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Sensory Evaluation, Biochemical, Bioactive and Antioxidant Properties in Fruits of Wild Blackthorn (*Prunus spinosa* L.) Genotypes from Northeastern Türkiye

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Abstract: Wild edible fruits are an important source for agriculture worldwide suffering from genetic erosion due to a severe genetic diversity reduction and domestication hindrance. In Türkiye, underutilized *Prunus spinosa* fruits are increasingly being considered as genetic resources and are marginally used by small farmers constituting a real safety valve for the sustainability of the processing plum value chain. Fruits of those plum genotypes differ in their biometric, processing and functional quality attributes. In this study, fruits of eight wild grown blackthorn (*Prunus spinosa*) genotypes were sampled from the Ispir district of the Erzurum province and subjected to sensory, morphological, biochemical and antioxidant characterization. Aroma, taste and juiciness were used as the criteria for sensory analysis, and a trained panel of ten experts established and evaluated the sensory characteristics of the fruits of the blackthorn. Fruit weight, fruit skin and flesh color as *L**, *a** and *b** values were the main morphological parameters. For biochemical and bioactive analysis, organic acids, SSC (Soluble Solid Content), vitamin C, total anthocyanins, total phenolic content and total antioxidant capacity were determined. Antioxidant capacity was determined by FRAP (ferric reducing antioxidant power) assay. The results indicated significant differences among genotypes for most of the traits. The fruit weight was found between 2.78–3.67 g. The skin *L**, *a** and *b** values were 13.11–16.12, 2.56–3.85 and 2.01–3.44, respectively. The flesh *L**, *a** and *b** values were in the ranges of 17.45–20.37, 4.88–6.73 and 4.12–5.66, respectively. The SSC content ranged from 18.66% to 21.07%. The total phenolic content (TPC), total anthocyanin content (TAC) and ferric reducing antioxidant power (FRAP) were between 372–504 mg GAE/100 g; 53–72 mg cy-3 g eq./100 g and 107–134 mmol Fe (II) eq./g, respectively. The dominant organic acid was malic acid for all genotypes and varied from 1.04 g/100 g to 1.52 g/100 g fresh weight base. The data showed that the analyzed blackthorns, particularly PS-5, PS-3 and PS-2 had bigger fruits indicate their suitability for fresh and dried consumption, PS-1 and PS-3 had higher juiciness, indicating their suitability for processing, and PS-4 and PS-6 had higher human health promoting compounds (higher total phenolic content and antioxidant capacity), making them suitable for future use as functional foods and as promising sources of natural antioxidants.

Keywords: plum; forgotten fruits; traits; composition

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1. Introduction

Fruits add value to earth's diversity and are fundamental to all life. They include high content of non-nutritive, nutritive, and bioactive compounds such as flavonoids, phenolics, anthocyanins, phenolic acids, and as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins, and minerals. With their rich nutrients, fantastic flavor and taste, excellent medicinal value and health care functions, wild edible fruits have been continuously well received by consumers as important economic crops [1–6].

Plum is one of the biggest family of genus *Prunus*, which has many different species distributed throughout the world. They found both wild and cultivated conditions. Central and eastern Europe is the main growing area of plums [7–10]. In eastern Europe, particularly the Balkan peninsula including Türkiye, has very rich plum genetic resources. The most widely distributed plum species in Türkiye are *Prunus domestica* L., *Prunus salicina* Lindl., *Prunus cerasifera* Ehrh., *Prunus insititia* L., and *Prunus spinosa* L. [11]. There was a great variation between and within species in particular for fruit and tree characteristics [12–14].

Türkiye is one of the most important diversity centers of wild plums. Seed propagated *P. cerasifera* and *P. spinosa* are abundant throughout temperate and semi-subtropical areas of Türkiye [11,12,15].

In Türkiye, local peoples have been using wild plum fruits for different purposes for centuries. Fruits can be consumed as unripe, fresh or either dried and processed into jam and marmalades. There are many different types of wild plum species and genotypes in particular rural areas in the country and one of these wild plum species is blackthorn (*Prunus spinosa* L.) [16].

It is a species of flowering plant in the rose family of the Prunoidae subfamily Rosaceae. Wild grown *Prunus spinosa* is an important part of the wild plant flora in some regions in Türkiye and has broad environmental adaptability. The blackthorn shrubs can grow on calcareous soils and shows drought and cold resistance. Wild *Prunus spinosa* shrubs are widely grown in various eco-geographical regions of Türkiye distributed from the high plateaus of Eastern Anatolia to the dry and very hot regions of Southeastern Anatolia regions, from Southeast Anatolia to the Mediterranean, Aegean and Central Anatolia regions [13]. It is referred to locally as güvem eriği, çakal eriği, ayı eriği, dağ eriği, kuş eriği, kum eriği, yaban eriği and domuz eriği in different regions in Türkiye.

Fruits of blackthorn are high in anthocyanins and are valuable sources of nutrients, vitamins, minerals, dietary fibers, phytochemicals, and especially of antioxidant compounds. The fruit of this species used as food and also has medicinal value. Thus, it is widely used for nutritional and medicinal purposes for centuries and possesses beneficial human health compounds [17–19]. In some regions of Türkiye, its fruits are also consumed as a compote because of increases resistance of body, provides blood-building effect and relieves rheumatic pain [18].

Different plant parts of the species including fruits, leaves, flowers have been used for treatment for mucosal lesions of mouth and pharynx due to its anti-inflammatory effect. *P. spinosa* plant extracts are used traditionally in the treatment of hypertension, diabetes and gastrointestinal disorders, as diuretic, in the regulation of menstruation for centuries [19].

The fruits of *P. spinosa* contain a variety of bioactive compounds such as anthocyanins (cyanidin-3-routinoside, peonidine-3-routinoside, cyanidin-3-glycoside), phenolic acids, ascorbic acid, flavonoids, tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol), ascorbic acid and β-carotene [20–22].

The plant is more recently gaining attention as a functional food and an underutilized source of bioactive compounds for application in the food and pharmaceutical industry in Türkiye. Previously, health-promoted compositions of a few *Prunus spinosa* genotypes were conducted. In addition, wild plums (*Prunus spinosa*) widely distributed throughout the Ispir district have not been investigated for their biochemical and antioxidant characteristics so far. Thus, the study investigates the content of compounds that might have health-promoting potential for a large number of blackthorn fruits from the Ispir district of Türkiye. The obtained data will provide more detailed information to the researchers about determining genotypes for multipurpose use such as for food, cosmetics, pharmaceutical, and functional product sectors.

2. Materials and Methods

2.1. Plant Materials

The plant material consists of 8 seed-propagated *P. spinosa* genotypes (Figure 1) sampled from Goc village belonging to the Ispir district of the Erzurum province of Türkiye in the year 2021. The village is very rich for wild grown Prunus species. During the field trip, health and high-yielded blackthorn genotypes were pre-selected and fruit samples were obtained from 8 different genotypes. The climate of the Ispir district is described as Humid Continental by the Köppen Climate System, abbreviated as Dfb. All genotypes are found in similar climate and soil conditions in Goc village.

Figure 1. Wildly grown blackthorn (*Prunus spinosa*) in the Ispir district.

2.2. Sensory Analysis of Fruits

A trained panel of ten experts evaluated three sensory features (taste, juiciness, and aroma) of blackthorn fruits for each genotype. The 0 to 9 bipolar hedonic scale was used to rate overall pleasantness of aroma, taste and juiciness, which were rated on a unipolar 0 to 9 intensity scale. For aroma, it was indicated as 0 = not detectable; 1 = just barely detectable; 3 = slight; 5 = moderate; 7 = intense; and 9 = extremely intense. For taste, the scale indicated 0 = not detectable; 1 = extremely sour; 3 = sour; 5 = sweet–sour; 7 = sweet; and 9 = extremely sweet. For juiciness, the scale indicated 0 = not detectable; 1 = extremely low; 3 = low; 5 = medium; 7 = high; and 9 = extremely high. Afterwards, the most evaluated characteristic was defined [23].

2.3. Morphological Traits

Fruit weights were determined with a digital balance (±0.01 g) (Scaltec SPB31, Scaltec Instruments GmbH, Goettingen, Germany) using 40 fruits per genotype. Each Prunus spinosa plant was given a code number. These code numbers were preceded by the abbreviation of the species name in Latin, the initials of the plant Latin name (PS) and finally the tree number. Accordingly, each identified genotype was named from PS-1 to PS-8.

The skin and flesh color of the blackthorn samples were measured using a chromameter (Minolta CR-400, Konika Minolta Inc. Tokyo, Japan). Colorimetric data were taken from both sides of the fruit after calibration with a black and white standard tile. The results were expressed in terms of L^*, a^*, and b^* values, where L^* represents luminosity or lightness (where 0 to 100 represents black to white), a^* represents chromaticity on a green (−) to red (+) axis, and b* represents chromaticity on a blue (−b) to yellow (+b) axis [13,14].

2.4. Biochemical Parameters

Fresh blackthorn juices were obtained from each genotype. Four replicates of 500 g each were used at full maturity. The fruits were washed, sliced into small pieces, and then crushed into juices with a juice presser. For each genotype, the juices were pressed in 4 replicate and stored at −80 °C immediately until further chemical analyses.

These homogenized samples were used for analysis of SSC, vitamin C, organic acids, total anthocyanins, total phenolics and antioxidant capacity.

SSC was determined by extracting and mixing one drop of juice from each fruit into a digital refractometer (Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan, Model RA-250HE) at 22 °C and expressed as % [24].

Vitamin C (ascorbic acid) was quantified using a reflectometer (RQFlex, Merck, Darmstadt, Germany) and expressed in a mg/100 g fresh fruit base.

In the study, citric acid, malic acid, succinic acid, and fumaric acid contents of organic acids were determined in blackthorn fruits. Organic acids in fruit extract were determined by HPLC analysis developed by Bozan et al. [25]. For the organic acid extractions, 1 g of the sample was mixed with 4 mL of 3% metaphosphorical acid. The mixture was placed in an ultrasound bath at 80 °C for 15 min and centrifuged at 5500 rpm for 15 min and the obtained mixture was filtered. The organic acids extract was analyzed using HPLC (Shimadzu LC 20A vp, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD 20A vp) and we used an 87 H column (5 µm, 300 × 7.8 mm, Transgenomic). Additionally, 0.05 M sulfuric acid was used as solvent. The operating conditions were: column temperature, 40 °C; injection volume, 20 µL; detection wavelength, 210 nm; flow rate 0.8 mL/min. The identification of organic acids and peak determination is based on peak retention times and the comparison of spectral data in accordance with standards. The identified acids were assessed in accordance with the corresponding standardized calibration curves.

Total phenolic content was determined using the Folin–Ciocalteu reagent in the modified method Singleton and Rossi [26]. In short, methanol was added to one gram of samples. Water, Folin–Ciocalteu, and 20% sodium carbonate were added to the insoluble portion of the suspension and stored in darkness for 2 h. Absorbance values for all samples used in the study were analyzed at 760 nm using a Multiscan GO microplate spectrophotometer (Thermo Scientific, Waltham, MA, USA). Gallic acid (GA) standards were prepared at determined concentrations. The obtained results are expressed in milligram gallic acid equivalents (GAE) per 100 g fresh fruit sample (FW).

The total anthocyanin content of fruits was determined using Krawczyk and Petri [27]. Anthocyanins were extracted from 2 g of fruits with 0.1% HCl (2 mL) in 96% ethanol. The mixture was centrifuged at 5.500 rpm for 10 min. Results were expressed as mg of cyanidin-3-glucoside equivalents/100 g of FW.

FRAP (Ferric Reducing Antioxidant Power) [28] assay was used for antioxidant capacity analysis. The results were expressed as mmol Fe (II) eq./g fresh weight. The FRAP assay is based on the reduction of ferric-2,4,6-tripyridyl-S-triazine (Fe (III)- TPTZ) complex into the blue-colored Fe (II) form in the presence of antioxidants. Firstly, the FRAP solution was prepared. The contents of the solution are as follows: 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl solution, and 2.5 mL of 20 mM ferric chloride ($FeCl_3.6H_2O$). The methanol extract was then filtered using filtration paper, and 1 mL of the extract was mixed with 1.5 mL of freshly prepared FRAP solution. Then, absorbance values were measured at 620 nm wavelength using Multiscan GO microplate spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Data Analysis

A statistical evaluation was employed to analyze the level of each parameter by using the software SPSS Statistics, version 19.0. All analyses were done in four replicates. For analysis of variance, the obtained data were used for means calculation. Duncan multiple range tests were performed at the significant level of $p < 0.05$. In addition, principal component analysis (PCA) was performed to determine the relationships among genotypes by using Statistica version 12.50 software.

3. Results and Discussion

3.1. Sensory Analysis

Table 1 shows sensory (aroma, taste and juiciness) properties of fruits of eight wild-grown blackthorn genotypes. Considering the genotypes, five genotypes had a high aroma (PS-1, PS-2, PS-3, PS-5, and PS-8), and the rest of the genotypes (PS-4, PS-6 and PS-7) had a moderate aroma. For taste evaluation, the majority of genotypes had a sweet taste (PS-1, PS-2, PS-4, PS-6 and PS-8) and three genotypes had a sweet-sour taste (PS-3, PS-5 and PS-7). For juiciness evaluation, five genotypes had moderate juiciness (PS-1, PS-2, PS-4, PS-6 and PS-8) and three genotypes had high juiciness (PS-3, PS-5 and PS-7) (Table 1). Kuru Berk et al. [24] used a number of Prunus spinosa genotypes for sensory analysis and found that among the genotypes, nine genotypes had medium aroma, six had high aroma and eight had very high aroma features.

Table 1. The sensory characteristics in the fruits of 8 blackthorn genotypes.

Genotypes	Aroma	Taste	Juiciness
PS-1	High	Sweet	Moderate
PS-2	High	Sweet	Moderate
PS-3	High	Sweet-Sour	High
PS-4	Moderate	Sweet	Moderate
PS-5	High	Sweet-Sour	High
PS-6	Moderate	Sweet	Moderate
PS-7	Moderate	Sweet-Sour	High
PS-8	High	Sweet	Moderate

More recently, the studies in different fruit species for special breeding purposes to obtain fruits that have high bioactive content as well high sensory profiles has been increased. The fruit flavor is a combination of the sensations of aroma and taste. The combination of sugars, acids, phenolics, and hundreds of volatile compounds contribute to the fruit flavor. However, flavor and aroma depend on the cultivar, edaphoclimatic conditions, agronomical practices, and postharvest handling. Besides aroma, other sensory properties, such as taste, juiciness, etc., are preferred by consumers [29,30]. Studies on different fruit species have shown that aroma has a very complex structure [31,32], and its perception in consumers is the resultant effect of the interaction among different volatile organic compounds (VOCs).

3.2. Morphological Traits

Table 2 shows the morphological evaluation (fruit weight and fruit skin and flesh color values) of eight blackthorn genotypes. The results indicate statistically significant differences ($p < 0.05$) among genotypes for all searched morphological parameters.

Table 2. Fruit weight and skin and flesh color indices of eight blackthorn genotypes.

Genotypes	Fruit Weight (g)	Skin			Flesh		
		*L**	*a**	*b**	*L**	*a**	*b**
PS-1	3.14 ± 0.12 e	14.56 ± 0.89 cd	3.12 ± 0.10 bc	3.16 ± 0.18 a	19.22 ± 1.09 ab	4.88 ± 0.32 b	4.43 ± 0.29 ab
PS-2	3.55 ± 0.17 c	15.67 ± 0.96 b	3.45 ± 0.14 b	2.85 ± 0.16 ab	17.68 ± 1.02 bc	6.34 ± 0.41 ab	5.18 ± 0.33 ab
PS-3	3.71 ± 0.16 b	13.88 ± 0.80 e	2.56 ± 0.11 d	3.44 ± 0.25 ab	17.45 ± 1.05 c	6.73 ± 0.29 a	5.66 ± 0.24 a
PS-4	2.95 ± 0.14 f	13.11 ± 0.77 f	2.83 ± 0.16 cd	3.28 ± 0.28 ab	19.04 ± 1.11 ab	5.12 ± 0.32 ab	4.33 ± 0.20 ab
PS-5	3.85 ± 0.22 a	16.03 ± 1.03 ab	3.85 ± 0.20 a	2.38 ± 0.19 ab	18.33 ± 1.03 b	5.56 ± 0.26 ab	4.98 ± 0.26 ab
PS-6	3.27 ± 0.20 d	14.44 ± 0.71 d	2.95 ± 0.17 c	2.56 ± 0.15 ab	18.56 ± 0.98 bc	5.80 ± 0.36 ab	4.49 ± 0.40 b
PS-7	2.67 ± 0.12 bc	16.12 ± 0.66 a	3.26 ± 0.24 bc	2.01 ± 0.17 b	20.37 ± 1.04 a	4.96 ± 0.39 b	4.12 ± 0.32 c
PS-8	2.88 ± 0.15 fg	14.86 ± 0.70 c	3.34 ± 0.26 bc	2.63 ± 0.12 ab	19.55 ± 1.02 ab	5.44 ± 0.34 ab	4.33 ± 0.35 ab

There were significant ($p < 0.05$) differences among the different letters in the same columns.

Considering the fruit weight of genotypes, the highest fruit weight was obtained from PS-5 as 3.85 g and followed by PS-3 as 3.71 g, PS-2 3.55 g while the lowest fruit weight was seen on PS-7 genotypes as 2.67 g (Table 2). Previously, Ozzengin et al. [33] reported the fruit weight of the wild-grown *P. spinosa* genotype as 2.40 g indicating close values with our results. There were few studies in the literature on the fruit weight of blackthorn. Smaller fruits have general characteristics of wild edible fruits including blackthorn. Kuru Berk et al. [24] also found variable fruit weight among a number of *Prunus spinosa* genotypes. The results clearly indicated that the fruit size of blackthorns is relatively small. It has been emphasized that smaller fruits are typically sweeter and more suitable for processing, especially to produce dried fruit such as prunes [34,35].

The genotypes exhibited skin *L**, *a** and *b** values between 13.11–16.12, 2.56–3.85 and 2.01–3.44, respectively. Flesh *L**, *a** and *b** values of eight blackthorn genotypes were in the ranges of 17.45–20.37, 4.88–6.73 and 4.12–5.66, respectively. Ozzengin et al. [33] found that wild blackthorn fruits had great variations in skin *L** (lightness), *a** (redness) and *b** (yellowness) values and they found skin *L**, *a** and *b** values were ranged from 15.47 to 27.58, from 2.59 to 7.04 and from 1.77 to 6.55, respectively. They also reported flesh *L**, *a** and *b** values in the range of 19.34 to 31.28, from 0.98 to 5.32 and from 4.86 to 14.59, respectively. Our *L**, *a** and *b** color indices results could be comparable with previous results [32,36]. The genotype PS-7 had the highest skin *L** (16.12), PS-5 had the highest *a** value (3.85) and PS-3 had the highest *b** value (3.44). For the flesh of fruits, the highest *L**, *a** and *b** were detected in genotype PS-7, PS-3 and PS-3 as 20.37, 6.76 and 5.33, respectively. Fruit color is a key characteristic, as it may be a guide to consumer acceptance and preference or rejection [33]. Therefore, it can also be concluded that the color of the product in which the fruit or its extract will be used is of utmost importance. Blackthorn fruits are characterized by very high anthocyanin content and concentrated on skins of fruits rather than flesh [24].

Fruit color, SSC and acidity are the best indicators of quality and maturation for all plum species, including blackthorn [13,23]. Naturally found in rural areas and grown as wild, blackthorn fruit colors are needed to attract and conscript animals to act as seed dispersers. Chromoplasts contribute the bright red, blue, orange, and yellow colors to many fruits and flowers [37,38].

3.3. Biochemical and Bioactive Content

Table 3 presents organic acid content in fruits of eight wild-grown blackthorn genotypes. All genotypes are characterized by high malic acid content in their fruits. The malic acid content ranged from 1.04 g/100 g to 1.52 g/100 g fresh weight base. The citric acid content was between 14.07 mg/100 g and 18.64 mg/100 g among genotypes and there were statistically significant differences among genotypes at $p < 0.05$ level (Table 3). PS-7 had the highest citric acid, and PS-2 had the highest malic acid content. The other detected organic acids in blackthorn fruits are succinic acid, which was between 1.95 and 3.44 mg/100 g, and fumaric acid, between 0.92 and 1.56 mg/100 g, respectively.

Table 3. Organic acid content in fruits of eight blackthorn genotypes.

Genotypes	Citric Acid (mg/100 g)	Malic Acid (g/100 g)	Succinic Acid (mg/100 g)	Fumaric Acid (mg/100 g)
PS-1	17.12 ± 0.80 ab	1.32 ± 0.09 ab	3.12 ± 0.14 ab	1.56 ± 0.12 NS
PS-2	17.80 ± 0.76 ab	1.52 ± 0.11 a	3.37 ± 0.18 ab	1.35 ± 0.10
PS-3	15.38 ± 0.90 bc	1.41 ± 0.08 ab	3.44 ± 0.19 a	1.12 ± 0.08
PS-4	14.07 ± 0.68 c	1.25 ± 0.10 ab	2.88 ± 0.20 ab	1.20 ± 0.13
PS-5	15.03 ± 0.96 bc	1.12 ± 0.10 b	2.50 ± 0.17 ab	0.92 ± 0.10
PS-6	14.68 ± 0.77 bc	1.44 ± 0.12 ab	3.20 ± 0.25 ab	1.44 ± 0.15
PS-7	18.64 ± 0.70 a	1.29 ± 0.09 ab	1.95 ± 0.14 b	0.97 ± 0.07
PS-8	16.50 ± 0.83 b	1.04 ± 0.07 ab	2.65 ± 0.18 ab	1.25 ± 0.12

There were significant ($p < 0.05$) differences among the different letters in the same columns; NS: Not significant.

Malic acid and succinic acid significantly varied among genotypes ($p < 0.05$), but there were no significant differences among genotypes for fumaric acid (Table 3). In contrast, Ozkan [16] reported that blackthorn fruits were dominantly characterized by high citric acid content (21.45–28.37%). Kuru Berk et al. [23] found that citric acid was dominant in blackthorn fruits and malic acid, succinic acid and fumaric acid also presented in its fruits with variable ratios.

Celik et al. [39] analyzed organic acid contents in blackthorn sampled from the Van province of eastern Türkiye and indicated that malic acid was the predominant organic acid, and had the highest value as 1.245 g/100 g of fresh fruit. They also found citric acid in fruits of *P. spinosa* L. as 27.61 mg/100 g, indicating similarities with our study. Ozcan [38] also found that *P. spinosa* fruits are rich for malic acid and followed by citric acid and fumaric acid, which is in agreement with our present results. Organic acids, which affect the taste in fruits, provide the sugar balance and forms the main ingredient of many compounds. In general, the dominant organic acid is malic acid in pome and stone fruit. Citric acid is found mostly in citrus fruits. Organic acids are an important compound of plum fruits. Organic acids are effective on properties such as color, taste and flavor of plum fresh fruits and products derived from plums. Organic acids are used as an energy source for carbon respiration metabolism for the production of sugars in plums. It is also used in the determination of maturity with sugars. Organic acids are associated with some heavy metals in the human body to form salts and regulate the rate acid-base in the blood. They also eliminate the negative effects of food and serve human health [40].

Table 4 presents SSC, vitamin C, total phenolic content, total anthocyanin content and total antioxidant capacity in fruits of eight blackthorn genotypes. SSC, vitamin C, total phenolic content, total anthocyanin content and total antioxidant capacity significantly differed among genotypes at $p < 0.05$ (Table 4).

Table 4. Soluble solid (SSC), vitamin C, total phenolic, total anthocyanin and total antioxidant capacity in fruits of eight blackthorn genotypes.

Genotypes	SSC (%)	Vitamin C (mg/100 g)	Total Phenolic (mg GAE/100 g FW)	Total Anthocyanin (mg cy-3 g eq./100 g)	FRAP (mmol Fe (II) eq./g)
PS-1	19.15 ± 0.88 bc	16.50 ± 0.84 b	498 ± 32 ab	60 ± 3.55 c	116 ± 6.13 c
PS-2	19.80 ± 0.79 b	18.90 ± 1.07 ab	450 ± 38 c	68 ± 3.80 b	125 ± 7.41 b
PS-3	18.66 ± 1.03 c	17.90 ± 0.90 ab	390 ± 29 f	72 ± 2.99 a	112 ± 5.95 cd
PS-4	20.70 ± 1.13 ab	20.44 ± 1.05 ab	504 ± 40 a	63 ± 2.67 bc	134 ± 7.23 a
PS-5	18.90 ± 1.04 bc	18.60 ± 1.10 ab	424 ± 39 d	68 ± 2.50 b	121 ± 6.56 bc
PS-6	21.07 ± 1.21 a	21.30 ± 1.05 a	482 ± 48 b	66 ± 3.03 bc	128 ± 6.20 ab
PS-7	18.40 ± 0.90 bc	19.50 ± 0.98 ab	404 ± 40 e	53 ± 2.60 d	116 ± 5.88 c
PS-8	21.00 ± 1.20 a	20.70 ± 1.04 ab	372 ± 29 g	56 ± 3.01 cd	107 ± 4.90 d

There were significant ($p < 0.05$) differences among the different letters in the same columns.

The highest SSC content was obtained from genotype PS-6 as 21.07% and followed by PS-8 (21.00%), PS-4 (20.70%), PS-2 (19.80%), PS-1 (19.15%), PS-5 (18.90%), PS-3 (18.66%), and PS-7 (18.40%), respectively (Table 4). Previously, the SSC content of fruit juices of a blackthorn genotype was determined as 20.62% [31], which indicated good agreement with our present SSC result. The SSC content of fruits change significantly with maturation and the ratio between SSC and titratable acidity is important for the practical determination of the ripening degree of the fruit. Soluble solid content (SSC) is also one of the most important quality indices of different fruits and it is mainly organic sugars, such as glucose, sucrose and fructose, which influence the taste and flavor of plum fruits [41]. The SSC content of plum cultivars was previously reported between 17.00–23.00% [24,41].

The eight blackthorn genotypes had vitamin C content between 16.50 mg/100 mg (PS-1) and 21.30 mg/100 g (PS-6) (Table 4). Ozkan [16] reported that fruits of a number of *Prunus spinosa* genotypes had vitamin C content between 16 and 26 mg/100 g fresh fruit. Nergiz and Yildiz [42] found that the vitamin C content in Victoria and Stanley plum cultivars was between 19 and 23 mg per 100 g. Celik et al. [39] found the vitamin C content in fruits of *Prunus spinosa* as 25.49 mg/100 g. The findings of the present study seem to be mostly consistent with the above studies. Horticulture crops including fresh fruits, vegetables and grapes are the major sources of vitamin C, therefore increasing its concentration will have an important impact in human nutrition. Sikora et al. [7] also found similar vitamin C content (23.84 mg/100 g FW) in fruits of Blackthorn wildly grown in Poland.

The total phenolic contents (TPC) in fruits of eight *Prunus spinosa* genotypes ranged between 372 mg GAE/100 g (PS-8 genotype) to 504 mg GAE/100 g (PS-4 genotype), respectively (Table 3). Previously, the total phenolic content of a wild grown blackthorn genotype was found to be 440 mg GAE/100 g FW [31], which indicates similarities with our present results. Cinquanta et al. [36] previously reported TPC between 520 and 750 mg GAE/100 g, which indicated higher values than our result. In Australia, Johnson et al. [43] have reported TPC of 65.00–160.00 mg GAE/100 g in six plum samples, which shows lower values than our result. The total content of polyphenolic compounds, expressed as gallic acid (GAE) equaled was found to be 599 mg GAE/100 g of fresh fruits in blackthorn grown in Poland [7]. Our results indicate that the fruits of wild grown blackthorn genotypes are rich in phenolic components. Previous studies also indicated that blackthorn fruits have functional properties [7,13,20,44].

The total anthocyanin content (TAC) and ferric reducing antioxidant power (FRAP) were between 53 and 72 mg cy-3 g eq./100 g and 107 and 134 mmol Fe (II) eq./g in the fruits of eight blackthorn genotypes, respectively (Table 4). PS-3 and PS-4 had the highest anthocyanin and antioxidant capacity. Ozzengin et al. [33] reported total anthocyanin content and total antioxidant capacity as 64.57 mg/100 g and 149 mmol Fe (II) eq./g, which shows similarity with our present results on the total anthocyanin content and total antioxidant capacity. Sikora et al. [7] also found similar total anthocyanin content (71.75 mg/100 g FW) in fruits of Blackthorn wildly grown in Poland. Katanic Stankovic et al. [44] found that blackthorn fruits are rich in antioxidants.

Studies on *Prunus spinosa* fruits in different parts of the world indicated the fruits' high antioxidant compounds [45–51]. Previous studies revealed that, as with other stone fruits, several factors were shown to influence the fruit antioxidant capacity, including species, cultivars, genotypes, accessions, geographic region, maturity stage, length of the fruit development period, etc. [52–54]. However, the genotype proved to be the most important factor influencing the fruits' anthocyanin content and antioxidant capacity [54–58].

3.4. Principal Compenent Analysis (PCA)

The PCA model was applied to all data to obtain clearer visualization and to identify any group patterns among the eight blackthorn genotypes (Figure 2). The analysis combined morphological, biochemical and bioactive characteristics. Overall, 69.19% of the variability observed in the characteristics was explained by the first three components (36.05%, 25.27% and 7.87% for PC1, PC2 and PC3, respectively) and the overall variance divided the analyzed cultivars into four distinct clusters. The first principal component (PC1), which explains 36.05% of the overall variance, is clearly identified with the fruit weight and SSC, while the second principal component (PC2) is related to the Vitamin C, total phenolics, total anthocyanins and citric acid. The factors that most contributed to PC1 (positive side) were: fruit weight and SSC. The PCA analysis carried out for the purposes of this study thus confirmed significant differences in the morphological, biochemical and bioactive content of wild blackthorn genotypes. PCA analysis also divided genotypes 4 groups into bigger fruited ones, sweeter ones or those with a higher content of polyphenolic compounds and antioxidant capacity (Figure 2). Kuru Berk et al. [23] used PCA (Principal Component Analysis) to determine the relationship between *Prunus spinosa* genotypes according to agro-morphological features and reported variation to be 63.50% for the first three components.

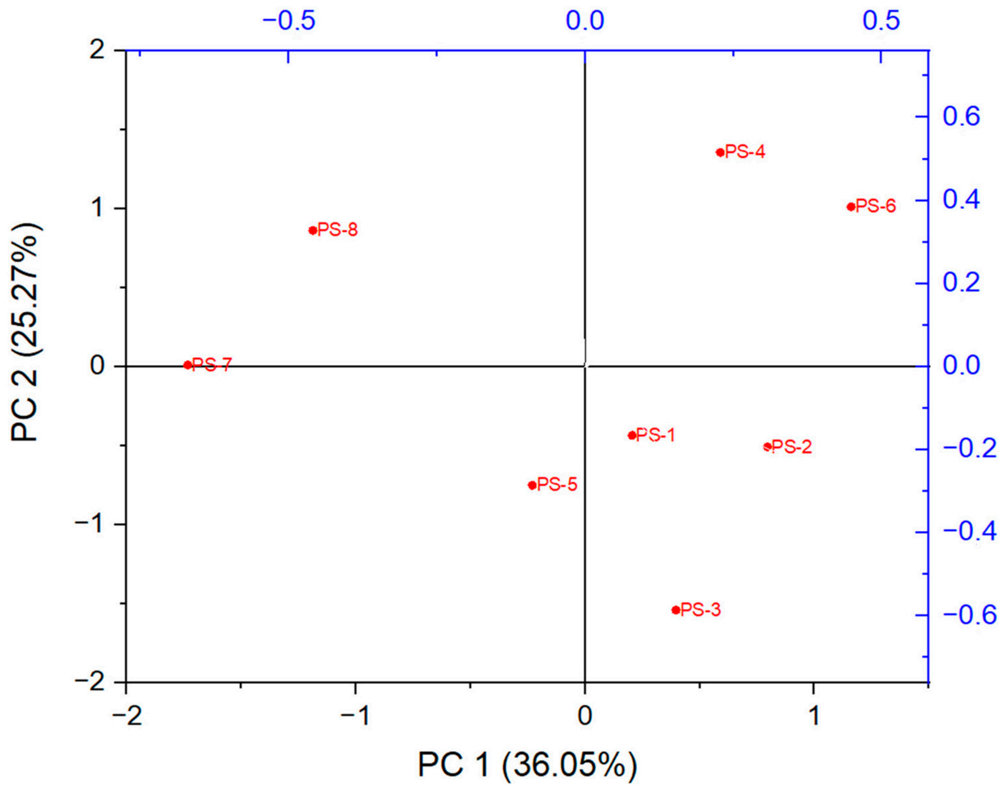

Figure 2. Distribution of blackthorn genotypes (PS-1 to PS-8) according to morphological, biochemical and bioactive characteristics determined by principal component analysis.

4. Conclusions

In this study carried out on *Prunus spinosa* genotypes, significant differences emerged between genotypes in terms of fruit sensory, morphological, biochemical and bioactive characteristics. The PS-5, PS-3 and PS-2 genotypes exhibited bigger fruits indicate their suitability for fresh and dried consumption. PS-1 and PS-3 showed higher juiciness indicated their suitability for processing and PS-4 and PS-6 had higher human health promoting compounds, making them suitable for future use in both pharmacological applications and use as functional foods. These genotypes could be important as promising sources of natural antioxidants as well. In addition, the studied blackthorn fruits are good nutritional sources that provide numerous opportunities for their application in the food industry for the development of new value-added foods or the improvement of existing food products. The obtained results support further evaluation of the functional food potential of blackthorn fruit. Finally, the manuscript highlights the potential applications of different blackthorn fruits in various industries, including the food, cosmetics, pharmaceutical, and functional product sectors.

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