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Research Article

Effects of harvest date on plant growth parameters and fruit phytochemical contents in different cultivars of chokeberry

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Abstract

Chokeberry, a type of berry fruit, has garnered attention in recent years due to its remarkable antioxidant properties. The rich bioactive components found in its fruits have been found to exert positive effects on human health. In this research, the growth, yield, and quality of two chokeberry cultivars ('Nero' and 'Viking') were evaluated, along with detailed biochemical parameters of the fruits, across different harvest dates ranging from mid-August to mid-September. During the experiment, it was observed that the 'Viking' cultivar exhibited more upright and taller plant growth than 'Nero'. There were no significant differences observed between the cultivars in terms of yield, total soluble solids (TSS), total phenolic content (TPC), total monomeric anthocyanins (TMA), and antioxidant activity. However, 'Nero' demonstrated superiority in phenolic acids (excluding ferulic acid), glucose, citric acid, quinic acid, and ascorbic acid content. As the harvest progressed from August 31 to September 15, there was an increase in the accumulation of fruit TSS, TPC, TMA, sucrose, glucose, fructose, sorbitol, epicatechin, neo-chlorogenic acid, caffeic acid, chlorogenic acid, and ferulic acid. Harvest dates did not impact antioxidant activity and anthocyanin compound values. Among the taste-related compounds, sorbitol was determined to be the most abundant, while sucrose exhibited a positive correlation with all individual sugars. Additionally, malic acid and quinic acid showed a positive correlation. The combination of the 'Nero' cultivar and the September 15th harvest date stood out as the most prominent in superior fruit bioactive compounds.

Keywords: *Aronia melanocarpa*; bioactive phytochemicals; color; taste-related parameters

Abbreviations: AA, antioxidant activity; TSS, total soluble solids; TPC, total phenolic content; TMA, total anthocyanin content; L*, lightness; a, redness=greenness; b, yellowness=blueness; C*, chroma; h°, hue angle; cv., cultivar; OD, observation date; HD, harvest date; Nr, Nero; Vk, Viking

Introduction

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From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Black chokeberry [*Aronia melanocarpa* (Michx.) Elliott] is a berry fruit crop that belongs to the *Rosaceae* family (Jeppsson, 2000; Kulling *et al.*, 2008). Fruits are a rich source of bioactive polyphenolic compounds (Jovanović *et al.*, 2023; Stach and Kolniak-Ostek, 2023). Its fruits, in particular, demonstrate superior properties in anthocyanins, flavonoids, phenolic compounds, vitamins, and minerals (Wu *et al.*, 2006; Platonova *et al.*, 2021; Zhang *et al.*, 2021). Due to their high antioxidant content, the fruits possess significant potential for promoting health and well-being (Torović *et al.*, 2023). Numerous studies have demonstrated the beneficial effects of black chokeberries, including their ability to strengthen the immune system and reduce the risks associated with conditions such as hypertension, obesity, glucose metabolism disorders, and thrombosis. Additionally, black chokeberries have been found to inhibit the development of various types of cancers (Sidor *et al.*, 2019; Gill *et al.*, 2021).

Chokeberry fruits are widely utilized in the food, pharmaceutical, and cosmetic industries (Jovanović *et al.*, 2023). Chokeberry fruits can be consumed fresh or processed into various forms, including dried fruits, juices, jams, fruit teas, marmalades, and dietary supplements (Torović *et al.*, 2023; Gurčik *et al.*, 2023).

Plant growth, yield, fruit quality, biochemical content, and taste of fruits are influenced by numerous factors, including variety, ecological conditions, cultural practices, and harvest dates. (Kawecki and Tomaszewska, 2006; Skupien *et al.*, 2008). The maturation of chokeberry fruits initiates with a change in color and an increase in the total soluble solids content. Specifically, the color change of the fruits begins in July and continues to increase throughout August. Likewise, the total soluble solid content of the fruits increases over time (Engin and Mert 2020). Therefore, the harvest date plays a significant role in determining the taste of chokeberry fruits. It has been observed that consumers' taste preferences vary over short intervals from July to September. Therefore, the phytochemical contents of the fruits can vary significantly, contributing to differences in taste (Jeppsson *et al.*, 2000).

The choice of cultivar also influences the taste and biochemical characteristics of the fruit. In Türkiye, the 'Nero' and 'Viking' cultivars are commonly grown. However, the comprehensive understanding of plant growth performance, taste, detailed fruit biochemical properties in these chokeberry cultivars, and the effects of different harvest dates remains limited under the growing conditions in Türkiye. Indeed, further studies are necessary to comprehend these factors more comprehensively. The present research aimed to investigate the plant growth, yield, pomological characteristics, and detailed phytochemical composition of chokeberry cultivars grown in the high-altitude area of Antalya, situated in the southern region of Türkiye. The study assessed the influence of two difference cultivars 'Nero' and 'Viking' and harvest dates on these aspects.

Materials and Methods

Research area and properties

The study was carried out at Akdeniz and Çukurova Universities during the growing season of 2019-2020. The trial area is located in Korkuteli, Antalya, in the southern part of Türkiye (latitude 37°01'-36' N, longitude 30°14'-52' E) at an altitude of 970 meters. Korkuteli district is known for its high elevation, and temperate conditions, including cold winters and dry summers, characterize this region's climate. The climate properties of the study area are presented in Table 1, while Table 2 provides information on soil properties.

Table 1. Climatic data in 2020

Parameter	Months											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Max. Temperature (°C)	9.3	10.4	14.8	18.2	24.1	26.5	33.9	33.7	31.9	25.0	16.9	12.5
Min. Temperature (°C)	-1.8	-0.2	2.1	5.5	9.5	12.2	17.4	16.4	14.8	9.7	2.3	1.8
Average Temperature (°C)	2.4	4.2	7.6	11.4	16.6	19.3	25.7	24.7	22.8	16.5	8.2	6.0
Average Relative Humidity (%)	70.3	75.1	66.3	65.5	54.2	53.1	35.7	35.8	41.7	58.2	64.5	87.2
Average Precipitation (mm=kg/m^2)	2.0	0.8	1.2	1.8	1.1	0.7	0.0	0.1	0.1	0.2	0.9	1.8
Max. Wind Speed (m/sn)	5.4	6.2	6.4	6.5	6.8	6.7	6.7	6.4	6.0	5.4	4.2	4.0
Average Wind Speed (m/sn)	1.2	1.3	1.4	1.3	1.4	1.3	1.3	1.2	1.2	0.9	0.9	0.8

Table 2. Soil properties of the study area

Parameter	Value	Comment
pH	7.86	Alkali
Lime	16.36%	High chalky
Organic matter	1.82%	Poor
Structure	64.90%	Clay loam
Salinity	0.051%	Without salt
P_2O_5 (Phosphorus)	10.72 ppm	Medium
K_2O (Potassium)	179.79 ppm	High
Ca (Calcium)	43.50 ppm	Rich
Mg (Magnesium)	406.50 ppm	Rich
Fe (Iron)	1.72 ppm	Little
Zn (Zinc)	1.16 ppm	Very high
Mn (Manganese)	0.78 ppm	Insufficient
Cu (Cupper)	2.54 ppm	Sufficient

Plant materials

Two chokeberry cultivars, 'Nero' and 'Viking', were selected as the plant materials for this study. The characteristics of these cultivars are described below:

Nero: The chokeberry plants used in the study grow in a shrub form, reaching a height of 1.5-2.0 meters. They are self-fertile, meaning they do not require pollinators for fruit production. The flowers are arranged in clusters, and each cluster typically contains 18-20 fruits. When ripe, the fruits have a black color and a round shape. The average fruit weight ranges from 1.1 to 1.6 grams (Poyraz Engin, 2020; Poyraz Engin and Mert, 2020).

Viking: The plant grows upright as a shrub and can reach a 2.0-2.5 m height. It is self-fertile. The number of fruits in the cluster ranges from 8 to 40. The fruit is flattened from the poles, purple-black in color. Fruit weight is between 1.0-1.4 g (Poyraz Engin, 2020; Poyraz Engin and Mert, 2020).

Growing conditions and evaluation parameters

For this research, 1.5-year-old seedlings obtained through tissue culture were utilized. The seedlings were planted in February 2019 at 160 cm X 300 cm intervals. A total of 250 plants were planted in the trial area. In this experiment, the 'Nero' and 'Viking' cultivars were subjected to examine morpho-physiological traits, including chlorophyll index, shoot diameter, bush height, leaf width, leaf length, and leaf number in a shoot. Furthermore, the study also evaluated the yield, pomological characteristics (fruit color, total soluble solids), and detailed biochemical properties of the fruits. These included measurements of total phenols, total monomeric anthocyanins, antioxidant activity, as well as taste-related parameters such as sucrose, glucose, fructose, sorbitol, citric acid, malic acid, quinic acid, ascorbic acid. Additionally, the content of various phenolic acids such as catechin, epicatechin, o-coumaric acid, neo-chlorogenic acid, caffeic acid, chlorogenic acid, ferrulic acid, rutin, and anthocyanin compounds like cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-xyloside were also evaluated. These assessments aimed to provide a comprehensive understanding of the biochemical composition and properties of the fruits.

In this study, the analysis of morpho-physiological traits was conducted monthly from May to October. Pomological analyses were performed on fruits collected on four different harvest dates (18 August, 21 August, 30 August, and 15 September). Biochemical studies were conducted on fruits from two different harvest dates (30 August, 15 September). Collecting data at various time points and harvest dates enabled a comprehensive assessment of the plant's development, fruit characteristics, and biochemical composition. The experiment consisted of three replications and five plants in each replicate. Morpho-physiological traits were evaluated in five plants and pomological analyses in ten fruits in each replicate.

Determination of morpho-physiological traits

This study assessed the leaf chlorophyll index value using a chlorophyll meter (FieldScoot CM1000) (Adak, 2019). The shoot diameter, bush height, leaf width, leaf length, and leaf number in a shoot were measured and recorded.

Determination of pomological features

The fruit skin color was measured using a color meter, specifically the 3NH NR20XE Precision Colorimeter manufactured by Shenzhen Threenh Technology Co., Ltd. The measurements were taken in terms of L*, a*, b* values and Hue and Chroma values. These measurements objectively quantified the fruit's skin color characteristics (Selcuk & Erkan, 2015). The total soluble solids content (TSS) of the fruits was measured using a digital refractometer (REF121; Atago, Guangzhou, China) expressed as a percentage (%) (Mitcham et al., 1996).

Determination of fruit yield

The fruit yield was determined on average throughout the growing season and was presented as yield per plant. Since each replication consisted of five individual plants, the yield per plant was evaluated based on the overall plot yield.

Fruit extract to detail analyses

The black chokeberry fruits were kept in deep freezer (-65 °C, Hettich, Germany) until analyses. The analyses were conducted in one week in order to eliminate storage effect.

The frozen black chokeberry fruits were initially processed using a high-speed shredder (Waring, USA) to obtain a uniform fruit pulp. Afterwards, five grams of this pulp were placed in a 50 mL centrifuge tube and mixed with 45 mL of methanol solution (80%) (Agcam, 2022). The tubes were centrifuged (6000 rpm, 4 °C and 10 min, Hettich, Germany). The supernatants were used as stock for bioactive compound analyses. All the suggested analyses were performed using the stock solution obtained on the same day.

Determination of individual sugar and organic acids

The individual sugras and organic acids in black chokeberry samples were determined by following the HPLC method suggested by Sturm *et al.* (2003). Briefly, 1 g sample was transferred into 15 mL centrifuge tube and diluted up to 10 mL with ultra-pure water, and then mixed adequately. After that, the tubes were centrifuged at 6000 rpm and 4 °C for 5 min (Hettich, Germany). The supernatant was passed through nylon membrane filter (0.45 µm) and injected to the HPLC/PDA/RID system (Shimadzu, Japan). The chromatographic conditions as flow rate, oven temperature, mobile phase, column was 0.4 mL/min (isocratic flow), 50 °C, 5 mM H_2SO_4, and Concise-Coregel/Ion 300, respectively. The PDA detector was set at 210 and 244 nm for identification of organic acids. The identification of the peaks was performed by comparing retention times and spectral data with the certified standards. The calibration curves were used to quantify the peak concentrations. The individual sugars were expressed as g/100 g FW, while the organic acids as mg/kg FW.

The monomeric anthocyanin determination

The total monomeric anthocyanin content (TMA) was determined by following the spectrophotometric assay suggested by Giusti & Wrolstad (2001). The findings were presented regarding the amount of pelargonidin 3-glucoside equivalents per kilogram of fresh fruit. The following formulas were used for TMA content calculation:

$$A = (A_{\lambda_{520}} - A_{\lambda_{700}})_{pH=1} - (A_{\lambda_{520}} - A_{\lambda_{700}})_{pH=4.5} \qquad (Eq.1)$$

$$TMA\ \left({}^{mg}/_{kg}\right) = \frac{A.MW.DF}{\varepsilon.l} \times 1000 \qquad (Eq.2)$$

where A is the absorbance value, MW is the molecular weight of pelargonidin 3-glucoside (433.4 g/mol), DF is the dilution factor, ε is the molar absorptivity constant (22400 L/cm.mol), and l is the path length of the cuvette (1 cm).

The total phenolic compounds determination

The Folin-Ciocalteu method Abdullakasim *et al.* (2007) suggested was adapted for black chokeberry fruit extract. The quantity of phenolic compounds in the fruit samples was expressed as milligrams per kilogram of gallic acid equivalent (mg GAE/ kg).

Antioxidant activity analysis

The DPPH* method, as employed by Klimczak *et al.* (2007), was utilized to evaluate the antioxidant activity (AA) of chokeberry fruits.

To determine the antioxidant activity of black chokeberry fruits, Trolox equivalents were calculated using the DPPH* method and calibration curves generated from specific concentrations of Trolox solutions. The equations derived from these curves were employed to express the antioxidant activity of chokeberry fruits in terms of milligrams of Trolox equivalent per kilogram of fresh fruit (mg TE/kg).

Determination of individual phenolic compounds

Using HPLC/PDA (Shimadzu, Japan), the specific phenolic compounds present in chokeberry fruits were identified. A primary stock extract was filtered through a membrane filter (0.45 µm, PTFE) to prepare brown injection bottles. The chromatography parameters were applied as follows: a gradient flow rate of 1 mL/min; wavelengths at 280, 320, and 520 nm; XTERRA RP C18 (Waters, 5 µm, 4.6 × 250 mm) as a column at 30 °C; and 20 µL as injection volume. The employed gradient flow comprised of two mobile phases, namely 5 % formic acid (mobile phase-A) and a combination of 20% mobile phase-A with 80% acetonitrile (mobile Phase-B) with the elution profile previously suggested by Sarıdaş (2021) was used.

The identification and quantification of the distinct phenolic peaks were executed by matching retention time and spectrum with high-purity external standards. The concentration of the identified peaks was determined using calibration curves established from these external standards ($R^2 \geq 0.996$). A validation study has been carried out on the applied method for analyzing phenolic compounds, and the findings have been outlined in a previous study (Sarıdaş, 2021).

Statistical analysis

The study utilized a completely randomized design with combined repeated time (HD), comprising three replications. All obtained data were evaluated with SAS-based JMP 8.1 software. ANOVA was conducted to determine the differences in black chokeberry cvs. ('Nero' and 'Viking'), HD, and these interactions in all examined variables except for yield. Total yield evaluated by completely randomized design. The results were compared with the LSD test at a 5% significance level. At the end of the study, the relationship between parameters was evaluated with Principal Component Analysis (PCA) using XLStat software (Addinsoft, New York, USA).

Results

Morpho-physiological traits

The research study assessed morpho-physiological traits monthly throughout a 6-month vegetation period from May to October (Table 3). Although there were no statistically significant differences observed in the chlorophyll index, shoot diameter, leaf width, leaf length, and leaf number within shoot values between the cultivars, the bush height of 'Viking' (108 cm) was significantly longer, with a 17.5% increase, compared to 'Nero' (89.7 cm) ($p<0.001$) (Table 3). Observation dates have been found to significantly influence morpho-physiological traits, including shoot diameter, bush height, and leaf numbers ($p<0.001$).

As anticipated, a consistent increase in shoot diameter and bush height values was observed from May to October. Furthermore, the highest number of leaves was recorded during June and July. The analysis revealed that there was no significant interaction between cultivar (cv.) and harvest date (HD) for all morpho-physiological trait values.

Table 3. Morpho-physiological traits of two chokeberry cultivars at different observation dates

Treatments	Chlorophyll index	Shoot diameter (mm)	Bush height (cm)	Leave width (mm)	Leave length (mm)	Leave number in a shoot
Cultivar (cv.)	N.S.	N.S.	***	N.S.	N.S.	N.S.
'Nero' (Nr)	238.4	8.7	89.7 b	49.3	72.8	11.0
'Viking' (Vk)	231.6	9.2	108.0 a	52.1	75.5	10.1
Observation Date (OD)	N.S.	***	***	N.S.	N.S.	***
May (M)	243.0	6.6 d	85.8 d	43.7	62.4	7.8 b
June (Jn)	225.8	7.6 c	94.0 c	52.2	75.5	13.0 a
July (Jl)	253.8	9.0 b	98.3 bc	52.6	77.4	15.0 a
August (A)	233.7	9.8 ab	102.7 ab	53.8	78.2	8.3 b
September (S)	204.8	10.4 a	105.3 ab	50.9	75.7	10.0 b
October (O)	249.0	10.5 a	106.8 a	51.1	75.8	9.2 b
cvs. x OD	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Nr xM	243.3	6.4	77.3	37.4	54.4	8.7
Nr x Jn	243.7	7.2	84.7	51.9	73.2	13.7
Nr x Jl	257.0	8.6	88.0	49.5	83.2	17.0
Nr x A	234.7	9.4	94.3	52.2	76.3	7.7
Nr x S	179.6	10.3	96.0	52.3	74.8	10.0
Nr x O	231.3	10.4	97.7	52.5	74.9	9.0
Vk xM	242.7	6.7	94.3	49.9	70.3	7.0
Vk x Jn	208.0	7.9	103.3	52.6	77.8	12.3
Vk x Jl	250.7	9.5	108.7	55.6	71.7	13.0
Vk x A	232.7	10.2	111.0	55.4	80.0	9.0
Vk x S	230.0	10.4	114.7	49.6	76.5	10.0
Vk x O	266.7	10.6	116.0	49.6	76.7	9.3

(1): Differences between the means were shown with different letters within the same column.

(2): N.S.: Not Significant, ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

Pomological properties

In Table 4, the effects of cv. and HD on fruit skin color and total soluble solids (TSS) are presented. Concerning the cultivar, statistically significant differences were not observed for fruit skin color L*, a*, b*, C*, and TSS values. However, significant variations were detected specifically in terms of the h° value ($p<0.01$). Indeed, the h° value was higher in the 'Viking' cultivar than the 'Nero' cultivar. The TSS value was 18.6% for 'Nero' and 18.3% for 'Viking', with no significant differences between the cultivars. Additionally, based on Table 4, the highest a* and C* values were observed at the earliest HD ($p<0.01$). The progression from 18 August to 15 September resulted in a decrease in a* and C* values (Table 4).

The HD significantly influenced the TSS values to a greater extent. ($p<0.001$). It is observed that the TSS content increased significantly from August 18 to September 15. Specifically, the TSS value was 16.9% on August 18 and increased to 20.8% on September 15. Furthermore, the interactions between cvs. and HD were determined to be insignificant for both TSS and fruit color values.

Table 4. Pomological parameters of two chokeberry cultivar's fruits at different harvest dates

Treatments	L*	a*	b*	C*	Hue*	TSS
Cultivar (cv.)	N.S.	N.S.	N.S.	N.S.	**	N.S.
'Nero' (Nr)	16.4	3.3	0.8	3.1	10.1 b	18.6
'Viking' (Vk)	17.4	4.7	4.4	5.2	15.9 a	18.3
Harvest Date (HD)	N.S.	**	N.S.	**	N.S.	***
18 August (1st)	17.9	6.5 a	2.5	7.0 a	15.5	16.9 c
21 August (2nd)	16.8	2.8 bc	1.9	3.3 bc	12.7	17.3 bc
30 August (3rd)	16.7	5.3 ab	5.8	4.9 ab	11.3	18.7 b
15 Sept. (4th)	16.3	1.3 c	0.3	1.3 c	12.4	20.8 a
cv. x HD	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Nr x 1st	17.9	6.1	1.8	6.4	12.1	16.9
Nr x 2nd	14.3	1.2	0.7	1.2	7.2	16.9
Nr x 3rd	16.3	4.4	0.4	3.4	7.8	18.4
Nr x 4th	17.2	1.5	0.4	1.5	13.2	21.9
Vk x 1st	17.9	6.9	3.1	7.6	18.8	16.9
Vk x 2nd	19.3	4.5	3.2	5.4	18.2	17.5
Vk x 3rd	17.2	6.2	11.1	6.5	14.8	19.0
Vk x 4th	15.4	1.1	0.2	1.1	11.7	19.7

(1): Differences between the means were shown with different letters within the same column.

(2): N. S.: Not Significant, ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

Fruit yield

The total yield per plant of the 2.5-year-old 'Nero' and 'Viking' cultivars are presented the Table 5. No significant differences in terms of yield were observed between the 'Nero' and 'Viking' cultivars. However, 'Nero' demonstrated a higher yield level of 128.4 g per plant, whereas 'Viking' yielded 113.0 g per plant. It is important to emphasize that these yield values pertain to the first-year yield following the initial planting.

Table 5. Fruit yield of two chokeberry cultivars at first yield year

Cultivar	Yield (g/plant)
'Nero'	128.4
'Viking'	113.0
cv.	N.S.

(1): Differences between the means were shown with different letters within the same column.

(2): N.S.: Not Significant, ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

Total phenol, monomeric anthocyanin, and antioxidant activity

The study revealed that there were no statistically significant effects of cvs. on total phenol content (TPC), total monomeric anthocyanin (TMA) levels, and antioxidant activity (AA) (Table 6). However, it was observed that the effects of HD on TPC and TMA were statistically significant ($p<0.05$). During the period from August 31 to September 15, there was an increase in both TPC and TMA levels. The TPC ranged from 4027 to 4758 mg/kg FW, while TMA ranged from 960 to 1129 mg/kg FW. On the other hand, no statistically significant differences were observed in terms of AA between the different HD. Furthermore, the interaction between cvs. and HD was insignificant for TPC, TMA, and AA.

Table 6. Some important fruit quality parameters of two chokeberry cultivar's fruits at different harvest dates

Treatments	Total Phenol (mg/kg FW)	Total Monomeric Anthocyanin (mg/kg FW)	Antioxidant Activity (mgTE/100g FW)
Cultivar (cv.)	N.S.	N.S.	N.S.
'Nero' (Nr)	4622	1042	3479
'Viking' (Vk)	4163	1047	3667
Harvest Date (HD)	**	*	N.S.
31 August (1st)	4027 b	960 b	3615
15 September (2nd)	4758 a	1129 a	3531
cv. x HD	N.S.	N.S.	N.S.
Nr x 1st	4412	1022	3562
Nr x 2nd	4832	1062	3395
Vk x 1st	3641	898	3667
Vk x 2nd	4685	1197	3667

(1): Differences between the means were shown with different letters within the same column.

(2): N.S.: Not Significant, ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

Sugars and organic acid contents

Taste-related individual sugars and acids were shown in Table 7. It was determined that sorbitol is the most dominant taste-related compound in chokeberry fruits, followed by glucose and fructose. On the other hand, sucrose was found to be the least abundant sugar in chokeberry fruits.

Table 7. Taste-related parameters of two chokeberry cultivar's fruits at different harvest dates

Treatments	Sucrose (g/100 g FW)	Glucose (g/100 g FW)	Fructose (g/100 g FW)	Sorbitol (g/100 g FW)	Citric Acid (mg/kg FW)	Malic Acid (mg/kg FW)	Quinic Acid (mg/kg FW)	Ascorbic Acid (mg/kg FW)
Cultivar (cv.)	N.S.	*	N.S.	N.S.	*	N.S.	*	*
'Nero' (Nr)	0.11	3.68 a	3.29	6.33	1284 a	8511	3896 a	99.8 a
'Viking' (Vk)	0.11	3.58 b	3.23	6.19	918 b	8056	3265 b	92.6 b
Harvest Date (HD)	***	***	***	***	N.S.	N.S.	N.S.	N.S.
31 August (1st)	0.097 b	3.37 b	3.05 b	5.61 b	1049	8452	3740	95.5
15 September (2nd)	0.116 a	3.89 a	3.48 a	6.92 a	1153	8126	3421	96.9
cv. x HD	**	***	***	***	N.S.	N.S.	N.S.	N.S.
Nr x 1st	0.102 c	3.53 c	3.18 c	5.88 c	1245	8700	4082	96.9
Nr x 2nd	0.110 b	3.83 b	3.41 b	6.79 b	1324	8344	3711	102.6
Vk x 1st	0.093 d	3.20 d	2.92 d	5.34 d	853	8203	3399	94.1
Vk x 2nd	0.122 a	3.96 a	3.54 a	7.05 a	982	7908	3130	91.1

(1): Differences between the means were shown with different letters within the same column.

(2): N.S.: Not Significant, ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

Among the organic acids analyzed, malic acid had the highest concentration, followed by quinic and citric acid. Ascorbic acid values in chokeberry fruits varied depending on the interactions, ranging from 102.6 mg/kg FW to 91.1 mg/kg FW. According to these outcomes, the effects of cvs. on sucrose, fructose, sorbitol, and malic acid contents in fruits were not statistically significant. However, cultivars significantly affect glucose, citric acid, quinic acid, and ascorbic acid contents ($p<0.05$). When evaluating the collective results, it was observed that the 'Nero' cultivar had higher levels of glucose, citric acid, quinic acid, and ascorbic acid compared to the 'Viking' except malic acid.

The effects of HD on individual sugars were found to be statistically significant ($p<0.001$), indicating that the timing of harvest significantly impacted the levels of sucrose, glucose, fructose, and sorbitol in the fruits. Specifically, it was observed that the contents of these sugars increased progressively from 31 August to 15 September. On the other hand, the harvest date did not have a statistically significant impact on organic acids and ascorbic acid content. These findings align with expectations, as sugars accumulate as fruits mature and ripen.

The interaction between HD and cv. was found to have significant effects on individual sugars. Specifically, the combination of the 'Viking' cultivar with the harvest date of 15 September resulted in the highest sorbitol, glucose, fructose, and sucrose contents among the combinations.

Individual phenolic compounds contents

According to the results presented in Table 8, all phenolic contents of the 'Nero' cultivar were significantly higher than those of the 'Viking' cultivar, except ferulic acid. Specifically, 'Nero' exhibited higher levels of catechin (74.8 mg/kg FW), epicatechin (19.6 mg/kg FW), o-coumaric acid (2.34 mg/kg FW), neo-chlorogenic acid (527 mg/kg FW), caffeic acid (19.6 mg/kg FW), chlorogenic acid (642 mg/kg FW), and rutin (164 mg/kg FW). These findings indicate that the 'Nero' cultivar had a greater abundance of these phenolic compounds than 'Viking', highlighting its potential for higher antioxidant and health-promoting properties.

The results indicate that several phenolic compounds, including epicatechin, neo-chlorogenic acid, caffeic acid, chlorogenic acid, and ferulic acid, showed statistically significant differences between HD. It was observed that the levels of these phenolic compounds increased from August 31 to September 15. On September 15, the harvested fruits exhibited the following contents: 20.2 mg/kg FW of epicatechin ($p<0.05$), 535 mg/kg FW of neo-chlorogenic acid ($p<0.001$), 20.4 mg/kg FW of caffeic acid ($p<0.001$), 659 mg/kg FW of chlorogenic acid ($p<0.01$), and 16.3 mg/kg FW of ferulic acid ($p<0.05$). Regarding the interaction between cvs. and HD, it was found to be significant only for o-coumaric acid ($p<0.05$). Based on our findings, the highest concentration among the phenolic acids analysed in chokeberry fruits was found to be chlorogenic acid, followed by neo-chlorogenic acid, rutin, catechin, epicatechin, caffeic acid, ferrulic acid, and o-coumaric acid, in descending order.

The effects of cvs. and HD on the anthocyanin compounds in chokeberry fruits are presented in Table 9. According to the results, there were significant differences observed between the cultivars for cyanidin 3-glucoside ($p<0.05$) and cyanidin 3-xyloside ($p<0.01$) contents. Specifically, these anthocyanin compounds were higher in the 'Nero' cultivar than in the 'Viking' cultivar.

Based on the results, no statistical differences were observed between HD in terms of anthocyanin compound contents. This indicates that the anthocyanin contents in chokeberry fruits did not vary significantly with progressive harvest dates. However, it is essential to note that the interaction between cvs. and HD was significant for the cyanidin 3-xyloside content ($p<0.05$).

Table 8. Phenolic acids concentration of two chokeberry cultivar's fruits at different harvest dates (mg/kg FW)

Treatments	Catechin	Epicatechin	*o*-Coumaric acid	*Neo*-chlorogenic acid	Caffeic acid	Chlorogenic acid	Ferrulic acid	Rutin
Cultivar (cv.)	**	*	***	**	**	*	*	***
'Nero' (Nr)	74.8 a	19.6 a	2.34 a	527 a	19.6 a	642 a	13.7 b	164 a
'Viking' (Vk)	57.3 b	17.6 b	1.83 b	469 b	17.8 b	596 b	16.4 a	131 b
Harvest Date (HD)	N.S.	***	N.S.	***	***	**	*	N.S.
31 August (1st)	63.1	16.9 b	2.18	462 b	16.9 b	579 b	13.8 b	142
15 September (2nd)	69.0	20.2 a	1.99	535 a	20.4 a	659 a	16.3 a	153
cv. x HD	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.
Nr x 1st	74.3	18.9	2.34 a	486	17.9	614	12.4	157
Nr x 2nd	75.3	20.3	2.35 a	568	21.3	671	15.0	172
Vk x 1st	51.8	15.0	2.02 b	438	16.0	544	15.2	127
Vk x 2nd	62.8	20.1	1.64 c	501	19.6	648	17.6	134

(1): Differences between the means were shown with different letters within the same column.

(2): N.S.: Not Significant, ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

Table 9. The concentration of anthocyanin compounds of two chokeberry cultivar's fruits at different harvest dates (mg/kg FW)

Treatments	Cyanidin 3-galactoside	Cyanidin 3-glucoside	Cyanidin 3-arabinoside	Cyanidin 3-xyloside
Cultivar (cv.)	N.S.	*	N.S.	**
'Nero' (Nr)	792	33.9 b	216	21.9 b
'Viking' (Vk)	833	39.5 a	245	28.5 a
Harvest Date (HD)	N.S.	N.S.	N.S.	N.S.
31 August (1st)	773	35.2	222	23.7
15 September (2nd)	851	38.2	239	26.6
cv. x HD	N.S.	N.S.	N.S.	*
Nr x 1st	771	34.7	215	22.5 b
Nr x 2nd	812	33.2	217	21.3 b
Vk x 1st	775	35.6	228	24.9 b
Vk x 2nd	889	43.3	262	31.9 a

(1): Differences between the means were shown with different letters within the same column.

(2): N.S.: Not Significant, ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

Principal component analysis (PCA)

Principal component analysis (PCA) was conducted to assess the interrelation of all observation parameters with the combination of the cultivar (cv.) and harvest date (HD). The results of the PCA revealed that two components accounted for 93% of the total variation (Table 10). The PC1, responsible for 49% of the variation, mainly consisted of glucose, sorbitol, fructose, chlorogenic acid, and TPC. On the other hand, the variation on the second component (PC2) is rooted by *o*-coumaric acid, Cy-3-xyloside, and Cy-3-ara, respectively (Table 10). While the HD was effective in the formation of the first component (PC1), the cultivars were decisive in the second component (PC2) (Figure 1). As expected, the combination 'Nero' x 'September' was closest to the point of catechin, *neo*-chlorogenic acid, caffeic acid, and TPC content, which obtained the highest results by ANOVA. On the other hand, the 'Viking' x 'August' combination was placed the closest the AA content point, as observed in ANOVA. Thus, all results are summarized in one figure by using PCA. Sucrose has a significantly positive correlation with all individual sugars ($p \leq 0.05$) as it is seen in Figure 1. While malic and quinic acid had a positive correlation, these had a negative correlation with ferulic acid; as expected, they had placed on opposite sides of the plot. Again, there was observed a positive correlation between Cy-3-glu and Cy-3-ara, whereas these were placed on opposite side and significantly negatively correlated with *o*-coumaric acid ($p \leq 0.05$). In conclusion, HD and cv. are decisive in fruit quality change. In this context, the September harvested fruits performed higher than August's regarding all examined quality parameters. Also, choosing the right chokeberry cultivar seems important to obtain higher fruit quality parameters, as observed in PCA results.

Table 10. Cumulative variance and vector values obtained from principal component analysis using various chokeberry cultivar parameters collected from different harvest dates

Parameter	**Component matrix**[a]		
	1	2	3
TSS	0.449	0.080	**0.471**
Sucrose	**0.843**	0.137	0.020
Glucose	**0.970**	0.022	0.008
Fructose	**0.956**	0.033	0.011
Sorbitol	**0.966**	0.033	0.001
Citric	0.293	**0.676**	0.031
Malic	0.077	**0.749**	0.174
Quinic	0.020	**0.813**	0.167
Ascorbic	0.061	**0.820**	0.119
Cathechin	0.383	**0.508**	0.109
Epicathechin	**0.911**	0.028	0.061
o-coumaric acid	0.017	**0.983**	0.001
Neo-chlorogenic	**0.751**	0.199	0.050
Caffeic acid	**0.911**	0.056	0.033
Chlorogenic	**0.954**	0.044	0.002
Ferulic acid	0.167	**0.660**	0.172
Rutin	0.277	**0.723**	0.001
Cy-3-Gal	**0.537**	0.463	0.002
Cy-3-Glu	0.102	**0.832**	0.067
Cy-3-Ara	0.119	**0.877**	0.005
Cy-3-Xyl	0.054	**0.919**	0.027
TPC (mg/kg FW)	**0.936**	0.042	0.023
TMA (mg/kg FW)	**0.790**	0.131	0.079
AA-DPPH (mgTE/kg FW)	0.234	**0.681**	0.085
Contribution rate to variability (%)	49.0597	43.7971	1.7144
Cumulative contribution rate %	49.0597	92.8567	7.1433

[a] Values in bold correspond for each variable to the factor for which the squared cosine is the largest

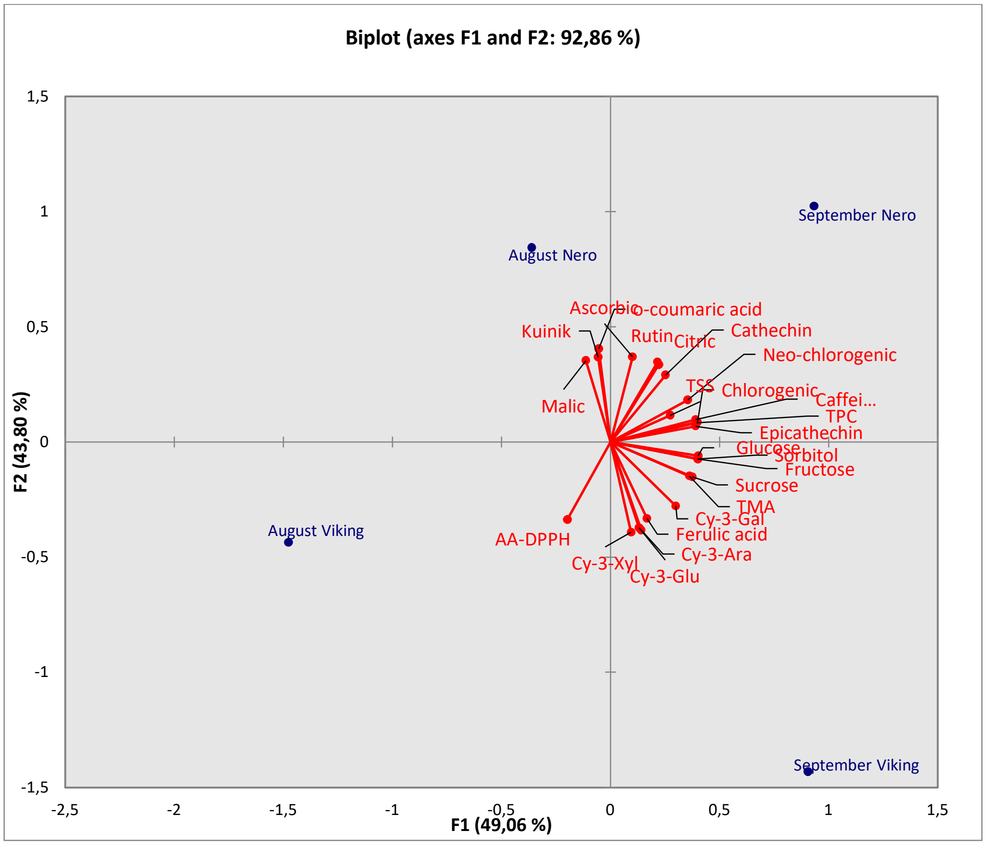

Figure 1. The principal component analysis (PCA) showing: interrelation of some fruit quality variables with the combination of cvs. and HD

Discussion

Limited research has been conducted on the morpho-physiological development of chokeberry plants, despite their varying growth, yield, and quality influenced by cultivar selection, soil management techniques, and ecological factors (Kawecki and Tomaszewska, 2006; Dragomir *et al.*, 2002; Đorđević *et al.*, 2022; Won *et al.*, 2018; Trenka *et al.*, 2020). For instance, Kawecki and Tomaszewska (2006) observed that the diameter of first-year shoots ranged from 2.2 to 4.0 mm, and the height of chokeberry bushes varied between 123 and 208 cm, depending on soil management techniques and years. In our study, we found similar bush height values as reported by Kawecki and Tomaszewska (2006). However, our results revealed higher shoot diameter values than their study (Table 3). This difference may be attributed to variations in plant age (Green *et al.*, 2023).

Taste is primarily determined by the total soluble solids-acid ratio. In this context, it is expected that the progressive harvest dates would have a positive effect on TSS content. Other researchers have also observed similar findings (Poyraz Engin and Mert 2020; Strik *et al.*, 2003). Besides, it can be thought that increasing TSS is due to increased sorbitol in our study (Bolling *et al.*, 2015). Skupien *et al.* (2008) stated that the highest TSS (18.7 %) and cyanidin-3-galactoside contents were determined in 'Hugin' shrubs within four chokeberry cvs. ('Galicjanka', 'Hugin', 'Nero', 'Viking'). On the contrary, our study obtained higher values than these research's results in 'Nero' and 'Viking' cvs. These differences can be rooted by altitudes, cultivation practices, and environmental conditions (Won *et al.*, 2018).

Our study's findings regarding yield values align with those reported by Strik *et al.* (2003), who identified 'Nero' cultivar as having the highest yield among chokeberry cultivars (‘Albigowa’, ‘Darbrowice’, ‘Egerta’, ‘Kutno’, ‘Nero’, and ‘Nowa Wies’). However, Kawecki and Tomaszewska (2006) stated that average yields ranged from 5.2 to 19.9 kg per plant from 10 to 15 years of plant age. In our present study, the lower yield can be attributed to the fact that it represents the first productive year after planting. The age of the plants can be considered the primary factor contributing to the observed differences in yield (Green *et al.*, 2023). Regarding the biochemical attributes, our research findings generally align with previous studies; however, it is important to note that some authors have reported different results (Jeppsson and Johansson, 2000; Poyraz Engin and Mert, 2020; Yang *et al.*, 2019; Mazilu *et al.*, 2022). Our study observed an increase in total phenolic content (TPC) and anthocyanin content from late August to mid-September. These findings are consistent with the understanding that these compounds tend to accumulate as the fruits mature and approach their optimal ripeness. In contrast to our findings, the study conducted by Yang *et al.* (2019) observed that the highest antioxidant content and activity (AA) were observed at the first stage of ripeness, specifically the red type. Tolic *et al.* (2017) reported a wide range of total phenolic content (TPC) and flavonoid content in chokeberry juices, ranging from 8834 to 11093 mg/L and 6993 to 9710 mg/L, respectively. These phenolic and flavonoid content variations can be attributed to ecological conditions, cultivar selection, growing conditions, and soil properties.

When evaluating the biochemical attributes of the fruits, including taste-related parameters and phenolic compounds, we observed variations that can be attributed to the cultivar (cv.) and harvest date (HD). Our findings are consistent with the results reported by Yang *et al.* (2019), which identified sorbitol as the dominant taste-related compound in chokeberry fruits. Our results also indicate that glucose and fructose are present in significant amounts, while sucrose content is relatively lower in comparison.

Regarding organic acids, our findings also align with a previous study (Yang *et al.*, 2019). Malic acid was the highest among the organic acids in chokeberry fruits, followed by quinic and citric acid. Our research revealed that the levels of sugars, except for glucose, were significantly influenced by the harvest date (HD) rather than the cultivar (cv.). This indicates that harvest timing is crucial in accumulating sugars in chokeberry fruits. We observed an increase in sugar levels from August to September, which is consistent with the findings reported by Bolling *et al.* (2015).

Another important part is the acid content of fruit that determines the taste. In this context, Jeppsson and Johansson (2000) observed that the malic acid content remained unchanged while the total acidity decreased in the initial part of their study. Similarly, in our research, the malic acid content ranged from 8126 to 8452 mg/kg depending on the harvest date, and there were no statistically significant differences, consistent with the findings of Jeppsson and Johansson (2000) (Table 7). Furthermore, our findings agree with those reported by Gao *et al.* (2022), who indicated that chokeberry fruit also contains a large amount of ascorbic acid, greatly promoting the degradation of cyanidin and its glycosides.

In our study, the highest anthocyanin compounds identified in chokeberry fruits were cyanidin 3-galactoside, followed by cyanidin 3-arabinoside, cyanidin 3-glucoside, and cyanidin 3-xyloside (Table 9). These findings are consistent with the results reported by Gao *et al.* (2022), who also identified these four glycosides of cyanidin in chokeberry fruits: cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, and cyanidin-3-O-xyloside. On the other hand, Jeppsson and Johansson (2000) determined September 8 as the best harvest date based on their evaluation of various attributes, including soluble solids, acidity, and anthocyanin content. Won *et al.* (2018) recommended the period from August 8 to 19 for the best levels of soluble solids content, acidity, and anthocyanin content. Andrzejewska *et al.* (2015) reported that the best harvest time for reducing sugars, ascorbic acid, and anthocyanin content fell between August 29 and September 1.

All phenolic contents of ‘Nero’ were significantly higher than those of ‘Viking’, except for ferrulic acid (Table 8). Moreover, some phenolic compounds, including epicatechin, neo-chlorogenic acid, caffeic acid, chlorogenic acid, and ferrulic acid, increased from August 31 to September 15. These findings differ from those reported by Yang *et al.* (2019), who observed a decrease in chlorogenic acid levels as the fruit maturity progressed. Therefore, our results demonstrate variations in phenolic profiles and their changes during fruit maturation, which may be influenced by factors such as cultivar, growing conditions, and harvest date.

Our study's findings align with these previous reports, further supporting the notion that September is a favorable time for harvesting chokeberry fruits to maximize their quality attributes. Additionally, under Antalya climatic conditions, we observed physical symptoms such as fruit shrinkage, deformation, and dehydration after mid-September. These observations indicate that the harvest season for chokeberry fruits cannot be extended beyond this period in this specific climate.

Conclusions

In recent years, the significance of chokeberry has been increasing due to its versatility and wide range of applications, including dried fruits, juices, jams, fruit teas, and marmalade. The fruit's high-quality content plays a vital role in ensuring the success and desirability of these various products. This study focused on observing the growth of chokeberry plants and assessing the quality of fruits from two important cultivars cultivated at the two harvesting date in the Korkuteli district of Antalya, Türkiye. The aim was to gather valuable insights into the plant development and fruit characteristics of these important chokeberry cultivars at different harvest dates. The results of our study demonstrate that the 'Nero' cultivar exhibited superior performance compared to the 'Viking' cultivar in terms of plant growth, fruit yield, and bioactive compounds. Furthermore, we identified September 15th as the best harvest date to maximize the levels of bioactive components in chokeberry fruits. These findings have significant implications, suggesting that chokeberry cultivation could be a promising alternative for fruit production in cool-temperate climate regions worldwide. Additional research should focus on improving the nutritional composition of chokeberry holds great promise for harnessing its full potential as a health-promoting fruit. Investigating strategies to enhance chokeberry's bioactive compounds, antioxidant capacity, and overall nutritional quality can contribute to its expanded cultivation and utilization in the food industry. By further enhancing the nutritional value of chokeberry, we can increase its availability and offer consumers a wider range of nutritious and beneficial fruit options.

Authors' Contributions

Conceptualization: N.Ü.; Data curation: M.A.S., E.A. and A.A.; Formal analysis: N.Ü., M.A.S. and E.A.; Investigation: N.Ü. and M.A.S.; Methodology: E.A., M.A.S. and A.A.; Supervision: S.P.K; Visualization: N.Ü. M.A.S. and E.A.; Writing-original draft: N.Ü. and M.A.S. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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