

**THE EFFECT OF COLD STORAGE
ON THE BIOACTIVE COMPONENTS AND PHYSICAL
PROPERTIES OF CAUCASIAN WHORTLEBERRY
(*Vaccinium arctostaphylos* L.). A PRELIMINARY STUDY**

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Abstract. In this study, antioxidant activity (AA), total phenolics (TP), total flavonoids (TF), individual phenolic compounds (IPCs), vitamin C and six other fruit characteristics including weight loss, flesh firmness, color, soluble solids content (SSC), dry matter and titratable acidity (TA) of Caucasian whortleberry fruits (*Vaccinium arctostaphylos* L.) were determined at harvest and at a week postharvest intervals throughout the cold storage at 0°C for 4 weeks. Significant decreases were observed in fruit weight and flesh firmness during the cold storage period. While L* and chroma values decreased significantly, an increase was observed in hue angle values. Significant increases were observed in dry matter, but decreases were observed in SSC, TA and vitamin C contents. Caucasian whortleberry fruits had quite high polyphenol contents. Total phenolics (TP), total flavonoids (TF), antioxidant activity (AA) (according to ABTS⁺, DPPH· and FRAP antioxidant tests) and individual phenolic compounds (IPCs) significantly decreased throughout the cold storage. Chlorogenic acid was the major phenolic in Caucasian whortleberry fruits. It was concluded that Caucasian whortleberry fruits with high phenolic compound and flavonoid levels might serve a potential antioxidant source.

Key words: Chlorogenic acid, flavonoids, flesh firmness, phenolics, weight loss

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INTRODUCTION

Antioxidant capacity of *Vaccinium* fruits are commonly related to flavonoids (especially anthocyanins), flavonols, tannins, carotenoids, phenolic acids, vitamin A, C and E-like antioxidative compounds of the fruits [Reque et al. 2014].

Turkey has a great diversity of plant species. Within this rich diversity, Caucasian whortleberry (*Vaccinium arctostaphylos* L.) has also a significant place. The fruit is locally known as 'Dal likapası', 'Çalı çileği', 'Ligarda', 'Çoban Üzüümü' and 'Çay Üzüümü'. Caucasian whortleberry fruits are quite rich in phenolic compounds and high antioxidant activity of the Caucasian whortleberry fruits collected from Eastern parts of Black Sea region was reported in previous studies [Yıldız 2012].

Daily antioxidant intake of individuals should be increased to improve the antioxidant potential and to reduce the oxidative stress level of the body [Kruger et al. 2014]. Consumers have recently started to use berry fruits with high antioxidant activity and bioactive compounds levels for diseases like atherosclerosis, cancer, cardio and cerebrovascular diseases, hypertension and diabetes [Hasanloo et al. 2011]. Berry fruits also neutralize free radicals and prevent the development of cancer, cardiovascular diseases and age-dependent alzheimer. They preserve nervous system and have the ability to reverse the decline in neural and cognitive functioning [Kruger et al. 2014].

Phenolic contents and consequently antioxidant activity levels of berry fruits change with the progress of fruit development and ripening, with postharvest drying and storage processes [Hasanloo et al. 2011, Lobos et al. 2014]. Since Caucasian whortleberry fruits are commonly consumed as fresh. There aren't any studies carried out about the changes observed in bioactive compounds (vitamin C, total phenolics, individual phenolics, total flavonoids and antioxidant activity) and other fruit quality parameters (weight loss, flesh firmness, color) throughout the cold storage of Caucasian whortleberry fruits.

The present study was conducted to determine the changes occurred in bioactive components and physical properties of Caucasian whortleberry fruits throughout 4-weeks of cold storage.

MATERIAL AND METHODS

Plant material. Caucasian whortleberry (*Vaccinium arctostaphylos* L.) fruit samples were harvested from an orchard located near Piraziz, Giresun, Turkey (40° 55' 17.34" N latitude, 38° 08' 49.02" E longitude and 240 m altitude). Fruits were collected from a single block with homogeneous plant condition and common technical practices (nutrition, irrigation and pruning). Caucasian whortleberry fruits were hand-harvested (2nd of August, 2014) by farm pickers according to commercial practices and normal harvest index (100% blue-black coloration). Uniform size and color Caucasian whortleberry fruits free from visual symptoms of any disease were selected. To determine the quality and physical parameters, and bioactive compounds, fruits were immediately transported at 15°C for 1 h to Research Laboratory of Horticulture Department of Ordu University, Ordu, Turkey.

Experimental design. To determine changes in fruit quality, physical and chemical parameters (weight loss, flesh firmness, color characteristics, soluble solids content, dry

matter and titratable acidity) and bioactive compounds (vitamin C, total phenolics, individual phenolics, total flavonoids and antioxidant activity) in each analysis date, approximately 200 g of Caucasian whortleberry fruits were weighted using a common scale (Radvag PS 4500/C/1, Poland) and then placed inside commercial polyethylene terephthalate (PET) vented clamshell containers (1 pint) (Shengxiang, China), which were snap-fitted and transferred to the cold storage at 0°C and 90 ±5% RH for 4 weeks of storage. The containers were randomly packed. At each sampling date, 5 packages (replications) were analyzed on a weekly basis for up to 4 weeks.

Weight loss and flesh firmness. Fruit weights were determined using a digital scale (±0.01 g) (Radvag PS 4500/C/1, Poland). Weight loss was determined by the difference between the initial and final weights of each replicate during cold storage and expressed as percent. Results were the means of five replicates for weight loss. Texture Analyzer, TA-TX Plus (Stable Microsystems, Godalming, UK), fitted with a 2.0 mm penetrometer probe, operating at a penetration speed of 10 mm s⁻¹ and a penetration depth of 3 mm, was used to measure flesh firmness (N mm⁻¹). Flesh firmness results were the average of 20 measurements in each replication.

Color characteristics. Changes in fruit color characteristics [L^* , a^* , b^* , chroma (C^*) and hue angle (h°)] were measured from one point over the equatorial section of fruit skin with a colorimeter (Minolta, model CR-400, Tokyo, Japan). Values of L^* , a^* and b^* were used to define a three-dimensional color space. The chroma value was calculated with the formula $C^* = (a^{*2} + b^{*2})^{1/2}$, and the hue angle with $h^\circ = \tan^{-1} b^*/a^*$. The results for fruit color characteristics (L^* , C^* and h°) were the average of 50 measurements in each replication.

Soluble solids content (SSC), dry matter, titratable acidity (TA) and vitamin C. Approximately 50 g of Caucasian whortleberry fruits from each replicate were juiced collectively and 3 individual samples were taken for soluble solids content and titratable acidity (TA). SSC was determined with a digital refractometer (PAL-1, McCormick Fruit Tech., Yakima, Wash) and expressed as percent. Dry matter was determined by drying at 70°C under vacuum. For TA, 10 ml of extract was taken from each sample, 10 ml of distilled water was added and the value corresponding to consume sodium hydroxide (NaOH) during the titration with 0.1 mol L⁻¹ sodium hydroxide to increase the pH of samples to 8.1 was expressed as g citric acid 100 g⁻¹. For vitamin C content, sufficient amount of extract was taken and resultant volume was completed to 5 ml with the addition of 0.5% oxalic acid. Ascorbic acid test strip (Catalog no: 116981, Merck, Germany) was taken from reclose tube, dipped into the solution for 2 seconds and reflectometer set (Merck RQflex plus 10) was started. The test strip was then shaken off to remove excess liquid over it, waited for 8 seconds and reading was performed until the end of 15th second. The resultant value was expressed as mg 100 g⁻¹. The results for soluble solids content, dry matter, titratable acidity and vitamin C were the average of 3 measurements in each replication.

Bioactive compounds. For bioactive compounds, approximately 75 g of Caucasian whortleberry fruits from each replicate were taken. Then these fruits were placed into tubes and stored at -80°C for analysis of bioactive compounds. Samples were thawed at room temperature (≈21°C) and homogenized in a food-grade blender. The resultant

slurry was centrifuged (12000 g) at 4°C for 30 min to separate the juice from the pulp. The freshly obtained juice was diluted with distilled water, divided into multiple sample aliquots and refrozen at -20°C until used in phenolics, flavonoids and antioxidant assay procedures. The results for total phenolics, individual phenolics, total flavonoids and antioxidant activity [according to 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1, 1-diphenyl-2-picryl-hydrazil (DPPH·) and ferric ions (Fe⁺³) reducing antioxidant power assay (FRAP) test] were the average of 3 measurements in each replication.

Total phenolics (TP). A portion of 300 µL from each sample was diluted with 4.3 mL distilled water and 100 µL Folin–Ciocalteu reagents were added. After an interval of 3 min, 2% Na₂CO₃ was added to 300 µL portions and the mixture was vortexed and incubated for 30 min. Absorbances were then read on a UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) at 760 nm. Gallic acid was used as the standard. The results were expressed as milligrams (mg) of gallic acid equivalents (GAE) per grams (g) of dry weight (dw) [Beyhan et al. 2010].

Total flavonoids (TF). The total flavonoid contents of fruit samples were determined according to colorimetric method [Chang et al. 2002]. Briefly, each extract (0.1 g) was dissolved in 1 ml of the appropriate solvent. This solution (0.1 ml) was mixed with 10% AlCl₃.6H₂O and 0.1 ml of 1 M potassium acetate (CH₃COOK). The absorbance of the reaction mixture was measured at 415 nm. Quercetin was chosen as a standard. The results were expressed as mg quercetin equivalents (QE) g⁻¹ dw.

Individual phenolic compounds (IPCs). Preparation of samples and standard solutions: All crude fruit samples were homogenized and 1000 mg slurry was accurately weighed and extracted with (5 mL) methanol in test tube for 6 h. After filtration through a syringe type filter (Chromtech, 13 mm, 0.22 µm), the filtrate was injected into the HPLC system for analysis. Accurately weighed solid portions of each standard were dissolved in methanol to prepare stock solutions. Working solutions were obtained by diluting the stock solutions with methanol. The final mixed standard solution contained 100 µg mL⁻¹ of each standard. The results were expressed as mg 100 g⁻¹.

Instrumentation and condition: High performance liquid chromatography [(HPLC), Perkin-Elmer Series 200; Perkin-Elmer, Norwalk, CT, USA] system equipped with a quaternary solvent delivery system (Series 200, analytical pump) and UV detector (Series 200, UV/Vis detector) was used at 280 nm. The analytes were separated on a Phenomenex Kromasil (Phenomenex Inc., Torrance, CA, USA) 100A C18 (250 mm × 4.60 mm, 5 µm) column. The column temperature was maintained at 26°C by using a water bath (Wisebath, WB-22, and Daihan Scientific, Seoul, Korea). The mobile phase consisted of acetonitrile (A) and water containing 2.5% formic acid (B). The following gradient conditions were used: initial 0–3 min, held at A–B (5:95, v/v); 3–8 min, linear change from A–B (5:95, v/v) to A–B (10:90, v/v); 8–13 min, linear change from A–B (10:90, v/v) to A–B (15:85, v/v); and 13–15 min, isocratic elution A–B (15–85, v/v); 15–22 min, linear change from A–B (15:85, v/v) to A–B (25:75, v/v); 22–37 min, linear change from A–B (25:75, v/v) to A–B (50:50, v/v); 37–40 min, isocratic elution A–B (100–0, v/v). The mobile phase flow rate was set at 1 mL min⁻¹ and the injection volume was 20 µL.

Antioxidant activity (AA). **ABTS⁺ radical scavenging activity:** 2 mM of ABTS⁺ [2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt] and 2.45 mM of K₂S₂O₈ solutions were prepared by 0.1 M of PO₄⁻³ buffer solution (pH 7.4). The ABTS⁺ and K₂S₂O₈ solutions were mixed in (1:2) ABTS- K₂S₂O₈ and incubated for 6 h in dark. The absorbance of the mixture was read at 734 nm and it was diluted with PO₄⁻³ buffer if the value was greater than 0.75. Finally, 20 µL samples were taken out of the mixture into tubes, 1 mL of ABTS⁺ – K₂S₂O₈ solution was added to each tube and buffer solution was added to make the total sample volume 4 mL. Following vortexing, they were incubated for 30 min and absorbances were read at 734 nm. The results were expressed as µmol Trolox equivalents (TE) per gram of dw [Pellegrini et al. 1999].

DPPH· free radical scavenging activity: The hydrogen atom or electron donation abilities of some pure compounds were measured by the bleaching of a purple colored methanol solution of DPPH. The free radical scavenging activities of methanol extract of fresh fruit of Caucasian whortleberry were measured by 1,1-diphenyl-2-picrylhydrazil (DPPH·) using the method of Blois [1958] wherein the bleaching rate of a stable free radical, DPPH· was monitored at a characteristic wavelength in the presence of the sample. An amount of 0.5 ml of 0.1 mM ethanolic solution of DPPH· was added to 3.0 ml of all the extract samples or standard antioxidants solution (50–500 µg mL⁻¹) in water. The mixture was shaken vigorously and kept standing at room temperature for 30 min. Then the absorbance of the mixture was measured at 517 nm. The results were expressed as µmol TE per gram of dw [Demirtas et al. 2013].

Ferric ions (Fe⁺³) reducing antioxidant power assay (FRAP): Portions of 120 µL were taken from the samples, 0.2 M of phosphate buffer (PO₄⁻³) (pH 6.6) was added to obtain a volume of 1.25 mL and then 1.25 mL of 1% potassium ferricyanide (K₃Fe(CN)₆) solution was added and undergone to vortexing. The samples were prepared with methanol. They were incubated at 50°C. Afterwards, 1.25 mL of 10% TCA (trichloro acetic acid) and 0.25 mL of 0.1% FeCl₃ were added to the samples. The absorbances of the resultant solution were read on an UV-Vis spectrometer at 700 nm. The results were expressed as µmol TE per gram of dw [Benzie and Strain 1996].

Statistical analysis. The normality of the data was confirmed by the Kolmogorov-Smirnov test and the homogeneity of variances by the Levene's test. The data sets were analyzed with ANOVA by using SAS Version 9.1 (SAS Institute, Cary, NC, USA) software. Duncan's multiple range test was used to compare treatments when ANOVA showed significant differences among means. The level of significance was set as 5%.

RESULTS AND DISCUSSION

Weight loss and flesh firmness. Changes observed in weight loss and flesh firmness of Caucasian whortleberry fruits throughout the cold storage is presented in Fig. 1. Significant weight losses were observed in Caucasian whortleberry fruits during the cold storage. About 40% of the total weight loss (2.60%) was observed during the initial first week of the storage. While the losses in flesh firmness were low at the beginning of the storage, the loss ratios increased from the 14th day of storage. While the flesh firmness was 1.31 N mm⁻¹ at the beginning, the value was 0.92 N mm⁻¹ at the end of storage.

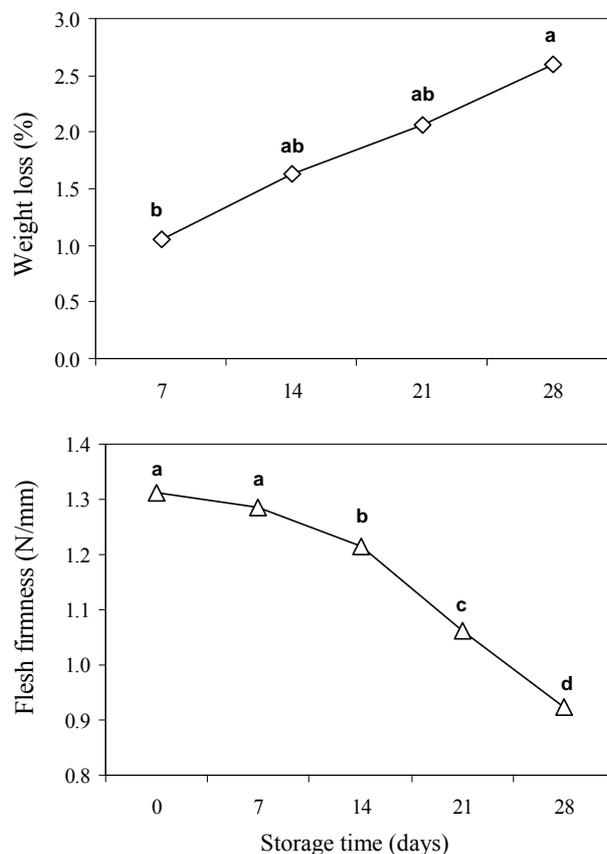


Fig. 1. Changes in weight loss and flesh firmness of whortleberry fruits at the time of harvest and throughout the cold storage at 0°C and 90% RH for 28 days. $n = 5$ for weight loss (five replications \times three different measurements for each replication). $n = 100$ for flesh firmness (five replications \times twenty fruits for each replication). Different letters above the line indicate significant differences according to Duncan's multiple range test at $P < 0.05$

Water loss is an expected phenomenon in fresh fruits and vegetables during the postharvest storage. Fresh fruits usually exhibit the symptoms of loss of freshness through 3–10% weight loss [Almenar et al. 2008]. The weight loss ratio (2.60%) of the present samples was lower than the specified values. Optimum storage temperature and relative humidity might have yielded such a low and stable weight loss in Caucasian whortleberry fruits. Mitcham and Mitchell [2002] reported low weight loss ratios for berry fruits stored at low temperature (0°C) and 90–95% relative humidity. In previous studies, weight loss ratio of blueberry fruits was reported as 0.98% by Duarte et al. [2009] and as 2.64% by Concha-Meyer et al. [2015]. Storage temperature, properties of packaging materials, damages over epidermis, physical damages and surface-volume relation of the product may designate the degree of loss to be observed during the cold storage period.

Long-term flesh firmness preservation is a critical issue for the preservation of market value and economic success of the product. Flesh firmness significantly decreased during the cold storage of the present study. Rapid losses were observed in flesh firmness of Caucasian whortleberry fruits especially after 14th day of the storage. Solubility of pectins of berry fruits increases and consequently various nutritional and sensory characteristics including flesh firmness change through the progress of ripening. Thus, Lobos et al. [2014] reported decreased cell wall components like pectin, cellulose and hemicelluloses of berry fruits through the enzymatic changes and fragmented cell wall and middle lamella. Duarte et al. [2009] indicated that losses in fruit flesh firmness were mostly related to storage atmospheric composition, fruit anatomic characteristics, cell size and cell wall thickness and also indicated that water loss might affect cell turgor. These metabolic reactions may also be presented as the primary reasons for the losses in flesh firmness of the present study.

Color characteristics. Changes observed in L*, chroma and hue angle values of Caucasian whortleberry fruit throughout the cold storage period are presented in Fig. 2. Hue angle values increased until the 28th day of storage. Contrarily, L* values significantly decreased until the 21st day and chroma values until the 14th day of storage. The decrease in L* value was more remarkable on 21st day and decrease in chroma value on 14th day of storage.

Homogeneous coloration indicates fruit quality and has significant impacts on consumer preference. Caucasian whortleberry fruits with dark skin color contain quite abundant anthocyanin pigments. While decreases were observed in L* and chroma values of the present study during the cold storage, an increase was observed in hue angle values. Çelik and Koca [2013] reported L* values of 6 different Caucasian whortleberry fruits at harvest as between 12.46–17.77. During the cold storage period, Zheng et al. [2003] reported decreased L* (from 32.0 to 30.1) and chroma (from 4.71 to 2.30) and increased hue angle values (from 301.2 to 341.2) for Duke blueberry fruits. The changes observed in color parameters of the present study were also similar to those findings. Lobos et al. [2009] indicated darkened (increased percentage of blue) skin colors for berry fruits with the progress of ripening. The decrease in L* value over the color scale indicates decreased color brightness and increase in hue angle indicates color darkness. Thus, it can be stated that ripening level of Caucasian whortleberry fruits progressed through the storage period.

Soluble solids content, dry matter, titratable acidity and vitamin C. The changes observed in SSC and dry matter values of Caucasian whortleberry fruits throughout the cold storage period are presented Fig. 3 and the changes in TA and vitamin C values are presented in Fig. 4. While significant decreases were observed in SSC values of Caucasian whortleberry fruits during the storage, increases were observed in dry matter values. SSC and dry matter values were respectively observed as 9.80 and 12.36% at the beginning of storage (0th day) and the values were 9.10 and 14.67% at the end of storage (28th days).

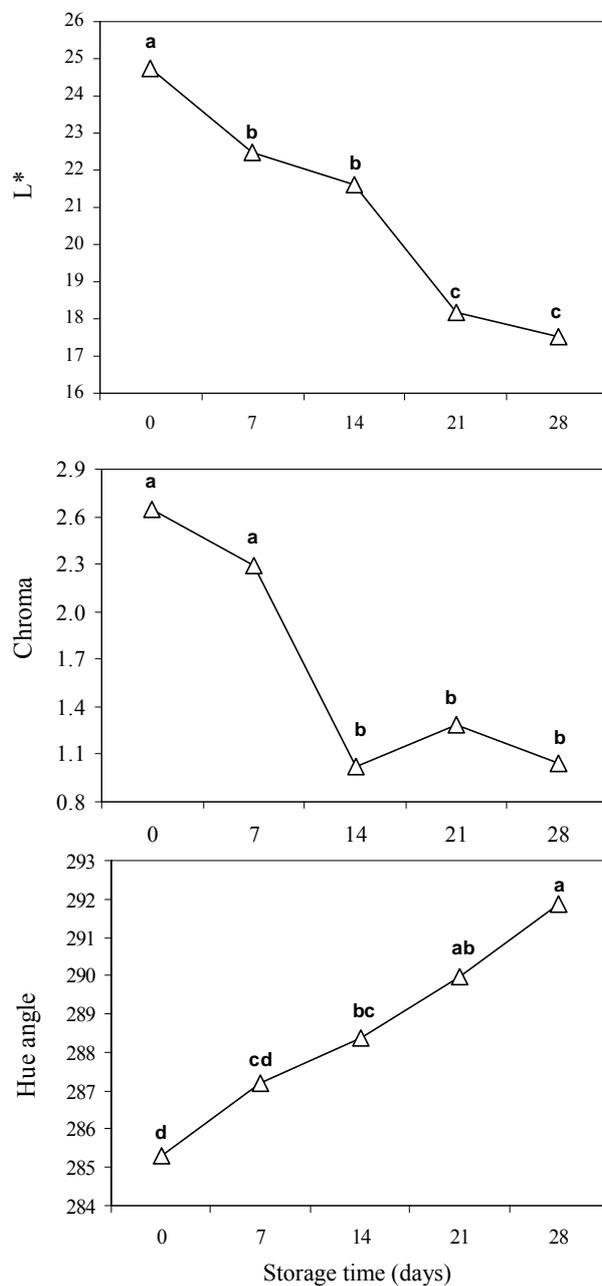


Fig. 2. Changes in color characteristics (L*, chroma and hue angle) of whortleberry fruits at the time of harvest and throughout the cold storage at 0°C and 90% RH for 28 days. n = 250 for color characteristics [L*, chroma and hue angle, (five replications × fifty fruits for each replication)]. Different letters above the line indicate significant differences according to Duncan's multiple range test at P < 0.05

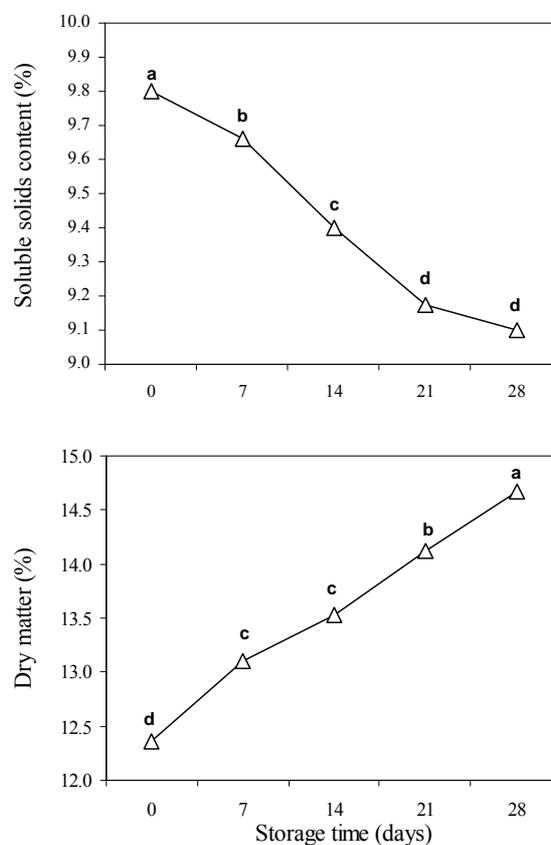


Fig. 3. Changes in soluble solids content and dry matter of whortleberry fruits at the time of harvest and throughout the cold storage at 0°C and 90% RH for 28 days. $n = 15$ for soluble solids content and dry matter (five replications \times three different measurements for each replication). Different letters above the line indicate significant differences according to Duncan's multiple range test at $P < 0.05$

The decrease in TA and vitamin C values until the 21st day of storage was found to be significant. TA values throughout the storage varied between 1.66% (0th day) and 1.24% (28th day). The decrease observed in vitamin C contents was higher and more remarkable on 7th and 21st day of storage. While vitamin C content was 1564.6 mg 100 g⁻¹ at the beginning of storage, the value decreased to 140.2 mg 100 g⁻¹ at the end of storage.

Sugars and acids are the basic substances of respiration metabolism of berry fruits throughout the cold storage. Thus, decreased SSC, TA and vitamin C values were mostly resulted from the consumption of sugar and acids throughout the cold storage. Zheng et al. [2003] reported that SSC values of Duke blueberry decreased from 9.8% to 8.5% and TA values decreased from 0.82% to 0.44% during the cold storage. Huang et al. [2015] reported TA and vitamin C contents of Elliott blueberry respectively as be-

tween 1.07–2.08% and between 5.55–17.58 mg 100 g⁻¹ fw. It was reported in previous studies that cultivar, level of ripening, storage temperature and duration, atmospheric composition of storage (O₂, CO₂) and ethylene synthesis might have significant impacts on decreases in biochemical composition throughout the cold storage. In measurements carried out at the estimated time of harvest, Çelik and Koca [2013] reported SSC, dry matter, TA and vitamin C content of 6 different Caucasian whortleberry fruits respectively as between 8.08–11.06%, 11.55–16.62%, 1.04–2.63% and 6.97–58.91 mg 100 g⁻¹.

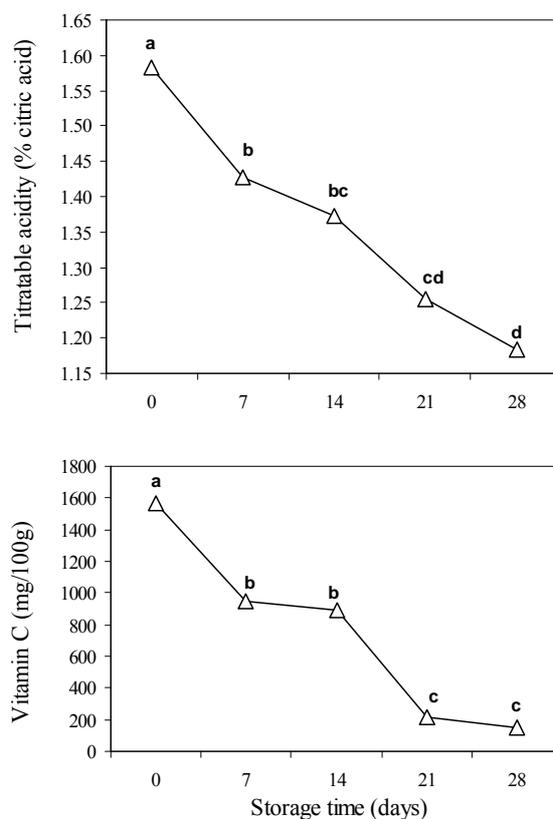


Fig. 4. Changes in titratable acidity and vitamin C of whortleberry fruits at the time of harvest and throughout the cold storage at 0°C and 90% RH for 28 days. n = 15 for titratable acidity and vitamin C (five replications × three different measurements for each replication). Different letters above the line indicate significant differences according to Duncan's multiple range test at P < 0.05

Total phenolics and total flavonoids. Changes observed in total phenolics (TP) and total flavonoids (TF) of Caucasian whortleberry fruits throughout the cold storage are presented in Fig. 5. Throughout the cold storage period, TP values decreased from 2.38 to 2.09 mg GAE g⁻¹ dw and TF values decreased from 0.025 to 0.017 mg QE g⁻¹ dw.

While the decrease in TF values observed until the 21st day of storage was significant, the decrease in TP values was significant throughout the entire storage period.

Phenolic compounds are preferred as dietary products for the treatment of several diseases because of their antioxidant characteristics. Berries are highly rich in bioactive compounds [Zheng et al. 2003; Huang et al. 2015]. Bioactive compounds (total phenolics, total flavonoids) decreased in this study throughout the cold storage. Such decreases were mainly due to oxidation of the main antioxidants including flavonoids and phenolics. Naczki and Shahidi [2006] reported that postharvest conditions (temperature, relative humidity) and fruit ripening levels might have significant impacts on total phenolics. Enzymatic reactions including fruit softening and aging observed throughout the storage might be effective in decrease in total phenolics [Ayaz et al. 1997].

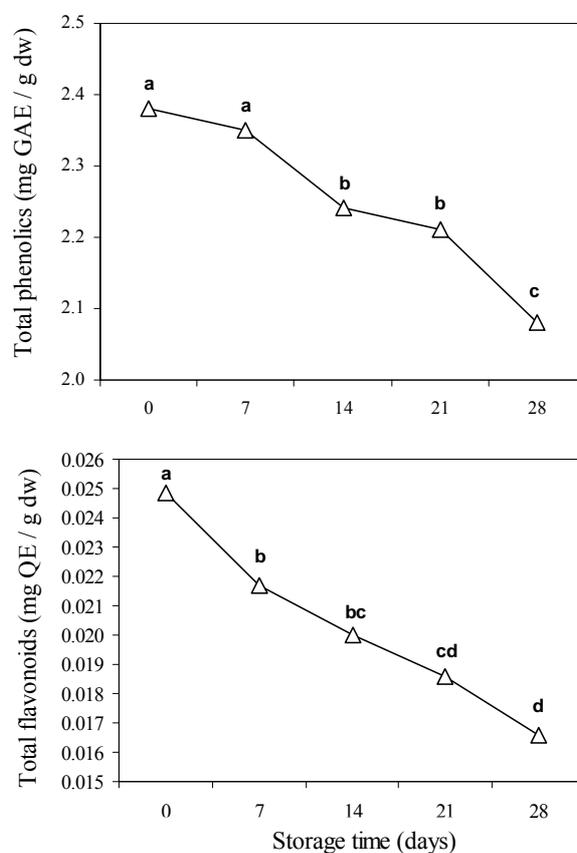


Fig. 5. Changes in total phenolics and flavonoids of whortleberry fruits at the time of harvest and throughout the cold storage at 0°C and 90% RH for 28 days. $n = 15$ for total phenolics and flavonoids (five replications \times three different measurements for each replication). Different letters above the line indicate significant differences according to Duncan's multiple range test at $P < 0.05$

Throughout the cold storage period, Wang and Chen [2010] reported decreased values for Duke blueberry species. Huang et al. [2015] reported the total phenolics of Elliott blueberry species during the cold storage as between 0.98–1.44 mg GAE g⁻¹ fw. In the measurements made at the estimated harvest time, Bunea et al. [2011] indicated TP and TF contents of wild and culture blueberry species respectively as between 424.84–819.12 mg GAE 100 g⁻¹ and 84.33–112.50 mg QE 100 g⁻¹. Hasanloo et al. [2011] reported TP contents of 4 different Caucasian whortleberry varieties as between 9.48–42.73 mg GAE g⁻¹ dw and TF contents as between 2.04–2.93 mg QE g⁻¹ dw; Özgen et al. [2014] indicated TP contents of 6 different Caucasian whortleberry genotypes as between 3.89–5.78 mg GAE g⁻¹ fw; Saral et al. [2015] reported TP and TF contents of 4 different Caucasian whortleberry genotypes respectively as between 11.54–20.74 mg GAE g⁻¹ dw and between 1.18–2.20 mg QE g⁻¹ dw; Koca and Karadeniz [2009] reported TP contents of 6 different whortleberry, 4 different blueberry and 10 different blackberry varieties respectively as between 2.64–3.79, 1.73–3.05 and between 0.77–8.20 mg GAE g⁻¹ fw. Current findings comply with those early findings for berry fruits.

Individual phenolic compounds. The changes observed in individual phenolic compounds (IPCs) of Caucasian whortleberry fruits throughout the cold storage period are provided in Table 1. Decreases were observed in entire IPCs throughout the cold storage. The decrease in gallic acid, protocatechuic acid, chlorogenic acid, vanillic acid and *p*-coumaric acid until 28th day, in rutin and ferulic acid until 21st day, in quercetin until 14th day and in 4-hydroxybenzoic acid until 7th day of storage were found to be significant. Chlorogenic acid was the major phenolics of Caucasian whortleberry fruits.

Table 1. Changes in individual phenolics of whortleberry fruits at the time of harvest and throughout the cold storage at 0°C and 90% RH for 28 days

Individual phenolics (mg 100 g ⁻¹)	Storage time (days)				
	0	7	14	21	28
Gallic acid	13.6 a	11.2 b	10.7 b	10.4 b	6.3 c
Protocatechuic acid	255.6 a	220.1 b	216.8 b	213.3 b	163.9 c
Chlorogenic acid	16742.0 a	14499.0 b	13862.0 b	11489.0 c	10862.0 d
Vanillic acid	101.9 a	98.6 a	98.6 a	98.3 a	80.9 b
4-hydroxybenzoic acid	230.5 a	191.4 b	184.6 b	181.3 b	180.9 b
Ferulic acid	28.0 a	25.3 ab	23.0 b	21.9 c	21.9 c
<i>p</i> -coumaric acid	41.9 a	19.4 b	18.6 b	17.8 b	12.7 c
Quercetin	75.9 a	52.4 b	48.6 bc	42.7 c	41.7 c
Rutin	305.8 a	267.8 b	267.1 b	230.9 c	215.2 c

n = 15 for individual phenolic compounds (five replications × three different measurements for each replications). The difference between mean values shown on the same row with same letter is not significant according to Duncan's Multiple Range test at P < 0.05

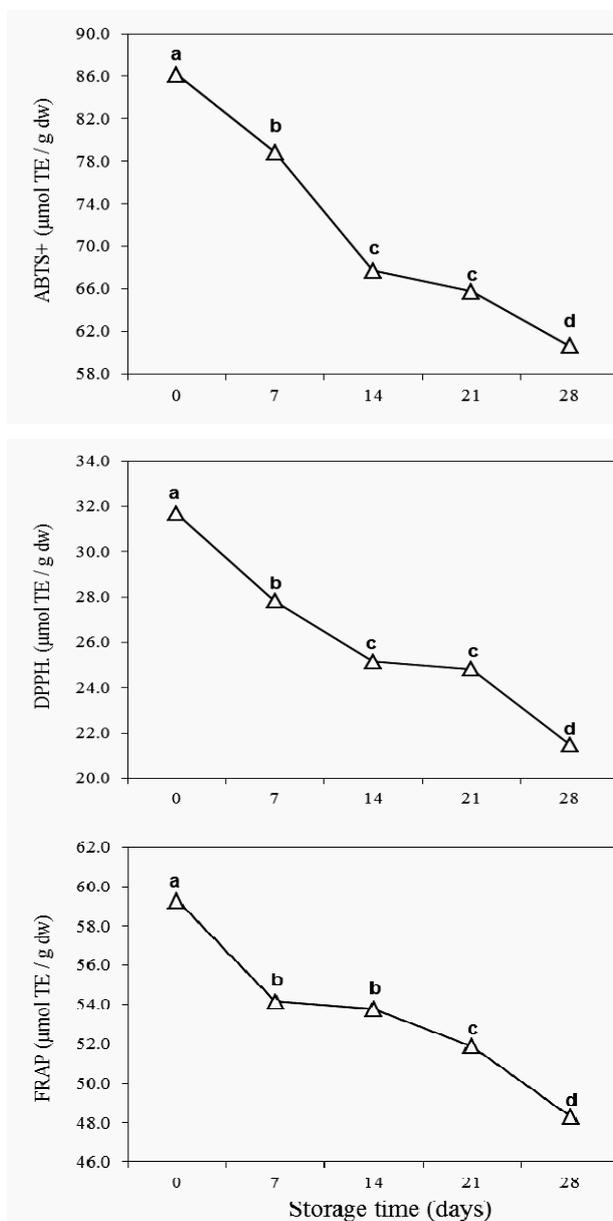


Fig. 6. Changes in antioxidant activity (according to ABTS⁺, DPPH and FRAP) of whortleberry fruits at the time of harvest and throughout the cold storage at 0°C and 90% RH for 28 days. n = 15 for antioxidant activity (five replications × three different measurements for each replication). ABTS: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl-hydrate, FRAP: ferric reducing antioxidants power. Different letters above the line indicate significant differences according to Duncan's multiple range test at P < 0.05

Chlorogenic acid was determined as the major phenolics in Caucasian whortleberry fruits of the present study. Wang and Chen [2010] reported chlorogenic acid contents of Duke blueberry fruits during 14 days of cold storage as between 74.2–55.3 $\mu\text{g g}^{-1}$ and indicated decreased contents throughout the cold storage. Ayaz et al. [2005] indicated Caucasian whortleberry fruits with potential phenolics acids as a well dietary source and reported that the fruits were rich especially in gallic, *p*-coumaric, caffeic, ferulic, chlorogenic, *p* and *m*-hydroxybenzoic, syringic and sinapic acid. Huang et al. [2012] also indicated that blueberry fruits were rich in *p*-hydroxybenzoic and vanillic acids. Decreases were observed in phenolic acids of the present study throughout the cold storage period. Ayaz et al. [2001] related such decreases with oxidative enzymes formed through the cellular breakdowns in fruit mesocarp during the ripening of fruits. Gonçalves et al. [2004] indicated that various factors (postharvest conditions, fruit development and ripening) might have significant impacts on phenolic compounds variability and concentration even of the same fruit species.

Antioxidant activity. Changes observed in antioxidant activity (according to ABTS⁺, DPPH[•] and FRAP) of Caucasian whortleberry fruits throughout the cold storage period are presented in Fig. 6. Significant decreases were observed in antioxidant activity according to ABTS⁺, DPPH[•] and FRAP tests. While antioxidant activity according to ABTS⁺, DPPH[•] and FRAP tests were respectively observed as 86.13, 31.67 and 59.27 $\mu\text{mol TE g}^{-1}$ fw at the beginning of storage (0th day), the values decreased throughout the storage respectively to 60.65, 21.48 and 48.31 $\mu\text{mol TE g}^{-1}$ fw on 28th day of storage.

Current findings revealed that Caucasian whortleberry fruits were rich in phenolics and flavonoids, but these values decreased throughout the storage and consequently all antioxidant tests (according to ABTS⁺, DPPH[•] and FRAP) indicated decreased antioxidant activity levels throughout the cold storage period. During the cold storage, Reque et al. [2014] reported decreased antioxidant activities for blueberry fruits (from 3322.98 to 1846.69 g fw g^{-1} DPPH[•]); Huang et al. [2015] for Elliott blueberry species [(according to DPPH) from 79.93 to 67.50 $\mu\text{mol TE g}^{-1}$ fw]. In measurements made at the anticipated harvest date, Koca and Karadeniz [2019] reported antioxidant activity of Caucasian whortleberry fruits according to FRAP test as between 52.88–70.41 $\mu\text{mol g}^{-1}$ fw; Hasanloo et al. [2011] according to DPPH and FRAP tests respectively as between 0.14–0.49 mg mL^{-1} and between 10.70–49.41 mmol g^{-1} dw; Özgen et al. [2014] according to TEAC and FRAP tests respectively as between 13.8–19.5 and between 14.9–23.4 $\mu\text{mol TE g}^{-1}$ fw.

Also, Koca and Karadeniz [2009] reported antioxidant activity of 4 different blueberry and 10 different blackberry species according to FRAP test respectively as between 7.41–13.69 and between 35.05–43.44 $\mu\text{mol TE g}^{-1}$ fw; Saral et al. [2015] reported antioxidant activity of 4 different Caucasian whortleberry genotypes according to FRAP test as between 130.7–299.5 $\mu\text{mol Fe g}^{-1}$ dw. Huang et al. [2015] indicated that antioxidant activity of berry fruits largely depend especially on phenols, flavonoids, anthocyanins and vitamin C. Thus, changes in phenolics were parallel to changes observed in antioxidant activity of the present study.

CONCLUSIONS

Losses were observed in quality parameters throughout the cold storage period. The current findings also revealed postharvest losses in quality parameters (color, firmness, weight) and bioactive compounds (phenolics, vitamin C) of Caucasian whortleberry fruits. The loss in flesh firmness and vitamin C was especially faster after the 14th day of storage. Caucasian whortleberry fruits were found to be highly rich in antioxidant activity.

The present study is the first study dealing with cold storage performance of Caucasian whortleberry fruits. Thus, there is a need for further studies investigating new technologies to reduce the postharvest losses in fruit quality parameters and bioactive compounds of Caucasian whortleberry fruits.

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WPLYW PRZECHOWYWANIA W CHŁODNI NA BIOAKTYWNE SKŁADNIKI I WŁAŚCIWOŚCI FIZYCZNE BORÓWKI KAUKASKIEJ (*Vaccinium arctostaphylos* L.). BADANIE WSTĘPNE

Streszczenie. Zbadano aktywność antyoksydacyjną (AA), całkowitą zawartość fenoli (TP), flawonoidów (TF), indywidualnych związków fenolowych (IPC), witaminy C oraz sześć innych cech, takich jak utrata wagi, zawartość miąższu, barwa, zawartość rozpuszczalnych substancji stałych (SSC), sucha masa oraz kwasowość (TA) owoców borówki kaukaskiej (*Vaccinium arctostaphylos* L.) podczas zbioru oraz z tygodniowymi przerwami po zbiorach w czasie całego okresu przechowywania w chłodni w temperaturze 0°C przez 4 tygodnie. Zaobserwowano istotny spadek masy owoców i zawartości miąższu podczas okresu przechowywania w chłodni. Wartości barwy zmniejszyły się istotnie, natomiast zaobserwowano wzrost wartości kąta odcienia. Zaobserwowano istotny wzrost suchej masy, ale spadek został zanotowany w zawartości SSC, TA oraz witaminy C. Owoce borówki kaukaskiej miały dość wysoką zawartość polifenoli. Całkowita zawartość fenoli (TP), flawonoidów (TF), aktywność antyoksydacyjna (AA) (według testów antyoksydacyjnych ABTS⁺, DPPH[·] i FRAP) oraz indywidualne związki fenolowe (IPC) istotnie zmniejszyły się w okresie przechowywania w chłodni. Kwas chlorogenowy był głównym fenolem w owocach borówki kaukaskiej. Wyciągnięto wniosek, że owoce borówki kaukaskiej o wysokim poziomie związków fenolowych i flawonoidów mogą służyć za potencjalne źródło antyoksydantów.

Słowa kluczowe: kwas chlorogenowy, flawonoidy, zawartość miąższu, fenole, utrata masy

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