Hindawi
Journal of Food Processing and Preservation
Volume 2023, Article ID 2802241, 13 pages
https://doi.org/10.1155/2023/2802241

WILEY | Hindawi

Research Article

Changes in some Physical and Chemical Properties of Black Mulberry (*Morus nigra* L.) Concentrate during Storage

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Received 27 December 2022; Revised 20 February 2023; Accepted 10 March 2023; Published 13 April 2023

Academic Editor: Charalampos Proestos

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The current study investigated changes in color parameters, 5-hydroxymethylfurfural (HMF), total monomeric anthocyanin (TMA) and total phenolic content (TPC), phenolic profile, antioxidant, and antimicrobial activities of black mulberry concentrate produced from dried black mulberry fruit at the beginning (day 0), on days 20, 40, and 60 during 60-day storage at two temperatures ($20 \pm 2^{\circ}$C and $4 \pm 2^{\circ}$C). It was revealed that black mulberry concentrate was a rich source in terms of TPC (550.06 μg GAE/mg) and antioxidant activity (IC_{50}) (ABTS, 8.03 μg/ml; DPPH, 10.38 μg/ml) at the beginning of storage. At the same time storage time had a significant ($p < 0.01$) effect on HMF, a^*, b^*, C^*, H°, TPC, DPPH, and ABTS, storage temperature had a significant ($p < 0.01$) effect on HMF, TMA, and DPPH. It was found that the HMF content increased during storage, and this increase was higher at room temperature. During storage, a decrease was recorded in a^*, b^*, C^*, and H° values. TPC increased during storage, and this increase was higher at refrigerator temperature. TMA was determined to increase significantly at refrigerator temperature. While DPPH antioxidant activity decreased during storage, antioxidant activity determined by ABTS increased. Chlorogenic acid and kaempferol-3-glucoside were identified in black mulberry concentrate, and it was revealed that the amounts of these compounds decreased during storage at both storage temperatures. It was determined that the dried black mulberry concentrate did not exhibit antimicrobial activity against the microorganisms tested in the study.

1. Introduction

The mulberry belongs to the genus *Morus* of the family Moraceae. Three species (white (*Morus alba*), red (*Morus rubra*), and black (*Morus nigra*) mulberry) are grown extensively in Turkey. The homeland of black mulberry is Iran and the Caucasus. The black mulberry tree is approximately 3-15 m in height and has a dense spreading crown structure, which is broad and round. Black mulberry fruit is a rich source of organic acids, sugar, vitamins, and minerals and takes an important place in nutrition. Black mulberry fruit can meet a significant part of vitamins B and C, iron, and calcium minerals that should be taken daily [1]. Black mulberry provides numerous benefits to human health not only with its nutritional qualities and aroma but also due to its phytochemical compounds (flavonoids, tannins, polyphenols, and anthocyanins). Black mulberry fruit and juice have numerous medicinal features such as antidiabetic, anti-inflammatory, antihyperglycemic, antiviral, antioxidant, hypolipidemic, hepatoprotective, neuroprotective, and positive health effects on arthritis, hypertension, lipid profile, and rheumatism [2–4]. Apart from mulberry fruit, its roots, leaves, bark, and tree branches are also used in the food, health, and cosmetic industries due to their valuable bioactive compounds [5].

However, fresh black mulberry fruit can be stored in the refrigerator only for a few days due to the short harvest season, high moisture content, and storage sensitivity [6]. Therefore, since mulberry fruit cannot be consumed fresh in sufficient amounts, it can be processed into various

products (juice, syrup, jam, marmalade, molasses, natural dye, and alcoholic beverages) and consumed out of season [3, 7, 8]. The mulberry has been cultivated in Turkey for more than 400 years to obtain traditional products, e.g., "mulberry pekmez," "mulberry pestil," and "mulberry kome" [9, 10]. Because black mulberry fruit is the fruit that can be named a superfood and be used industrially for commercially valuable edible products, it is very important to acquire a product with a composition closest to fresh fruit. Pure and fresh mulberry juice can remain fresh for three months in a cold storage environment, and bottled juice can stay fresh for six months to one year at ambient temperature [7]. However, the durability of black mulberry juice and its storage increase the production cost. Hence, the procedure of obtaining a concentrate is applied to fruit juices in order to save on cooling, storage, and transportation costs and facilitate their storage. Black mulberry juice concentrate is a sweet and delicious product in which the water in şıra (fermented juice) obtained from the black mulberry fruit is removed to a certain extent by physical methods and its shelf life is extended. Black mulberry concentrate, produced using industrial and traditional methods, is obtained by thickening the şıra obtained after the fresh or dried black mulberry fruit is separated from foreign materials such as leaves, soil, and insects to a certain consistency in open boilers or vacuum boilers [11].

Based on the current literature, there are limited studies on the quality of black mulberry concentrate during storage. Therefore, this study is aimed at determining changes in the color parameters, 5-hydroxymethylfurfural (HMF), total monomeric anthocyanin (TMA) and total phenolic content (TPC), phenolic profile, antioxidant, and antimicrobial activities of the black mulberry concentrate produced from black mulberry fruit, whose nutritional and functional properties had been determined in previous studies stored for 60 days at different temperatures (20 ± 2°C and 4 ± 2°C) during storage (on days 0, 20, 40, and 60).

2. Materials and Methods

Dried black mulberry fruit was acquired from the Yusufeli District of Artvin Province in Turkey and processed into concentrate. Black mulberry concentrate (C) was stored at two temperatures (20 ± 2°C and 4 ± 2°C) for 60 days and analyzed at the beginning of storage (day 0) and on days 20, 40, and 60. It was coded as black mulberry concentrate stored at room temperature (ROT) and black mulberry concentrate stored at refrigerator temperature (RET).

2.1. Concentrate Production. The black mulberry concentrate was produced by making some changes according to the method of Boranbayeva et al. [12]. Dried black mulberry fruits were cooked in a stainless steel pot at 65°C for 2 hours by adding water (1 kg of dried black mulberry/2 l of water). After the cooked mulberries were rested for a while, they were placed in cheesecloth and were pressed on which 40 kg of pressure was applied until the flow of black mulberry juice stopped, and pulp and black mulberry juice were obtained. Black mulberry juice (şıra) obtained by pressing was placed in a separate stainless steel pot and concentrated up to 60 Brix at 95°C. The black mulberry concentrate was filled into presterilized jars with the hot filling method, their lids were closed, and the jars were turned upside down and left for one day at room temperature to cool down and determine whether the lid had been removed. The production flow chart of black mulberry concentrate is shown in Figure 1.

2.2. Color Parameters and 5-Hydroxymethylfurfural (HMF). The samples' L^*, a^*, and b^* values were identified with a colorimeter (Konica Minolta CR-400, Korea) according to the Hunter color system, and C^* and H° values were calculated using the said values [13, 14]. 5-Hydroxymethylfurfural (HMF) was found by a spectrophotometric method [15, 16].

2.3. Determination of the Total Monomeric Anthocyanin Content. İnitially, 12.5 g of the samples was weighed, and 10 ml of acidified methanol was added. After vortexing the tubes for 3 minutes, the supernatant was separated by centrifugation at 4420 g and 4°C for 15 minutes in a cooled centrifuge (Hettich Mikro 22R, Germany). This procedure was repeated several times until the residue became colorless, and it was filtered through ordinary filter paper. The extracted samples were then mixed separately with sodium acetate (pH 4.5) and potassium chloride (pH 1.0) buffers and filtered, following which absorbance values were found at 515 nm and 700 nm in a UV-vis spectrophotometer (PG Instruments, TV60). The monomeric anthocyanin content was calculated with the following equation below [17].

$$\text{Total monomeric anthocyanin}\left(\frac{\text{mg}}{\text{l}}\right) = \frac{\text{A} \times 1000 \times \text{MA} \times \text{SF}}{\mathcal{E} \times \text{L}}. \quad (1)$$

A means absorbance difference: $(A_{515}\text{-}A_{700})pH_{1.0}\text{-}(A_{515}\text{-}A_{700})pH_{4.5}$.

L means layer thickness of the reading cuvette (L = 1 cm).

DF means dilution factor.

MA means molecular weight.

$\mathcal{E}$ means molar absorbance.

2.4. Extraction Preparation for the Determination of Total Phenolic Content and Antioxidant Activity. For extraction, 25 ml of the sample was taken, 75 ml of 90% ethanol-water mixture was added to it, and it was shaken for 6 hours in an orbital shaker (Orbital Shaker SSL1, UK). Afterward, the mixture was filtered through filter paper and evaporated at 50°C in a rotary evaporator. The sample, from which ethanol was removed, was rinsed with distilled water, completed to 25 ml, and transferred to the tube. The extracts transferred to the tube were stored at -20°C until analysis [18].

2.5. Determination of Total Phenolic Content. Ten milliliters of the extract prepared for analysis was taken, and 10 ml of 90% ethanol-water (90 : 10) mixture was added to it and mixed in an orbital shaker for half an hour. One ml of the extract was taken from this mixture, and 46 ml of distilled water and then 1 ml of Folin-Ciocalteu solution were added

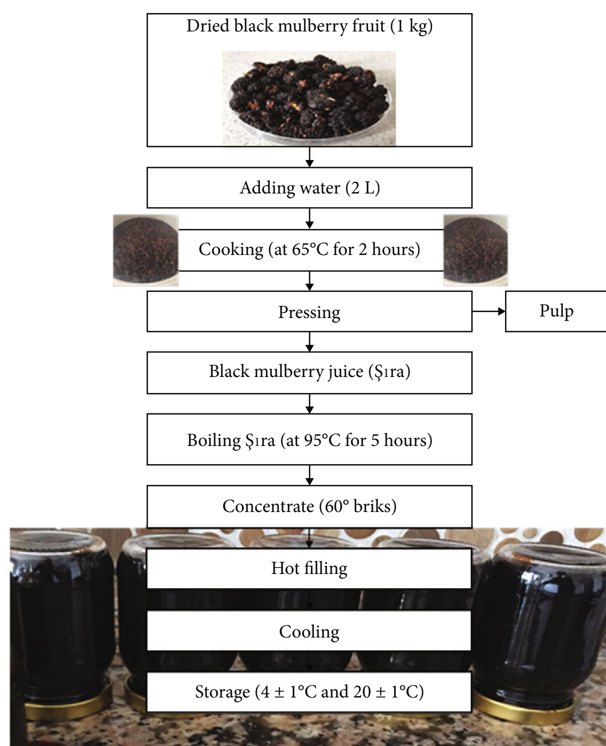


Figure 1: Production flow chart of black mulberry concentrate.

to it and left for 3 minutes. Afterward, 3 ml of 2% Na_2CO_3 solution was added to it and mixed at 210 rpm for 2 hours in an orbital shaker. The samples' absorbance was read in a UV-vis spectrophotometer (PG Instruments, TV60) at a wavelength of 760 nm [19].

2.6. Determination of Antioxidant Activity

2.6.1. Determination of Antioxidant Activity by the DPPH Method. The method is based on measuring decreases in color at 517 nm as a result of the inhibition of the DPPH radical. 10, 20, and 30 μl were taken from the samples into tubes, and their volumes were completed with ethanol so that the total amount would be 2000 μl in each tube. After adding 500 μl of the DPPH radical, the samples were left to incubate at room temperature (20 ± 2°C) for 30 minutes in the dark, and the reading was performed at 517 nm in the spectrophotometer (PG Instruments, TV60). The results were calculated as the IC_{50} value, in other words, the concentration providing the inhibition of 50% of the radical [20]. A low IC_{50} value refers to high antioxidant activity, while a high IC_{50} value refers to low antioxidant activity [16, 21].

$$\text{DPPH} \cdot \text{inhibition}\ (\%) = \left[A_{\text{control}} - A_{\text{sample(DPPH)}}/A_{\text{control}}\right] \times 100. \quad (2)$$

In the equation, A_{control} means absorbance value of DPPH·solution and A_{sample} means absorbance value of the sample solution.

2.6.2. Determination of Antioxidant Activity by the ABTS Method. The $ABTS^+$ method is a method for determining the activity of both water- and fat-soluble antioxidants [15]. $ABTS^+$ radical scavenging activity was identified following the method introduced by Re et al. [22]. First, $ABTS^+$ (2,2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) solution was prepared. This solution was mixed with 2.45 mM potassium persulfate and kept in the dark at room temperature for 12-16 hours after mixing. Black mulberry, şıra, and black mulberry concentrated extracts were taken into tubes (10-30 μl), and the volume in each tube was made up to 1500 μl with ethanol. Then, 0.5 ml $ABTS^+$ radical solution was added to every tube and incubated in a dark environment for half an hour. Absorbances were recorded at 734 nm against the blank consisting of ethanol [21, 23, 24].

$$\text{ABTS}^+\ \text{inhibition}(\%) = \left[A_{\text{control}} - A_{\text{sample(ABTS)}}/A_{\text{control}}\right] \times 100. \quad (3)$$

A_{control} means absorbance value of $ABTS^+$ solution.
A_{sample} means absorbance value of the sample.

2.7. Extract Preparation for Antimicrobial Activity. For extraction, 5 g of the samples was weighed, 25 ml of 80% methanol-water (80 : 20) was added, and the samples were shaken at 210 rpm for 2 hours in an orbital shaker. Then, after vortexing the mixture for 3 minutes, it was centrifuged (with Hettich Mikro 22R, Germany) at 4°C at 5180 g for 10 minutes to achieve phase separation and filtered through Whatman 1 filter paper. After filtering, 25 ml of 80% methanol was added to the remaining part and centrifuged (with Hettich Mikro 22R, Germany) for 10 minutes. This procedure was carried out three times. After the filtrate obtained was subjected to evaporation until the methanol was removed, the remaining part was stored at -20°C until analysis.

2.8. Determination of Antimicrobial Activity by the Well Diffusion Method. The agar well diffusion method was used to analyze antimicrobial activity [25]. The Food Microbiology Laboratory, Department of Food Engineering, Faculty of Agriculture, Atatürk University, provided microorganisms. The Microbial Identification System (Sherlock Microbial Identification System version 4.0, MIDI Inc., Newark, DE, USA), API (bioMerieux, Craponne, France), Biolog (MicroStation™ ID System, Biolog Inc., Hayward, CA, USA), and classical identification test from Bergey's Manual of Determinative Bacteriology Holt et al. [26] confirmed the identification of the microorganisms utilized in the current research. It was ensured that the eight strains tested in the experiment consisted of two yeasts (*Candida albicans* ATCC 1223 and *Saccharomyces cerevisiae* BC 6541), two molds (*Aspergillus niger* ATCC 16888 and *Penicillium roqueforti* BC 111), two Gram (-) bacteria (*Escherichia coli* BC 1402 and *Pseudomonas aeruginosa* ATCC 9027), and two Gram (+) bacteria (*Bacillus cereus* ATCC 33019 and *Staphylococcus aureus* ATCC 29213), and thus, the effect on different cell types was examined.

Table 1: The color values and HMF content of the black mulberry concentrate.

	L^*	a^*	b^*	C^*	$H^°$	HMF (mg/kg)
Storage time (A), day						
0	21.11 ± 0.51	0.46 ± 0.10^a	1.62 ± 0.28^{ab}	1.68 ± 0.29^{ab}	74.30 ± 1.31^b	6.10 ± 0.85^b
20	21.34 ± 0.28	0.39 ± 0.03^{ab}	1.75 ± 0.10^a	1.79 ± 0.10^a	77.34 ± 1.16^{ab}	9.26 ± 5.64^b
40	21.22 ± 0.83	0.32 ± 0.10^{bc}	1.50 ± 0.15^b	1.53 ± 0.16^b	78.21 ± 2.93^a	11.88 ± 5.03^{ab}
60	20.78 ± 0.54	0.28 ± 0.05^c	1.00 ± 0.13^c	1.04 ± 0.11^c	73.94 ± 4.35^b	18.44 ± 10.36^a
Significance	Ns	**	**	**	**	**
Storage temperature (B)						
Room temperature (20 ± 1°C)	21.37 ± 0.50^a	0.37 ± 0.10	1.51 ± 0.30	1.55 ± 0.32	76.22 ± 1.88	15.21 ± 9.06^a
Refrigerator temperature (4 ± 1°C)	20.85 ± 0.54^b	0.35 ± 0.10	1.43 ± 0.37	1.47 ± 0.37	75.68 ± 4.22	7.63 ± 2.55^b
Significance	*	Ns	Ns	Ns	Ns	**

a-c means with different letters in the same column are significantly different ($p < 0.05$); ns: not significant ($p > 0.05$); $^*p < 0.05$; $^{**}p < 0.01$.

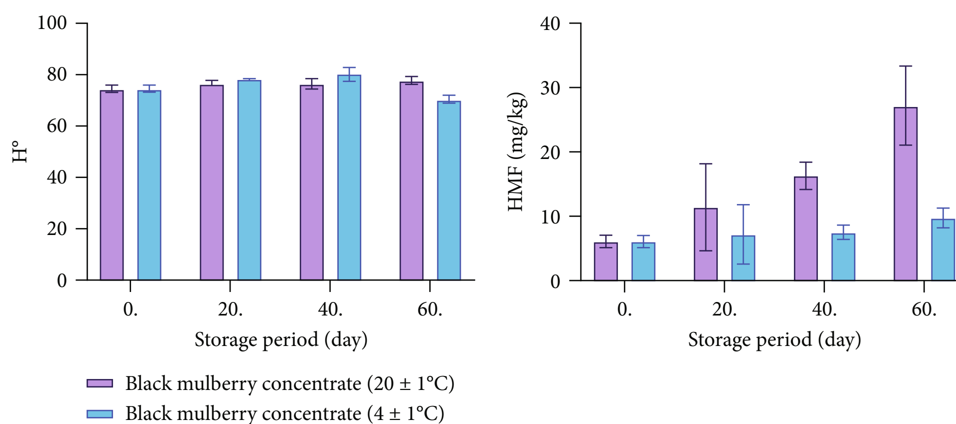


Figure 2: Changes in H° and HMF during storage.

100 μl of each bacteria, yeast, and mold suspension prepared at standard density was spread on the nutrient agar (Merck, Darmstadt, Germany) prepared for bacteria and on the potato dextrose agar (Merck, Darmstadt, Germany) prepared for yeasts and molds with the help of sterile Drigalski spatulas. Afterward, a total of 8 wells, each 5 mm in diameter, at distances equal to each other, were opened in the Petri dishes for the extracts and antibiotics. 20 μl of positive control (ofloxacin for bacteria and amphotericin B for yeasts and molds) and 20 μl of negative control (DMSO) were placed in the middle wells, while 20 μl of each of the prepared extracts were placed in the surrounding wells.

2.9. Purification and Determination of Phenolic Profiles. The HPLC determined the phenolic substance profile from the extracts prepared by modifying the extraction method developed by Coklar and Akbulut [27]. Purification was applied to the concentrate before analyzing of phenolic compound profiles. One milliliter extract was loaded into a reversed-phase C18 SPE cartridge (Agilent, USA) conditioned with preacidified water and methanol. Acidified water was passed through the sample-loaded cartridge with the help of a vacuum manifold (Supelco) to remove sugar and organic acids; phenolic compounds were obtained by passing methanol. After the methanol phase containing phenolic compounds was evaporated under a vacuum at 85 rpm at 35°C, it was dissolved in methanol in a certain proportion and transferred to vials by passing through an injector tip filter (Sartorius AG, Goettingen, Germany) with a pore size of 0.45 μm. The phenolic profile was determined by HPLC (Agilent 1260 Infinity Series). A C18 column (5 μm, 250 × 4.6 mm i.d.) was utilized for separation. The mobile phase flow rate was 1 ml/min, and acetic acid : water and acetic acid : acetonitrile : water were used in the gradient flow. Detection was performed in a DAD detector at wavelengths 306, 330, and 360 nm (resveratrol, chlorogenic acid, and kaempferol-3-glucoside).

2.10. Statistical Analysis. The data acquired in the research were subjected to correlation and variance analyses using the IBM SPSS Statistics 22 package program (IBM, Armonk, New York). Duncan's multiple comparison test revealed a significant difference between the groups at levels of $\alpha = 0.01$ and 0.05 levels. Principal component analysis (PCA) was carried out with SIMCA 14.1 (MKS UMETRICS, Umea, Sweden) to identify homogeneous pestil groups based on phenolic and anthocyanin compounds.

Table 2: Total monomeric anthocyanin, total phenolic content, and antioxidant activity of the black mulberry concentrate.

Sample	Storage time (A), day	Total phenolic content (μg GAE/mg)	Total monomeric anthocyanin (mg/L)	DPPH (IC_{50}, μg/ml)	ABTS (IC_{50}, μg/ml)
Black mulberry concentrate	0	550.06 ± 8.99[b]	107.71 ± 27.66	10.38 ± 0.57[b]	8.03 ± 0.83[b]
	20	569.14 ± 27.93[b]	177.01 ± 82.99	11.67 ± 0.85[b]	9.11 ± 0.36[a]
	40	540.30 ± 64.03[b]	181.88 ± 91.99	12.68 ± 1.69[b]	7.63 ± 0.15[b]
	60	638.93 ± 79.90[a]	182.58 ± 58.74	28.18 ± 14.16 a	7.59 ± 0.41[b]
	Significance	**	Ns	**	**
	Storage temperature (B)				
Black mulberry concentrate	Room temperature (20 ± 2°C)	539.79 ± 38.28[b]	128.03 ± 48.19[b]	18.60 ± 13.46 a	8.04 ± 0.75
	Refrigerator temperature (4 ± 2°C)	609.43 ± 65.50[a]	196.56 ± 78.65[a]	12.86 ± 2.82[b]	8.14 ± 0.84
	Significance	**	*	**	Ns
	A X B	**	Ns	**	Ns
	Storage time (A), day				
BHA	0	—	—	10.51 ± 1.31	8.06 ± 0.09
	20	—	—	11.67 ± 3.91	8.08 ± 0.09
	40	—	—	11.98 ± 0.03	7.99 ± 0.08
	60	—	—	13.03 ± 0.06	8.15 ± 0.09
BHT	0	—	—	22.92 ± 2.02	7.94 ± 0.92
	20	—	—	26.05 ± 1.85	7.95 ± 0.93
	40	—	—	10.31 ± 1.05	7.87 ± 0.87
	60	—	—	20.64 ± 1.10	8.02 ± 0.97
Trolox	0	—	—	9.35 ± 0.01	7.78 ± 0.40
	20	—	—	9.20 ± 0.34	7.80 ± 0.40
	40	—	—	9.25 ± 0.70	7.73 ± 0.38
	60	—	—	9.42 ± 0.74	7.85 ± 0.42
α-Tocopherol	0	—	—	12.96 ± 0.20	7.81 ± 0.48
	20	—	—	12.68 ± 0.04	7.83 ± 0.48
	40	—	—	12.±0.35	7.75 ± 0.46
	60	—	—	13.13 ± 0.63	7.88 ± 0.51

a-b means with different letters in the same column are significantly different ($p < 0.05$); Ns: not significant ($p > 0.05$); $^{*}p < 0.05$; $^{**}p < 0.01$.

3. Results

3.1. Color Intensity and 5-Hydroxymethylfurfural (HMF). HMF, formed in the acid-catalyzed dehydration reaction of hexoses, is a process pollutant of numerous fruit juices and many other sugar-containing food products. It is metabolized into potentially toxic and carcinogenic compounds in the human body [9]. Therefore, the HMF content in foods is important. Our study found that storage temperature and storage time had a significant ($p < 0.01$) effect on the samples' HMF content (Table 1). As seen in Figure 2, the samples' HMF content increased during storage and the black mulberry concentrate stored at room temperature (6.10-27.16 mg/kg) had higher HMF content than the black mulberry concentrate stored at refrigerator temperature (6.10-9.72 mg/kg) (Figure 2).

Kılıç [28] observed an increase of 17% and 34% in the HMF contents of black grape concentrates stored at different temperatures for 8 months at 5°C and 20°C, respectively, compared to baseline. Likewise, Dincer et al. [9] stated that the HMF content increased with the increased storage time and temperature during the storage of black mulberry concentrate. Boranbayeva et al. [12] also indicated that the HMF content increased linearly with storage temperature and time in black mulberry concentrate. Karataş and Şengül [11] found that the HMF content increased after 6 months

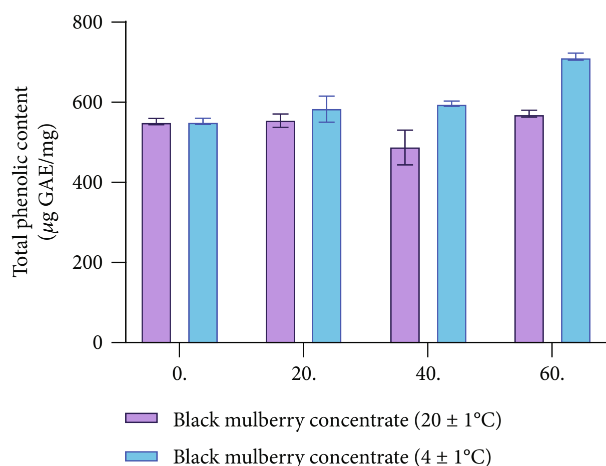


Figure 3: Changes in the total phenolic content during storage.

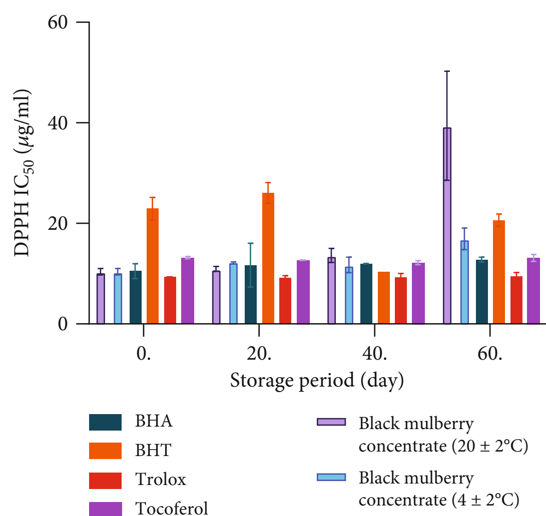


Figure 4: Changes in the DPPH (IC_{50}) activity during storage.

of storage of mulberry molasses. Erceyes [29] revealed that while sour cherry juice concentrate did not contain HMF at the beginning of storage, HMF was formed in all samples after ten months of storage. Tokbaş [30] reported that the HMF content increased in black mulberry jam and marmalade during the four-month storage. Toker et al. [31] revealed a positive correlation between HMF and temperature and time. In general, the increase in the HMF content originates from Maillard reactions occurring in heat-treated foods depending on ambient temperature, pH, and water activity [32, 33]. A significant ($r = 1.000$) positive correlation was determined between the HMF content and $H°$ value at the $p < 0.05$ level.

It was found that the impact of storage temperature on the L^* value, which indicates the lightness/darkness of the food color, was statistically significant ($p < 0.05$), whereas storage time did not impact it ($p > 0.05$). The L^* value of black mulberry concentrate was 21.37 in the samples stored at room temperature and 20.85 in those stored at refrigerator temperature (Table 1). No study investigating the color value of black mulberry concentrate during storage was found in the literature. In parallel with our study, some studies on the storage of sour cherry juice concentrate and various molasses samples have reported that the L^* value decreased with storage [29, 34, 35]. Statistical analysis also indicated a significant ($p < 0.01$) negative correlation ($r = -0.85$) between the L^* value and TPC.

Statistically significant ($p < 0.01$) changes occurred in the a^* and b^* values of the concentrates during storage, but the impact of storage temperature was not significant ($p > 0.05$). It is thought that the decrease in the a^* value at the end of storage originated from the decomposition of anthocyanins, giving the concentrate its red color. In contrast, the reduction in the b^* value originated from Maillard reaction products formed by the reaction of reducing sugars and amino acids in heat-treated foods (Table 1). In our study, changes in a^* and b^* values with storage were compatible with the results of previous studies conducted with different products (various molasses and sour cherry juice) [29, 31, 34, 35]. Şimşek and Artık [36] stated that the decrease in the b^* value at the end of storage might be due to HMF formation, Maillard reaction products, and the conversion of leukoanthocyanins into phlobaphen. As seen in 1, storage time had a very significant ($p < 0.01$) impact on the C^* and $H°$ values, and the effect of storage temperature was insignificant ($p > 0.05$). Since the C^* value decreased during storage and approached 0, it was concluded that black mulberry concentrate gained a more opaque appearance. Likewise, previous studies conducted with various fruit products have shown that the C^* value decreased with storage [14, 29, 35]. An increase was detected in the $H°$ value of the concentrate on days 20 and 50 of storage compared with the baseline. It is considered that the elements causing turbidity in the environment caused the color to be perceived as darker. Erceyes [29] reported that the $H°$ value increased due to the increase of turbidity in the environment with the storage of sour cherry juice concentrates.

3.2. Total Phenolic Content. Storage temperature and storage time had a statistically significant impact on the total phenolic content (TPC) of the concentrates at the $p < 0.01$ level (Table 2).

The TPC of the concentrates stored in the refrigerator was higher than those stored under room conditions. A statistically significant increase took place in TPC on the 60th day of storage (Figure 3). Likewise, Piljac-Zegarac et al. [37] stored various fruit juices at +4°C and recorded an increase in the total phenolic content during storage. Erceyes [29] stated that an increase and decrease occurred in the total phenolic content in some varieties in sour cherry juice concentrate samples during storage and that the total phenolic content increased at the end of the storage in comparison with the beginning. Hojjatpanah et al. [8] indicated that the phenolic content of mulberry juice was approximately 1450 mg GAE/100 g. It can be considered that the increase in the total phenolic content at the end of storage at both storage temperatures was caused by the phenolic properties of the compounds formed as a result of the Maillard reaction.

Table 3: Changes in the phenolic substance profile of black mulberry concentrate.

Phenolic substances (μg/ml)	Mulberry concentrate (C)						
		Room temperature (20 ± 2°C)			Refrigerator temperature (4 ± 2°C)		
	Day 0	Day 20	Day 40	Day 60	Day 20	Day 40	Day 60
Resveratrol	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Kaempferol-3-glucoside	71.55	Nd	24.43	Nd	73.79	34.00	Nd
Quercetin	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Chlorogenic acid	892.40	75.70	66.50	84.20	791.40	75.80	51.20

Nd: not detected.

3.3. Total Monomeric Anthocyanin Content. Whereas storage temperature had a statistically significant ($p < 0.05$) impact on the total monomeric anthocyanin content, storage time did not affect it ($p > 0.05$). In the present research, the total monomeric anthocyanin content was 128.03 mg/l in the black mulberry concentrate stored at room temperature and 196.56 mg/l in the concentrate stored at refrigerator temperature (data not shown). The total monomeric anthocyanin content increased during storage, but this increase was statistically insignificant (Table 2). Likewise, Boranbayeva et al. [12] found that the total anthocyanin content increased when the black mulberry concentrate was stored for 8 months at various temperatures. In their study, Dincer et al. [9] reported that the anthocyanin content of black mulberry juice concentrates decreased significantly during storage. Still, there was no significant ($p > 0.05$) change in the anthocyanin content of the osmotically distilled samples stored at 4°C.

3.4. Antioxidant Activity

3.4.1. DPPH. Compared with the standard antioxidants of BHA, BHT, trolox, and α-tocopherol, the DPPH·activity of the black mulberry concentrate was found to be similar to the standard antioxidants of BHA and α-tocopherol (Table 2). Storage temperature, storage time, and the interaction of storage temperature × storage time had a very significant ($p < 0.01$) effect on the DPPH$^{\cdot}$ radical scavenging activity. The IC_{50} value of the black mulberry concentrates stored at room temperature was 18.60 μg/ml, and the IC_{50} value of the concentrate stored at refrigerator temperature was 12.86 μg/ml (Table 2). Parallel to this result, the amounts of TMA and TPC were found to be higher than at room temperature. For this reason, it can be said that compounds with antioxidant properties are better preserved at refrigerator temperature (Table 2). Table 2 demonstrates that the antioxidant activity of the black mulberry concentrate stored at room temperature was lower than that of the concentrate stored in the refrigerator.

Upon comparing the antioxidant activity of black mulberry concentrate with standard antioxidants, the DPPH activity of the concentrate was found to be similar to the standard antioxidants of BHA, BHT, trolox, and α-tocopherol (Table 2). Moreover, a very significant positive correlation ($r = 0.904$) was identified at the $p < 0.01$ level between the DPPH·IC_{50} value and the HMF content.

Table 2 and Figure 4 show that the IC_{50} value increased during storage and reached the highest value statistically on day 60. Likewise, Erbay [38] determined that antioxidant activity decreased in black mulberry concentrate stored for 65 days.

Menevşeoğlu [39] reported that antioxidant activity decreased as a result of the storage of strawberry juice concentrate. It can be thought that the decrease in antioxidant activity at the end of storage in the study may have originated from the breakdown or oxidation of compounds with high antioxidant activity during storage since the breakdown of some phenolic substances during storage causes a decrease in antioxidant activity [9]. Furthermore, Karadeniz et al. [40] reported a high level of correlation between the total phenolic contents of various fruits and their antioxidant activities.

3.4.2. ABTS^{+}. While storage temperature had a statistically significant ($p < 0.01$) effect on the total monomeric anthocyanin content, storage time did not have a significant impact on it ($p > 0.05$). Increases and decreases were detected in antioxidant activity determined by the ABTS+ method during storage, and there was a significant ($p < 0.01$) increase in antioxidant activity at the end of the storage compared to the beginning (Table 2). The ABTS+ antioxidant activity of black mulberry concentrate was revealed to be close to BHA, BHT, trolox, and α-tocopherol, which are used as standard antioxidants (Table 2). In his study on the kinetics of nonenzymatic browning reactions in carob molasses during storage, Özhan [35] revealed that antioxidant activity increased and decreased during storage and found that antioxidant activity increased at the end of storage in comparison with the beginning. The researchers reported that they detected an increase in antioxidant activity in previous studies carried out with grape juice and sour cherry juice concentrate [28, 29].

3.5. Phenolic Substance Profile. The present study is aimed at determining resveratrol, kaempferol-3-glucoside, quercetin, and chlorogenic acid in black mulberry concentrate; however, resveratrol, one of the most significant phenolic components of quercetin and berry fruits, could not be detected (Table 3). Representative chromatograms for phenolic compounds of black mulberry concentrate are given in Figure 5. On the other hand, Arslan [41] determined the resveratrol content in black mulberry fruit as 32 μg/g. The kaempferol-3-glucoside content in black mulberry concentrate was found as 71.55 μg/ml in the beginning. On day 40 of storage, the kaempferol-3-glucoside contents of the concentrates were better preserved than the concentrates stored

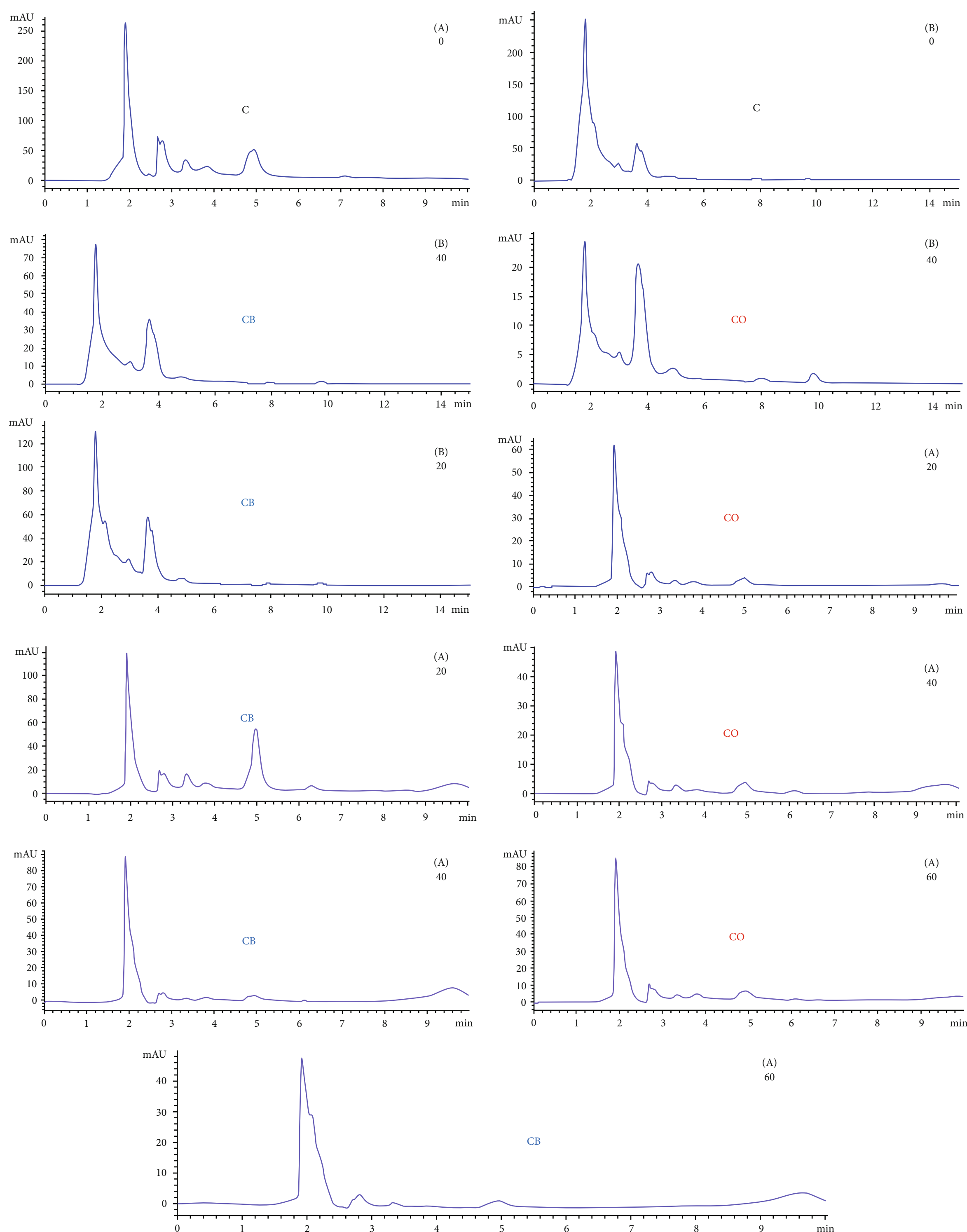


Figure 5: Representative chromatograms for phenolic compounds of black mulberry concentrate. A: 330 nm (chlorogenic acid), B: 360 nm (kaempferol-3-*O*-glucoside) 0: beginning; 20: day 20; 40: day 40; 60: day 60; C: black mulberry concentrate; CO: black mulberry concentrate stored at room temperature; and CB: black mulberry concentrate stored at refrigerator temperature.

under refrigerator conditions. Kaempferol-3-glucoside could not be detected on day 60 of storage (Table 3).

Kwaw et al. [42] revealed that the kaempferol content was 38.μg/ml in unprocessed mulberry juice and varied between 38.57-40.73 μg/ml in mulberry juice processed by different methods (Table 3). Tomas et al. *[42]* determined the quercetin-3-glucoside content as 36 mg/100 g in fresh fruit, as 38 mg/100 g in pasteurized black mulberry juice,

Table 4: Antimicrobial activity of black mulberry concentrate.

Samples		*E. coli*	*S. aureus*	*B. cereus*	*P. aeruginosa*	*S. cerevisia*	*C. albicans*	*A. niger*	*P. roqueforti*
Black mulberry concentrate		—	—	—	—	—	—	—	—
Black mulberry concentrate day 20	Room temperature (20 ± 2)	—	—	—	—	—	—	—	—
	Refrigerator temperature (4 ± 2)	—	—	—	—	—	—	—	—
Black mulberry concentrate day 40	Room temperature (20 ± 2)	—	—	—	—	—	—	—	—
	Refrigerator temperature (4 ± 2)	—	—	—	—	—	—	—	—
Black mulberry concentrate day 60	Room temperature (20 ± 2)	—	—	—	—	—	—	—	—
	Refrigerator temperature (4 ± 2)	—	—	—	—	—	—	—	—
Ofloxacin		10*	20*	12*	20*	—	—	—	—
Amphotericin B						—	2.50*	—	—

(-) indicates the absence of inhibition zone diameter. (∗): inhibition zone diameter unit (mm). Ofloxacin; antibiotic for bacteria, amphotericin B; antibiotic for yeasts and molds.

and as 21 mg/100 g in the black mulberry pulp. In their study, Kwaw et al. [42] found that the quercetin content was 276.28 μg/ml in unprocessed mulberry juice, and the quercetin content varied between 295.08-29.60 μg/ml in mulberry juice processed with different methods. It was stated that kaempferol glucosides in black mulberry concentrate had antioxidant, antimicrobial, and anticancer effects and reduced the risk of chronic diseases, particularly cancer [44, 45].

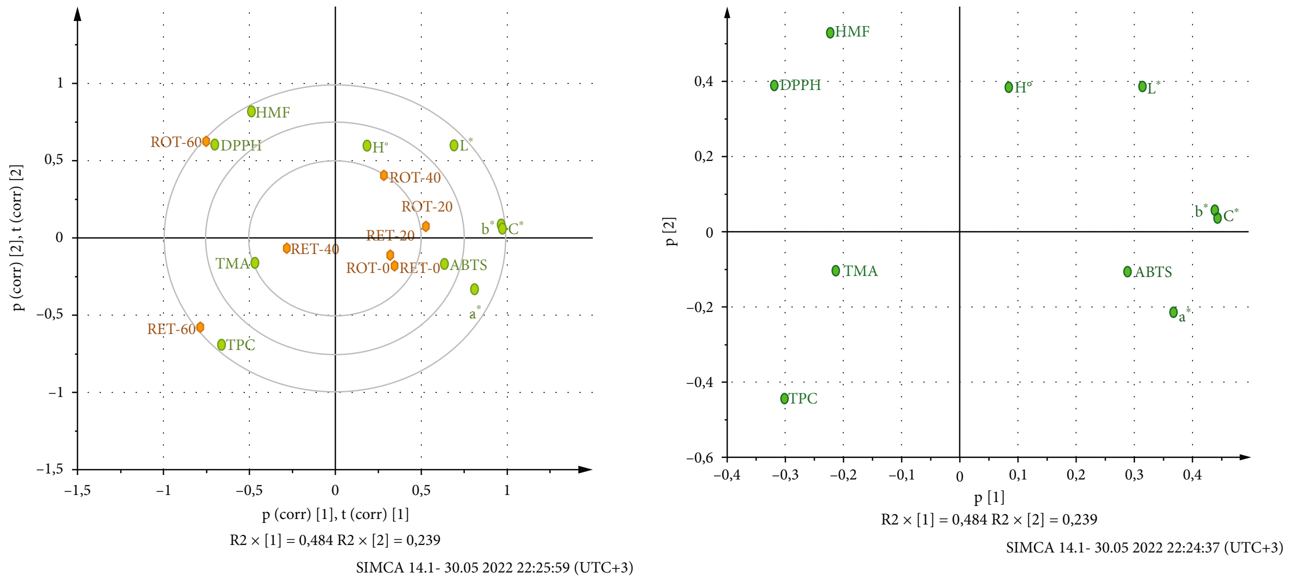
The initial chlorogenic acid content in black mulberry concentrates was found as 892.40 μg/ml. The chlorogenic acid content in the concentrates at room and refrigerator temperatures was determined as 75.70-791.40 μg/ml, 66.50-75.80 μg/ml, and 84.20-51.20 μg/ml, respectively, on days 20, 40, and 60 of storage (Table 3). Chlorogenic acid displays anticancer properties due to its cytotoxic properties and prevents the growth of cancerous cells in cancer types, e.g., leukemia, colon, breast, and prostate cancers [46]. In the research examining the antioxidant capacity and bioactive content of mulberry species, Gundogdu et al. [47] found the highest chlorogenic acid content of black mulberry samples as 53.13 mg/100 g. In another research, Gundogdu et al. [48] determined the chlorogenic acid content in black mulberry fruit as 3.11 mg/g. Kwaw et al. [42] found that the chlorogenic acid content was 90.50 μg/ml in unprocessed mulberry juice and varied between 91.51-93.18 μg/ml in the black mulberry juice processed with various methods. Tomaş [49] determined the chlorogenic acid content as 84.90 mg/100 g in black mulberry juice and as 8.50 mg/100 g in black mulberry jam.

3.6. Changes in Antimicrobial Activity. Table 4 shows the antimicrobial activities of dried black mulberry concentrate (days 0, 20, 40, and 60) and ofloxacin and amphotericin B and inhibition zone diameters.

It was revealed that the stored black mulberry concentrate did not exhibit antimicrobial activity against the microorganisms tested in the current research (Table 4). It is considered that antimicrobial substances may be broken down in the dried black mulberry fruit depending on factors, such as temperature, time, drying method, and light, applied during the drying and storage of the black mulberry fruit, and thus, the antimicrobial activity could not be detected against the microorganisms tested in the study due to the absence of antimicrobial substances in the concentrate.

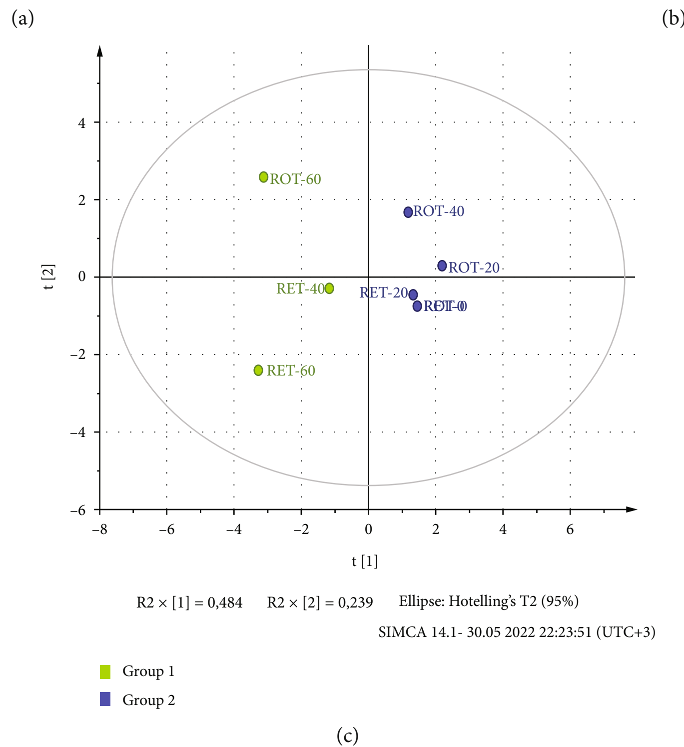
3.7. Principal Component Analysis. The black mulberry concentrates stored for 60 days were evaluated in terms of different storage temperatures, and the principal component analysis (PCA) was applied to show differences between them. Figures 6(a)–6(c) show the score scatter plot (a), loading scatter plot (b), and biplot (c) of the evaluations of the concentrates stored for 60 days in terms of different storage temperatures. The first two principal components (PC1 = 48.4% and PC2 = 23.9%) constituted 72.3% of the variance.

The samples could be classified into two main groups (Figure 6(a)). While the results of the black mulberry concentrate samples stored at room temperature for days 0, 20, and 40 and the results of the samples stored at refrigerator temperature for days 0 and 20 were located on the right side of PCA 1, the results of the remaining samples for days 40 and 60 were located on the left side (Figure 6(b)). Thus, it was observed that the samples at the end of the storage time were clearly separated from the initial samples. On the contrary, samples stored at room temperature were located on the right side of PCA 2, whereas samples stored at refrigerator temperature were located on the left side of PCA 2 (Figure 6(b)). In this way, samples stored at different temperatures were also separated from each other. The 60th-day sample of black mulberry concentrate stored at room temperature and the DPPH antioxidant activity analysis result were closely



X
t (corr) [2]

(a) (b)



(c)

FIGURE 6: Score scatter plot (a), loading scatter plot (b), and biplot (c) of the principal component analysis (PCA) (PC1 versus PC2) for the attributes of black mulberry concentrate during 60 days of storage.

located. Because it is known that the antioxidant activity value increases with a decrease in antioxidant activity, it was seen that the sample with the lowest antioxidant activity was ROT-60 (Figure 6(c)). Moreover, the 40th-day result of the concentrate stored in the refrigerator was closely located with the total monomeric anthocyanin analysis. The 60th-day result was situated closely with the total phenolic substance analysis result (Figure 6(c)). This shows that the anthocyanin content and the total phenolic content of the samples stored at refrigerator temperature were better preserved than those stored at room temperature. A positive correlation was revealed between the C^* value and b^* value and between HMF and DPPH. On the other hand, a negative correlation was determined between total phenolic content and the L^* value (Figures 6(c) and 7).

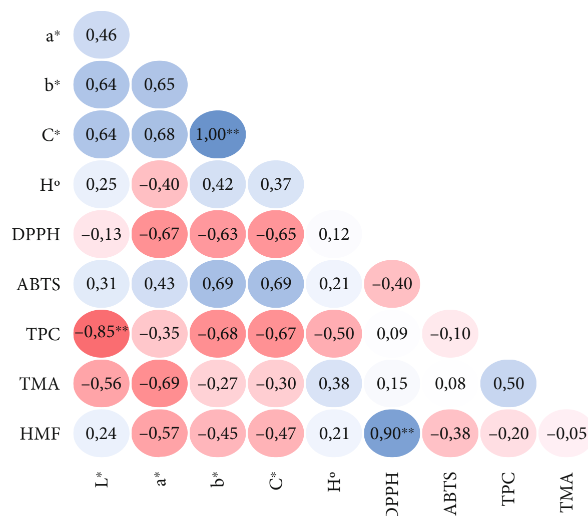


Figure 7: Pearson's correlation coefficients between some physical and chemical properties of black mulberry concentrate.

4. Conclusion

The current research determined that black mulberry concentrate was a source of phenolic substances and natural antioxidants. The total phenolic content, the total monomeric anthocyanin content, and the HMF content increased during the storage of the concentrate at room temperature and refrigerator temperature for 60 days, whereas the antioxidant activity decreased with storage. While the HMF content increased less in the black mulberry concentrate stored at refrigerator temperature, the total phenolic and monomeric anthocyanin content was better preserved. Therefore, it is necessary to cook under a vacuum and store at low temperatures in order to reduce HMF formation during production and storage in sugary products, to preserve the phenolic content and antioxidant activity. Chlorogenic acid and kaempferol-3-glucoside were identified in black mulberry concentrate, and the amount of the said compounds decreased during storage. The concentrate produced from dried black mulberry did not display antimicrobial activity against the microorganisms tested in the study. This is thought to originate from the fact that antimicrobial substances were broken down. Hence, the selection of raw materials and the procedures applied are essential for the functional properties of a product to be produced, and appropriate procedures should be selected.

Data Availability

The derived data supporting the findings of this study are available from the corresponding author on request.

Conflicts of Interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Oğuzhan Yavaş is responsible for the conceptualization, methodology, data curation, visualization. Memnune Sengul is responsible for the project administration, methodology, visualization, investigation, writing original draft and editing. Melek Zor is responsible for the investigation, resources, and writing of the original draft.

Acknowledgments

This research was supported by the Atatürk University Research Center (BAP: 2019/7235).

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