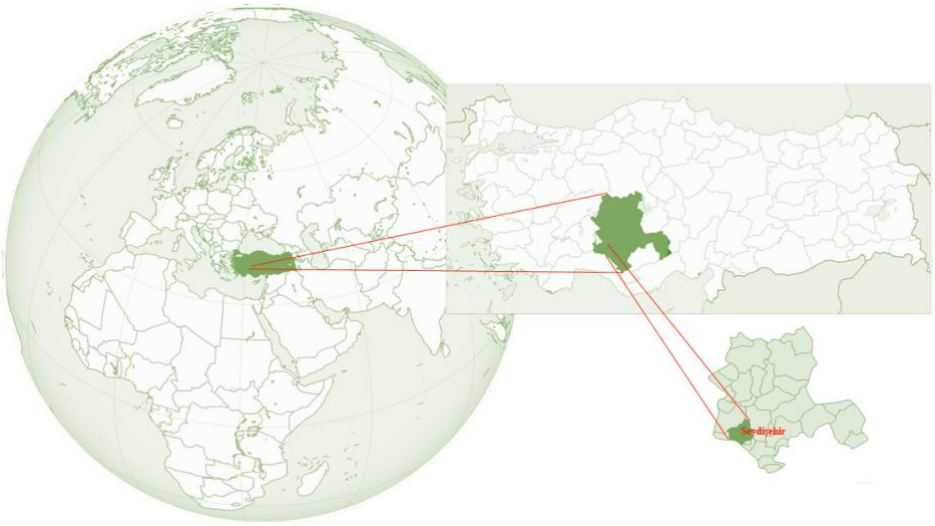

Figure 2. Location of the region from which 5 different blackthorn genotypes were obtained.

Şekil 2. *Beş farklı çakal eriği genotipinin elde edildiği bölgenin konumu.*

Table 1. The coordinate and altitude information of the blackthorn genotypes

***Çizelge* 1.** *Çakal eriği genotiplerinin koordinat ve rakım bilgileri*

Genotypes	Latitude (N)	Longitude (E)	Altitude (m)
B01T	*37°24'53.1"N*	*31°50'21.7"E*	1118
B02T	*37°25'04.6"N*	*31°49'56.3"E*	1122
B03T	*37°25'00.6"N*	*31°50'06.0"E*	1122
B04T	*37°24'56.8"N*	*31°50'30.8"E*	1117
B05T	*37°24'53.6"N*	*31°50'25.0"E*	1119

Fruit weight and color

Pericarp and pulp colors belonging to the blackthorn genotypes fruits were determined using Lovibond (TR 300; Amesbury, UK) to categorize color values as a* (red), b* (yellow) and L* (brightness). L brightness value, 0 black, 100 white, a red, -a green; b defines yellow and -b defines blue. Chroma and hue angle values were calculated from the following equations: chroma = $(a^2+b^2)^{1/2}$ and Hue = $\tan^{-1}$ $^{(b/a)}$ (McGuire, 1992). Each blackthorn genotype's fruits were individually weighed using a precision scale with an accuracy of 0.01 g, and this process was repeated in five separate replicates, each comprising 20 fruits, to determine mean values.

Phytochemical properties

In each blackthorn genotype, twenty fruit samples were squeezed, and the resulting juices were subjected to centrifugation at 2000 rpm for five minutes. The pH level and total soluble solids content (TSSC) of the juices were determined using a digital refractometer from A. KRÜSS Optronic and a pH scale from HANNA Instruments, respectively. For titratable acid (TA) (%), 6 g of fruit juice underwent titration with 0.1 M NaOH using an Automatic Potentiometric Titrator (AT-510; KEM Kyoto Elect., Tokyo, Japan) until reaching pH 8.2. TA was expressed as a percentage of malic acid.

The total phenolic content in extracts from the blackthorn genotypes was determined using the Folin-Ciocalteu method (Singleton et al., 1999). Each sample (0.5 g, unpeeled) was weighed into 50 mL capped test tubes and homogenized for 30 seconds with 2500 µl of methanol. After homogenization, the samples were centrifuged at 2000 rpm for 5 minutes. Filtrates from the supernatant of each extract (50 µl, in triplicate) were transferred to screw-cap test tubes, followed by the addition of 250 µl of Folin Ciocalteu reagent and 750 µl of sodium carbonate solution (20%). After vortexing the tubes, they were incubated at room temperature in the dark for 2 hours. Subsequently, the UV-VIS spectrophotometer was employed to measure the absorbance of the mixture at 760 nm. The total phenolic content in samples was finally expressed as gallic acid equivalents mg GAE/100g of blackthorn extract.

To assess antioxidant content, a method based on modifications to the technique developed by Hatano et al. (1989) was employed. Each sample, weighing 0.5 g, was placed into a 50 mL capped test tube and homogenized with 2500 µl of methanol for a duration of 30 seconds. Following the homogenization process, the samples underwent centrifugation at 2000 rpm for a period of 5 minutes. Filtrates from the upper phase of each extract (50 µl, in triplicate) were transferred to screw-capped test tubes and mixed with a DPPH (2,2-diphenyl-1-picrylhydrazyl) solution diluted in methanol (1950 µl) to a concentration of 0.2 mM. Following vortexing, the tubes were incubated at room temperature in darkness for thirty minutes until the color changed from dark purple to light yellow. Subsequently, using a UV-VIS spectrophotometer, the absorbance of the mixture was determined at 517 nm. A control solution was prepared by mixing methanol with the DPPH radical reagent solution. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH (\%)} = [\ (\text{A517 control} - \text{A517 sample}) / \text{A517 control}] \times 100$$

Analysis of volatile organic compounds (VOCs)

Ripe fruit juice samples were transferred into glass tubes for analysis. Volatile organic compounds (VOCs) were extracted using the HS-SPME/GC/MS (Headspace Solid Phase Micro Extraction/Gas Chromatography Mass Spectrometry) technique, where organic substances in the headspace were adsorbed by the syringe. The syringe then desorbed the volatile organics into the injector part of the GC-MS using a polar column. Analysis was conducted for seventy minutes using an Innowax column (30 m x 0.250 mm, 25 microns) in HS-GC/MS (Perkin Elmer) with a polar column. Compounds were identified by correlating peaks detected in the GC with those of reference compounds or mass spectra stored in the computer memory, facilitated by the Wiley and NIST Library Scanning Software (Urek, 2016).

Statistical analysis

The significance of differences between the genotypes was determined with one-way ANOVA, followed by Tukey's HSD test for multiple comparisons. Additionally, Principal Component Analysis (PCA), which illustrates the distances between the blackthorn genotypes on a two-dimensional graph, was conducted using JMP 13 based on the covariance matrix of the coefficients, using the values of volatile organic compounds.

RESULTS AND DISCUSSION

Some fruit properties of the blackthorn genotypes

The fruit weight, pericarp color and pulp color profile results of the genotypes examined in the study are presented in Table 2. The highest mean fruit weight was determined in B03T with 2.67 g and the lowest in B05T with 1.31 g. The L* values in pericarp color measurements in blackthorn genotypes ranged from 18.36 to 19.42. Values expressing the transition from green to red were found between 2.23 and 2.40. The b* values varied between 0.16 and 0.54. Pulp color values of the genotypes examined in the study were also measured. The L*, which expresses the brightness, was measured between 17.65 and 18.91 values. The a* values were found to be between 3.11 and 4.18, and the b* values were found to be between 0.54 and 0.81 for pulp color. Also, the chroma values were calculated between 2.63 and 2.97 for pericarp color, and between 5.16 and 8.90 for pulp color. Claudia et al. (2017) in a study conducted on 15 different blackthorns in Romania, it was reported that the fruit weights of genotypes ranged from 1.10 g to 4.20 g. The fruit weights of the genotypes examined in this study varied among these values. The hue angle value represents true color, which is effective for visualizing the color appearance of fruits (Rojas-Graü et al., 2006). Among the genotypes, the highest hue angle value for pericarp color was determined in B03T and B02T for pulp color (Table 1).

Table 2. Some fruit properties of the blackthorn genotypes

Çizelge 2. *Çakal eriği genotiplerinin bazı meyve özellikleri*

Genotypes	Fruit weight (g)	Pericarp color					Pulp color				
		L*	a*	b*	Chroma	Hue angle	L*	a*	b*	Chroma	Hue angle
B01T	1.79	19.21	2.34	0.35	2.80	8.51	18.25	3.32	0.75	5.79	12.73
B02T	1.42	19.25	2.39	0.16	2.87	3.83	18.91	3.11	0.81	5.16	14.60
B03T	2.67	18.36	2.23	0.54	2.63	13.61	17.65	3.41	0.72	6.07	11.92
B04T	1.77	19.35	2.40	0.42	2.97	9.93	18.29	4.18	0.58	8.90	7.90
B05T	1.31	19.42	2.38	0.33	2.89	7.89	18.51	3.19	0.54	5.23	9.61

Total soluble solids, pH, titratable acid, total phenols and DPPH of blackthorn genotypes

Total soluble solids (TSS) are used for quality control purposes, especially in the production stages of foods. In fruits, it is a parameter used for processes such as monitoring maturity and harvest time and continuous monitoring of the processing processes of foods such as fruit juice and canned food. TSS values were found between 11.9% and 13.2% in 5 different blackthorn genotypes examined in the study (Table 3).

To determine food quality and reliability, various analyzes are carried out on raw materials and the end of the products. From these analyzes, pH is used to determine acidity, one of the important quality criteria in foods. The pH of the blackthorn genotypes were determined between 3.35 and 4.22 (Table 3). In a study conducted by Kuru Berk et al. (2020), it was reported that the pH of *Prunus spinosa* L. subsp. dasyphylla (Schur) Domin genotypes varied between 3.17-4.13. The results obtained from our study are similar. Titratable acid values of 5 blackthorn genotypes were found to be between 0.83 and 1.30 (Table 3).

The total phenol content examined in the fruits of the blackthorn genotypes was determined by the Folin-Ciocalteu method and results are indicated in Table 3. Experimental results show that the total phenol content in the examined extracts ranges from 356.92 mg GAE/100 g to 387.56 mg GAE/100 g. Veličkovic et al. (2014) reported that the content of total phenol compounds in fresh blackthorn ranged from 15.33 mg GAE/g to 20.94 mg GAE/g. In another study, they determined that the total phenol content in blackthorn genotypes were 117 mg GAE/100 g and 407 mg GAE/100 g (Erturk et al., 2009).

Leaf and flower extracts of *P. spinosa* are also reported to be rich in phenolics (Wolbiś et al., 2001; Olszewska & Wolbis, 2001, 2002). Lovrić et al. (2017) and Elez-Garofulić et al. (2018) also confirm the phenolic richness in the flowers of blackthorn. In addition, it has been reported that blackthorn fruit extracts have high phenolic content, which is rich in anthocyanins (Popović et al., 2020).

Blackthorn is one of the wild fruit species with high antioxidant levels. In this study, the antioxidant activity of the blackthorn fruit extracts was determined by DPPH method. All tested extracts exhibited potent scavenging activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals ranging from 65.13% to 77.06%. Thanks to the antioxidant property of this species, it neutralizes the free radicals formed in the body and has a preventive effect against the damages of free radicals caused by various sources such as external factors and inadequate and unhealthy diet (Baysan, 2021).

Table 3. Total soluble solids, pH, titratable acid, total phenols and DPPH values of the blackthorn genotypes

Çizelge 3. *Çakal eriği genotiplerinin suda çözünebilir kuru madde, pH, titre edilebilir asitlik, toplam fenol ve DPPH değerleri*

Genotypes	Total soluble solids (%)	pH	Titratable acid (%)	Total phenols content (mg GAE/100 g)	DPPH (%)
B01T	13.2±0.01	3.85±0.12	1.04±0.22	387.56±0.03	72.38±0.13
B02T	12.3±0.03	3.35±0.06	0.83±0.21	375.89±0.16	65.13±0.20
B03T	13.02±0.02	4.22±0.23	0.90±0.13	366.5±0.12	74.34±0.16
B04T	11.9±0.02	4.14±0.19	0.87±0.24	356.92±0.16	77.06±0.21
B05T	12.7±0.03	4.19±0.10	1.30±0.09	373.23±0.11	68.39±0.21

Volatile organic compounds of blackthorn genotypes

Organic compound analyses of the blackthorn genotypes were performed with HS-SPME/GC/MS. According to these results, 30 different compounds were determined in 7 groups as a result of the volatile organic compound analysis. Six compounds from alcohols, 1 compound from terpenes, 6 compounds from aldehydes, 3 compounds from esters, 2 compounds from ketones, 8 compounds from acids and 5 other compounds were identified. Statistically significant differences were found among the genotypes in the identified compounds (Table 4).

Six compounds from the alcohol group, one of the volatile organic compounds, were determined in blackthorn genotypes. These compounds are 1-Octanol, (Z)-Hex-3-en-1-ol, Dihydrocitronellol, α-Terpineol, Linalool, and Nerolidol. In the study, it is the B03T genotype in which 6 alcohol groups were determined among 5 different blackthorn genotypes. Nerolidol alcohol compound was determined in all genotypes (Table 4). This alcohol compound is a sweetening and flavoring agent as an additive in foods. It is also used as a fragrance component in perfumes, cosmetics, soaps and detergents (Chan et al., 2016). Spadaccino et al. (2021), 14 compounds were detected in the alcohol group in blackthorn, and 1-Octanol values were determined to be close to each other.

In the study, only 1-Limonene was detected in B01T and B03T genotypes in the terpene group in the blackthorn genotypes (Table 4). Limonene is a compound commonly found in citrus fruits and is used as an additive to perfumes, soaps, foods, chewing gums and beverages due to its pleasant lemon-like sweet scent (Lappas & Lappas, 2012). In addition, this compound has been used in different treatments with its antibacterial, hepatoprotective, anticancer, gallstone dissolving and stomach acid-neutralizing effects (Argon et al., 2019; Kvittingen et al., 2021).

Six aldehydes were determined from volatile organic compounds. They are 2 Octenal, 2-Decenal, (E)-, 2-Dodecenal, (E)-, 2-Heptenal, (Z)-, Hexanal and Nonanal. There are many studies on the use of detected Hexanal to extend the shelf life of fresh fruits and meats (Shahidi & Pegg, 1994; Lanciotti et al., 1999; Brunton et al., 2000; Sharma et al., 2010). In a study, it was determined that the Hexanal content of blackthorn fruits was close to our study (Spadaccino et al., 2021). It was determined that the genotype richest in aldehyde content was B02T (Table 4).

In the genotypes examined in the study, 2-Butenedioic acid (Z)-, dibutyl ester, Neryl butyrate, Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester were determined within the ester group. The genotype with the highest ester component was determined as B03T (Table 4).

Two ketone group compounds (2-Propanone, 1-hydroxy- and Nonyl methyl ketone) were detected in 5 different blackthorn genotypes used in the study. B03T is the genotype with the highest values among the 5 genotypes in terms of 2-Propanone, 1-hydroxy- and Nonyl methyl ketone content. Values and statistical differences of other genotypes are presented in Table 4. Mikulic Petkovsek et al. (2016) reported some wild *Prunus* species the total amount of acid in the fruits of *P. spinosa* was higher than that of *P. avium, P. mahaleb* and *P. padus.* In this research, Decanoic acid, Undecanoic acid, Dodecanoic acid, Heptanoic acid, Hexanoic acid, Octanoic acid, Nonanoic acid and Tetradecanoic acid were determined in blackthorn genotypes. The synonym of Nonanoic acid is Pelargonic acid. This acid was determined in high amounts in all blackthorn genotypes. Ammonium pelargonate, the ammonium salt of Pelargonic acid, is a herbicide (Chitwood, 2002). Among the genotypes, the highest total acid value was determined in B05T, while the lowest was determined in B03T. Statistically significant differences were observed among the genotypes concerning acid compounds (Table 4).

Table 4. Retention times and volatile organic compounds of the blackthorn genotypes

Çizelge 4. *Çakal eriği genotiplerinin retansiyon süreleri ve uçucu organik bileşikleri*

R. time	Alcohols			B01T	B02T	B03T	B04T	B05T
	Compound Name	Syn	Molecular Formula					
29.008	1-Octanol	Octanol	$C_8H_{18}O$	0.42±0.04^{c}	nd	5.46±0.27^{b}	7.34±0.17^{a}	nd
14.749	(Z)-Hex-3-en-1-ol	Leaf alcohol	$C_6H_{12}O$	nd	1.68±0.13^{a}	1.54±0.09^{a}	nd	0.84±0.08^{b}
25.276	Dihydrocitronellol	Tetrahydrogeraniol	$C_{10}H_{22}O$	nd	nd	2.85±0.20^{a}	2.73±0.09^{a}	nd
22.878	α-Terpineol	Terpineol	$C_{10}H_{18}O$	1.10±0.06^{b}	nd	2.17±0.22^{a}	0.73±0.12^{c}	nd
19.150	Linalool	Phantol	$C_{10}H_{18}O$	0.34±0.11^{c}	0.72±0.14^{b}	2.45±0.17^{a}	nd	nd
35.821	Nerolidol	Peruviol	$C_{15}H_{26}O$	1.45±0.17^{a}	0.51±0.05^{c}	0.93±0.10^{b}	0.47±0.09^{c}	0.73±0.06^{bc}
Total Alcohols				**3.31±0.19**	**2.91±0.21**	**15.4±0.42**	**11.27±0.46**	**1.57±0.14**
		Terpen						
8.955	l-Limonene	S- (-)-Limonene	$C_{10}H_{16}$	0.37±0.05^{b}	nd	0.78±0.06^{a}	nd	nd
Total Terpen				**0.37±0.05**	**nd**	**0.78±0.06**	**nd**	**nd**
		Aldehydes						
15.893	2 Octenal	(E)-Oct-2-enal	$C_8H_{14}O$	1.24±0.12^{c}	2.64±0.25^{b}	3.16±0.20^{b}	5.13±0.28^{a}	1.26±0.11^{c}
21.534	2-Decenal, (E)-	Decenal	$C_{10}H_{18}O$	0.69±0.15^{b}	6.72±0.27^{a}	1.02±0.09^{b}	0.67±0.14^{b}	0.81±0.11^{b}
24.155	2-Dodecenal, (E)-	2-dodecenal	$C_{12}H_{22}O$	0.40±0.05^{cd}	4.80±0.37^{a}	0.81±0.20^{c}	1.49±0.17^{b}	nd
12.951	2-Heptenal, (Z)-	(Z)-2-Heptenal	$C_7H_{12}O$	0.55±0.13^{c}	1.57±0.24^{b}	4.16±0.25^{a}	nd	0.34±0.08^{c}
6.369	Hexanal	Caproaldehyde	$C_6H_{12}O$	4.74±0.41^{a}	2.96±0.29^{b}	2.19±0.19^{c}	1.53±0.16^{cd}	1.35±0.11^{d}
14.885	Nonanal	Pelargonaldehyde	$C_9H_{18}O$	0.68±0.18^{c}	4.09±0.30^{a}	nd	1.21±0.15^{b}	0.99±0.16^{bc}
Total Aldehydes				**8.30±0.59**	**22.78±1.26**	**11.34±0.81**	**10.03±0.78**	**4.75±0.11**

Table 4. Continued

Çizelge 4. *Devamı*

R. time	Esters			B01T	B02T	B03T	B04T	B05T
	Compound Name	**Syn**	**Molecular Formula**					
31.708	2-Butenedioic acid (Z)-, dibutyl ester	Dibutyl maleate	$C_{12}H_{20}O_4$	0.35±0.07^{d}	nd	4.32±0.32^{c}	18.15±0.76^{a}	13.26±0.41^{b}
35.119	Neryl butyrate	Neryl butanoate	$C_{14}H_{24}O_2$	nd	nd	1.92±0.31^{a}	2.39±0.20^{a}	0.50±0.14^{b}
26.847	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	2,4,4-Trimethyl-1,3-pentanediol 1-isobutyrate	$C_{12}H_{24}O_3$	3.55±0.47^{d}	13.6±0.38^{c}	34.55±2.93^{a}	26.46±1.54^{b}	nd
Total Esters				**3.90±0.54**	**13.6±0.38**	**40.79±2.98**	**47.00±2.10**	**13.76±0.48**
		Ketons						
12.730	2-Propanone, 1-hydroxy-	Hydroxyacetone	$C_3H_6O_2$	2.01±0.28^{b}	0.81±0.10^{c}	2.86±0.18^{a}	1.00±0.10^{c}	2.34±0.32^{ab}
28.012	Nonyl methyl ketone	2-Undecanone	$C_{11}H_{22}O$	1.62±0.27^{b}	0.45±0.10^{c}	2.76±0.26^{a}	2.63±0.25^{a}	nd
Total Ketons				**3.63±0.29**	**1.26±0.19**	**5.62±0.17**	**3.63±0.31**	**2.34±0.32**
		Acids						
35.361	Decanoic acid	Capric acid	$C_{10}H_{20}O_2$	3.32±0.35^{c}	5.32±0.57^{a}	3.64±0.30^{bc}	2.69±0.36^{bc}	4.03±0.25^{b}
37.965	Undecanoic acid	Hendecanoic acid	$C_{11}H_{22}O_2$	7.04±0.19^{a}	1.65±0.12^{b}	nd	nd	nd
41.336	Dodecanoic acid	Lauric acid	$C_{12}H_{24}O_2$	15.14±0.70^{a}	5.45±0.15^{b}	2.02±0.20^{c}	1.81±0.12^{c}	1.23±0.28^{c}
28.884	Heptanoic acid	Enanthic acid	$C_7H_{14}O_2$	0.81±0.16^{b}	0.68±0.15^{b}	nd	0.88±0.11^{b}	1.30±0.24^{a}
26.554	Hexanoic acid	Caproic acid	$C_6H_{12}O_2$	1.42±0.18^{d}	3.01±0.24^{b}	2.58±0.38^{bc}	4.54±0.39^{a}	2.07±0.16^{cd}
31.114	Octanoic acid	Caprylic acid	$C_8H_{16}O_2$	6.35±0.29^{c}	8.53±0.48^{b}	2.86±0.32^{e}	4.17±0.22^{d}	12.74±0.73^{a}
33.236	Nonanoic acid	Pelargonic acid	$C_9H_{18}O_2$	31.49±1.09^{b}	30.54±1.23^{b}	13.04±0.19^{c}	11.71±0.58^{c}	45.04±2.54^{a}
51.846	Tetradecanoic acid	Myristic Acid	$C_{14}H_{28}O_2$	0.77±0.15^{a}	0.83±0.17^{a}	nd	nd	nd
Total acids				**66.34±2.32**	**56.01±2.09**	**24.14±0.79**	**25.8±0.99**	**66.41±2.61**
			Other Compounds					
1.492	Methane, tetranitro-	Tetranitromethane	CN_4O_8	0.93±0.22^{b}	nd	1.00±0.15^{b}	nd	10.14±0.18^{a}
29.314	Morpholine, 4-octadecyl-	4-Octadecylmorpholine	$C_{22}H_{45}NO$	8.19±0.78^{a}	3.46±0.21^{b}	0.93±0.19^{c}	1.15±0.09^{c}	1.04±0.12^{c}
20.279	Hexadecane	Cetane	$C_{16}H_{34}$	0.52±0.13^{a}	nd	nd	nd	nd
1.490	l-Alanine ethylamide, (S)-	Alanine ethylamide	$C_5H_{12}N_2O$	4.51±0.38^{a}	nd	nd	nd	nd
24.024	Octane, 1,1'-oxybis-	Dioctyl ether	$C_{16}H_{34}O$	nd	nd	nd	1.12±0.11^{a}	nd
Total Other Compounds				**14.15±0.66**	**3.46±0.21**	**1.93±0.32**	**2.27±0.17**	**11.18±0.27**

nd; not detected.

^{a,b,c,d}; data with different letters in each column are significantly different ($p \leq 0.05$).

Some other compounds were also detected in the blackthorn genotypes used in the study. These are Methane, tetranitro-, Morpholine, 4-octadecyl-, Hexadecane, 1-Alanine ethylamide, (S)-, Octane, 1,1'-oxybis-. Data for these compounds and statistical differences between the genotypes are presented in Table 4. Also, the sum of the peak areas obtained by HS-SPME-GC-MS samples of 5 *P. spinosa* genotypes is presented in Figure 3.

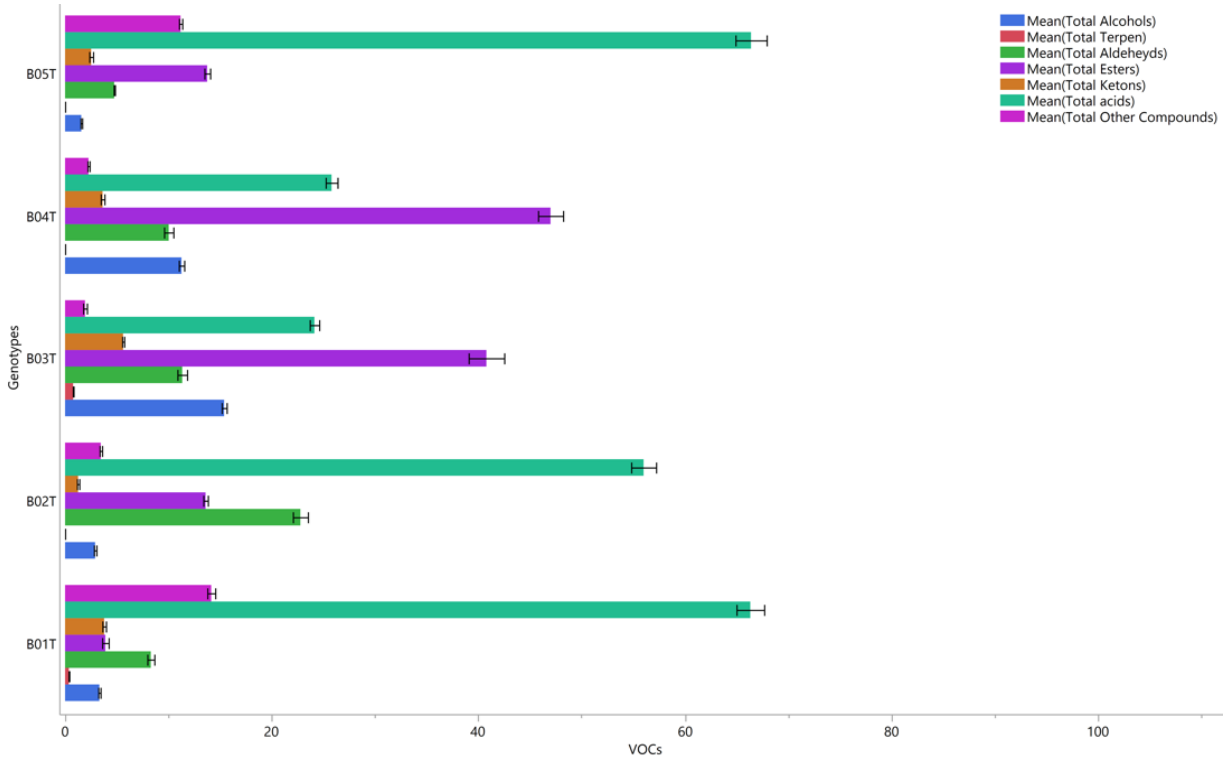

Figure 3. Sum of the peak areas obtained by HS-SPME-GC-MS samples of the *P. spinosa* genotypes.

Şekil 3. *P. spinosa genotiplerinin HS-SPME-GC-MS örneklerinden elde edilen pik alanlarının toplamı.*

A Principal Component Analysis (PCA) was performed, which reduces the multidimensional nature of the data and provides a two-dimensional map for both genotypes and VOCs to explain the observed variance. PC1 explained 26.8% of the total variation, while PC2 explained 39.0%. These two components explain a large proportion of 65.8% of all variation. The score plot clearly showed the differences between the genotypes. While B02T and B05T were in the same cluster, other genotypes (B01T, B03T and B04T) were diversified (Figure 4). As can be seen in Figure 4, all chemical components are separated from each other.

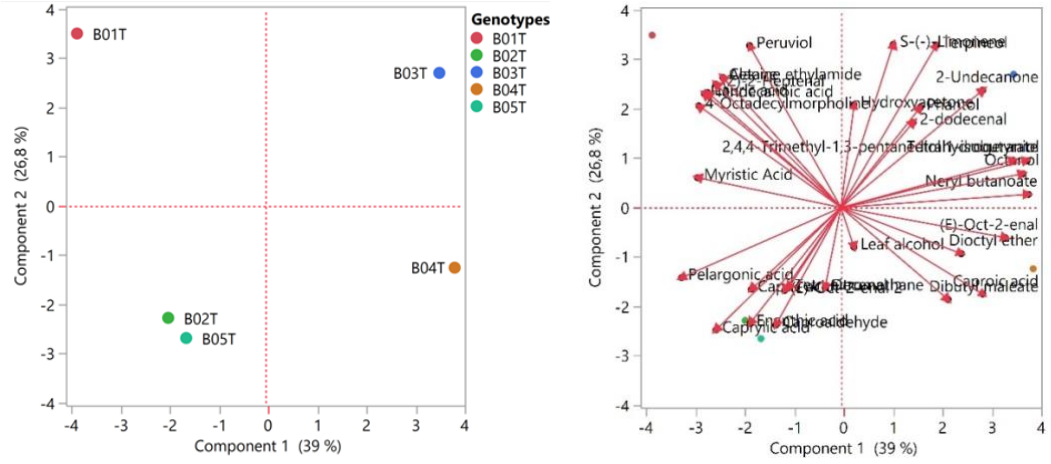

Figure 4. Score plot of principal component analysis of VOCs analyzed in different genotypes of *P. spinosa* and chemical classes.

Şekil 4. *P. spinosa 'nin farklı genotiplerinde analiz edilen Uçucu organik bileşiklerin PCA analizi skor grafiği ve kimyasal sınıfları.*

CONCLUSION

In this study, some fruit traits, total soluble solids, pH, titratable acid, total phenol and DPPH analyses were performed on the blackthorn genotypes grown naturally in the Seydişehir district of Konya province of Türkiye. As a result of the findings, it was revealed that there were differences among the genotypes. B03T, with an average fruit weight of 2.67 g, exhibited significantly higher fruit weight compared to other genotypes. Among the genotypes, B01T stood out for its total soluble solids, B01T for total phenolic content and B04T for antioxidant content. Based on the values of volatile organic compounds, PCA analysis revealed that genotypes B02T and B05T were closely associated with each other while other genotypes were distinctly separated from these two.

In conclusion, detailed information was obtained through volatile organic analyses on five different blackthorn genotypes. It was determined that the contribution of acids was high in all essential oils in the blackthorn genotypes. VOC components detected in the study; it is used in the development of functional components in food (flavor and sweetener), cosmetics, cleaning products, pharmacy and agriculture (plant growth regulator, herbicide). The findings also provide important contributions to obtaining deeper information about the chemical structure of *P. spinosa* and understanding the differences among the genotypes.

Data Availability

Data will be made available upon reasonable request.

Author Contributions

Conception and design of the study: ŞBB, ZS, AÖ, NEK, MS; sample collection: ŞBB, ZS, AÖ; analysis and interpretation of data: ŞBB, ZS, AÖ; statistical analysis: ŞBB, ZS, AÖ; visualization: ŞBB, ZS, AÖ; writing manuscript: ŞBB, ZS, AÖ, NEK, MS.

Conflict of Interest

There is no conflict of interest between the authors in this study.

Ethical Statement

We declare that there is no need for an ethics committee for this research.

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