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Functional characteristics of ice creams enriched with *Aronia melanocarpa* and *Sambucus nigra L.* extracts

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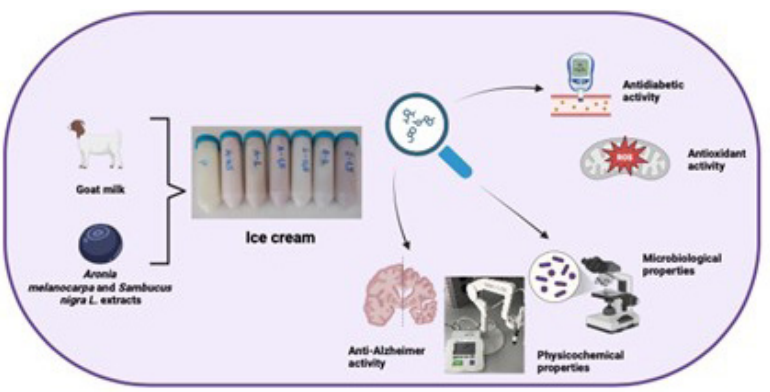
Abstract: In this study, the effects of *Aronia melanocarpa* and *Sambucus nigra* L. extracts added at different concentrations (0.5%, 1%, and 1.5%) on the physicochemical, sensory, microbiological, antioxidant capacity (DPPH• and ABTS•$^{+}$ radical scavenging activity, TPC, TFC, and TAC) antidiabetic (α-amylase and α-glucosidase inhibition), and anti-Alzheimer properties (AChE inhibitory activity) of ice cream were investigated. According to the statistical analysis results, extract addition did not have an effect on the total dry matter, fat and ash ratios of the ice cream samples ($p > 0.05$), but it had an effect on the protein ratio, viscosity, and first drip time at the level of $p < 0.05$. The addition of extract positively affected the DPPH• radical scavenging activity IC_{50} and ABTS•$^{+}$ radical scavenging activity IC_{50} values of the ice cream samples, and also increased the TPC, TFC, and TAC values. Indeed, a decrease in the IC_{50} values of DPPH• and ABTS•$^{+}$ radical scavenging activities indicates an increase in antioxidant activity. The highest TPC, TFC, and TAC values were detected in the A-1.5 sample (sample containing 1.5% *A. melanocarpa* extract) as 749.33 mg GA/kg, 434.70 mg QE/kg, and 290.97 mg/kg, respectively. Similarly, the highest α-amylase inhibitory activity (23.08%), the highest α-glucosidase inhibitory activity (10.41%) and the highest AChE inhibitory activity (14.70%) were observed in the A-1.5 sample. Extract addition had no effect on microbiological properties and sensory properties except texture. The results of this study showed that *A. melanocarpa*, *S. nigra* L. extracts can be used as a natural additive with antioxidant, antidiabetic and anti-Alzheimer activities in ice cream production.

Key words: Ice cream, *Aronia melanocarpa*, *Sambucus nigra* L., antioxidant properties, antidiabetic activity, anti-Alzheimer activity

Graphical abstract



1. Introduction

Ice cream is a frozen mixture of various components such as sweeteners, dairy components, emulsifiers, stabilizers, and flavorings. Ice cream is rich in macronutrients such as fat, protein, and carbohydrates and micronutrients such as vitamins A, D, E, and the mineral calcium, but is generally not rich in natural pigments and some natural antioxidants such as vitamin C, polyphenols, and carotenoids (Erkaya et al., 2012; Mohammed et al., 2022). In recent years, goat's milk has emerged as a more valuable alternative to cow's milk due to its unique properties and is also preferred in ice cream production (Güzeler et al., 2019). Goat milk

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plays an important role in human nutrition due to its high digestibility (small fat globules), low allergenic properties and biofunctional compounds (some serum proteins and polyunsaturated fatty acids) (Türkmen, 2017). However, lately there has been a consumer trend toward purchasing healthier products. As a result of studies conducted with ginger juice and paste (*Zingiber officinale*) (Gabbi et al., 2018) and dried Besni grape (*Vitis vinifera* L.) (Kavaz et al., 2016), it has been stated that consumers want to purchase ice creams with higher bioactivity and fewer artificial ingredients. For this reason, manufacturers choose to add functional components such as phenolics, antioxidants, and phytosterols to food products to attract the attention of consumers (Shaviklo et al., 2011).

Less well-known fruit species, such as *A. melanocarpa*, are becoming the focus of attention of researchers but also the public, especially due to their high antioxidative activity. *A. melanocarpa* is valuable as a great source of phenolic acids, flavonoids, and polyphenols. It has been reported to contain > 71 mg flavonols per 100 g fresh weight (Slimestad et al., 2005), 6.5–8.5 mg total anthocyanins per 1 g dry weight (Strigl et al., 1995), 40.1 mg of GAE/g dw total phenolics (Kähkönen et al., 1999). These fruits and their products have great potential for health promotion as they reduce the risk factors of metabolic syndrome. *A. melanocarpa* has shown positive effects in common diseases such as hypertension, dyslipidemia, glucose metabolism disorders, obesity, proinflammatory states, and thrombosis risk. It has the potential to inhibit the development of several types of cancer, including cancer stem cells, leukemia, as well as bowel and breast cancer (Sidor et al., 2019). *S. nigra* L. contains various bioactive components such as terpenoid compounds and polyphenolic compounds (Ferreira et al., 2022). *S. nigra* L. exhibits various health functions, including anticancer, antiinflammatory, antiinfluenza, antidiabetic, antimicrobial, and neuroprotective activities, as well as cardiovascular protection. It also has great potential to be transformed into functional foods (Liu et al., 2022).

Diabetes, which results from carbohydrate metabolism associated with defects in insulin secretion, can be defined as the presence of glucose in the blood well above normal levels. Basically, proper management of diabetes is based on controlled release of glucose in the bloodstream, controlling hyperglycemia and improving the level of antioxidant compounds in the body. Some drugs that inhibit α-amylase and α-glucosidase enzymes such as acarbose, emiglitate, voglibose, and miglitol are used in the treatment of diabetic patients, but their side effects such as diarrhea and gastrointestinal disorders are of concern. Therefore, it is important to identify alternative natural sources of antidiabetics that inhibit α-amylase and α-glucosidase. α-amylase and α-glucosidase are enzymes that hydrolyze carbohydrates, and inhibition of these enzymes causes a decrease in the rate of glucose absorption in the digestive tract and, as a result, the postprandial plasma glucose increase is blunted (Leroux-Stewart et al., 2004; Shirkhan et al., 2024). According to data, approximately 52 million people are affected by Alzheimer's disease, a neurodegenerative disease characterized by cognitive impairment, neuropsychiatric disorders, and mood changes (Maccioni et al., 2022). In Alzheimer's disease, pathological features such as losses in the cholinergic system in the forebrain, intercellular neurofibrillary networks from β-amyloid, accumulation of amyloid plaques, and decreases in the neurotransmitter acetylcholine are observed. However, due to the regulation of processes such as memory and learning in the cholinergic system, anticholinesterase activity forms the basis of anti-Alzheimer's drugs. Cholinesterase inhibitors are known to play a role in increasing cholinergic transmission by inhibiting the AChE (acetylcholinesterase), which hydrolyzes acetylcholine. Therefore, AChE inhibition in the treatment of Alzheimer's disease is effective in the treatment of the disease by reducing β-amyloid levels in the brain and increasing the utilization of acetylcholine (Eröz Poyraz et al., 2021).

In the literature, there are many studies on functional ice cream production using various fruit extracts (da Silva et al., 2020; Boyanova et al., 2022; Rad et al., 2022; Ahmed and Ayodele, 2023; Shadordizadeh et al., 2023; Slimani et al., 2025). However, to the best of our knowledge, there is no study in the literature in which *A. melanocarpa* and *S. nigra* L. extracts were used in ice cream production and compared in terms of physicochemical, microbiological, sensory, and functional properties. On the other hand, while *S. nigra* L. has become more popular due to the COVID-19 pandemic (Setz et al., 2025), *A. melanocarpa* is unfortunately not widely consumed, although it has been proven to contain many beneficial components (Negreanu-Pirjol et al., 2023). Therefore, in this study, two fruits used for similar purposes and exhibiting relatively similar properties were preferred and their effects on ice cream production were compared. In addition, the study aimed to observe the effect of adding *A. melanocarpa* and *S. nigra* L. extracts to ice cream production on both the quality criteria of ice cream and the antioxidant, antidiabetic and anti-Alzheimer activities of the conventional ice cream mix. Therefore, the aim of this study was to investigate the possibility of using *A. melanocarpa* and *S. nigra* L. extracts in combination with goat milk in the production of a new functional ice cream due to their many beneficial effects. In this context, some physicochemical, sensory, microbiological, antioxidant, and phenolic properties, as well as α-glucosidase, α-amylase, and AChE inhibitory activities, of ice creams produced from goat milk using

0.5%, 1%, and 1.5% *A. melanocarpa* and *S. nigra* L. extracts were investigated.

2. Materials and methods

2.1. Materials

Cream and skimmed milk powder were obtained from the Pilot Milk Factory, Faculty of Agriculture, Atatürk University, Türkiye. Goat milk was sourced from a local farm in Erzurum. Commercial-grade granulated sugar, stabilizer, emulsifier, *A. melanocarpa*, and *S. nigra* L. were purchased from the local markets of Erzincan, Türkiye.

2.2. Preparation of fruit extracts

Fruits (*A. melanocarpa* and *S. nigra* L.) were washed with drinking water, mixed with water in a ratio of 1:3 (weight/volume) and kept in 30 °C water for 1 day, and hydrated fruits with approximately 45–50% water content were obtained. They were then blended with distilled water (1:3 w/v) at maximum speed for 5 min using a blender (model HR1337/00; Philips, Amsterdam, Netherlands). After the mixture was filtered through muslin cloth, the filtrate was centrifuged at 2500 × *g* for 5 min. The resulting supernatant was frozen at –84 °C in the freezer (Sanyo Electric Co., Osaka, Japan) and lyophilized at –50 °C under 5 mm Hg pressure (TRST-4-4 lyophilizer; Teknosem, İstanbul, Türkiye).

2.3. Production of ice cream samples

The mix prepared to contain 17% sugar (sucrose), 15% skimmed milk powder, 3.5% milk fat, 0.7% stabilizer, and 0.25% emulsifier was pasteurized in a hot water bath at 85 °C for 10 min. Then, it was cooled to 4 ± 1 °C and left to ripen for 24 h at the same temperature in the cold storage. Following maturation, the mix was divided into equal parts corresponding to seven groups, and two different extracts were used in three different ratios (0.5%, 1%, and 1.5%) and processed into ice cream. Control samples were produced without adding extract. Produced ice creams were divided into a sufficient number of sterile plastic containers for physical, chemical, microbiological, and sensory analysis and kept in a deep freezer (–20 °C).

2.4. Physicochemical analysis

Total dry matter, fat, protein, ash, pH, and overrun were determined according to Kurt et al. (2012), while melting rate and first drip time were determined according to Güven and Karaca (2002). Viscosity was measured using a Poulten RY-8 model viscometer (Poulten Selfe and Lee Ltd., Essex, UK) at 50 rpm with a No. 6 head (sample temperature 3 ± 1 °C), and the results were read directly in centipoise (cP) on the digital display of the instrument.

2.5. DPPH• (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

The method described by Bakirci et al. (2023) was used as the basis for determining DPPH• free radical scavenging activity. To measure the ability of DPPH• radicals to be scavenged by antioxidants, an ice cream sample (50 µL) was mixed with a methanolic solution of DPPH• (5 mL, 0.004%) and incubated for 30 min, and the decrease in color was measured as absorbance at 517 nm using a spectrophotometer. Radical scavenging ability was expressed as IC_{50} (half maximal inhibitor concentration) values, representing the sample concentration required to reduce DPPH• radicals by 50%. The decrease in absorbance indicates the ability of the samples to scavenge DPPH•.

2.6. ABTS•$^+$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid)) radical scavenging activity

The method given by Terzioğlu et al. (2023) was used. After the ABTS•$^+$ stock solution (7 mM ABTS•$^+$ and 2.45 mM $K_2S_2O_8$) was prepared by mixing and keeping in a dark environment for 16 h, it was diluted with ethanol to obtain an absorbance value of 0.70 ± 0.02 at a wavelength of 734 nm. Then, extract/frozen samples (15–45 µg/mL) were added to the ABTS•$^+$ solution, and after 30 min of incubation, the absorbance of all samples was measured at the same wavelength. The decrease in absorbance indicates the ABTS•$^+$ radical scavenging ability of the samples.

2.7. Total phenolic content (TPC)

The TPC content of ice cream samples was determined by modifying the method given by Terzioğlu et al. (2023). For this purpose, an ice cream sample (100 µL), Folin–Ciocalteu reagent (2 mL, 2 N), and distilled water (46 mL) were taken into a measured bottle and shaken for 3 min. Then, Na_2CO_3 solution (3 mL of 2%) was added and shaken. After the solution was mixed at regular intervals and kept at room temperature for 2 h, its absorbance was measured on a spectrophotometer (Optizen POP; Mecasys Co. Ltd., Daejeon, South Korea). Spectrophotometric analysis of the mixture was performed at a wavelength of 760 nm against gallic acid (reference standard).

2.8. Total flavonoid content (TFC)

TFC was determined by the spectrophotometric method described by Kumar et al. (2022). The calibration curve was drawn using quercetin as the reference standard. After adding $NaNO_2$ solution (150 µL, 5%), distilled water, and $AlCl_3$ (300 µL, 10%) to the ice cream sample (0.5 mL), it was incubated for 5 min. Then, NaOH solution (1 mL) and distilled water (5 mL) were added, and the absorbance at 510 nm was determined. TFC results were expressed as mg quercetin equivalent per kg.

2.9. Total anthocyanin content (TAC)

TAC was determined according to Ercisli et al. (2012). Absorbance readings were taken against pure water at the wavelengths where the samples showed maximum absorbance, using a spectrophotometer. Briefly, the absorbance of the extract was measured at 520 and 700 nm in pH 4.5 (acetic acid–sodium acetate, 1 M) and pH

1.0 (hydrochloric acid–potassium chloride, 0.2 M) buffers. TAC was calculated using the extinction coefficient (cyanidin 3-rutinoside) with a molar absorptivity of 28,800 M^{-1} cm^{-1} according to the following equation.

$$A=(A520-A700)pH1.0-(A520-A700)pH4.5$$

2.10. α-Amylase inhibition assay

An amount of sample (100 µL), sodium phosphate buffer (20 mM, pH 6.9, containing 6 mM sodium chloride), and α-amylase (100 µL) were placed in each well of a 24-well microplate. After incubation (10 min at 25 °C), 100 µL of 1% starch solution prepared in the same buffer was added and incubated again at the same temperature for the same duration. Following the incubation, 200 µL dinitrosalicylic acid color reagent was added, the mixture was kept at 100 °C for 5 min, and then cooled to 25 °C. An amount of sample (50 µL) was taken, mixed with 200 µL of distilled water, and the absorbance was measured at 540 nm. Each determination was performed in triplicate. The results were calculated using the equation below (Yuca et al., 2023).

$$\text{Inhibition (\%)} = (1-A_{sample}-A_{control})x100$$

2.11. α-Glucosidase inhibition assay

The α-glucosidase enzyme inhibition effect was determined using the method of Yuca et al. (2023) with some modifications. For this analysis, α-glucosidase solution (50 µL), p-nitrophenyl-α-D-glucopyranoside (50 µL), and the sample (50 µL) dissolved in phosphate buffer were placed in microplate wells and incubated for 30 min at 37 °C. The reaction was completed by adding sodium carbonate (50 µL, 0.1 M). The blank solution was prepared without adding the α-glucosidase enzyme solution. The absorbance of the blank solution and the samples at 400 nm was recorded. Measurements were repeated three times, and the results were calculated using the equation below (Yuca et al., 2023).

$$\text{Inhibition (\%)} = (1-A_{sample}-A_{control})x100$$

2.12. Acetylcholinesterase (AChE) inhibitory activity

AChE inhibition activity was measured in 96-well microplates using Ellman's method (Ellman et al., 1961). DTNB (5,5-dithio-bis(2-nitrobenzoic acid), 125 µL), AChE enzyme solution (25 µL) prepared in Tris-HCl buffer (pH 8.0), and the sample (50 µL) were placed in the microplate wells. A blank was prepared without adding the AChE enzyme solution to the same mixture. After incubation for 15 min at room temperature, acetylthiocholine iodide (ATCl, 25 µL) was added and incubated for another 10 min. Absorbance values were measured at a wavelength of 405 nm. The actual absorbance values were calculated by subtracting the absorbance values of the blanks from those of the samples, using the following equation.

$$\text{Inhibition (\%)} = 100-(A_{sample}-A_{control})x100$$

2.13. Microbiological analysis

Total aerobic mesophilic bacteria were counted after incubation on standard plate count agar (Merck KGaA, Darmstadt, Germany) at 30 °C for 48 h. For the enumeration of coliform group bacteria, incubation was carried out on violet red bile agar (Merck KGaA) at 32 ± 1 °C for 24 ± 2 h. Potato-dextrose agar (Merck KGaA) was used for yeast and mold enumeration. The pH of the medium was adjusted to 3.5 with 10% sterile tartaric acid, and the plates were incubated at 20–25 °C for 5 days (Richardson, 1985).

2.14. Sensory analysis

For this purpose, the color, taste, smell, texture, and overall acceptability of ice cream samples were evaluated by a panel of 12 faculty members from the Department of Food Engineering. Evaluations were scored on a 5-point scale (5, very good; 4, good; 3, slightly flawed; 2, flawed; 1, very poor)

2.15. Statistical analysis

The research was conducted using two different fruit extracts at three different rates and two replications, according to the completely randomized design. The results obtained were analyzed using the SPSS statistical software (version 12.0) (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Physicochemical analysis results

The physicochemical analysis results of ice cream samples are given in Table 1. According to the statistical analysis results, the addition of extract did not affect the total dry matter, fat, or ash ratios of the ice cream samples. Protein content, apparent viscosity, and first dripping had an effect at $p < 0.05$, while pH, complete melting time and overrun had an effect at $p < 0.01$. Total dry matter ratios of ice cream samples were between 36.88 ± 1.22% (control sample) and 38.38 ± 0.74% (S-1.5 sample). The fat contents varied between 3.55 ± 0.14% (P) and 3.39 ± 0.13% (S-1.5), and the ash content varied between 0.77 ± 0.01% (control sample) and 0.82 ± 0.04% (S-1.5). This stability may be attributed to the relatively low concentration of extract used (0.5–1.5%), which was likely insufficient to alter the intrinsic formulation of the ice cream matrix. Similarly, Çam et al. (2013) stated that there was no significant difference in dry matter and fat contents between ice cream enriched with pomegranate byproducts. Qayyum et al. (2017) reported that the minerals in watermelon seeds contributed to the increase in ash content of ice creams with watermelon seed flour addition. Protein ratios of ice cream samples varied between 2.90 ± 0.03% (control sample) and 2.74 ± 0.03% (S-1.5). This decline can be interpreted as a proportional dilution effect, since increasing extract

Table 1. Physicochemical analysis of ice cream samples.

Sample		Total dry matter (%)	Fat (%)	Protein (%)	Ash (%)	pH	Apparent viscosity (cP)	First dripping time (s)	Complete melting time (s)	Overrun (%)
Control sample	P	36.88 ± 1.22^{a}	3.55 ± 0.14^{a}	2.90 ± 0.03^{a}	0.77 ± 0.01^{a}	6.54 ± 0.04^{a}	1759.16 ± 321.44^{b}	1110.00 ± 124.45^{d}	5170.50 ± 137.89^{e}	22.92 ± 3.90^{d}
Aronia melanocarpa	A-0.5	37.52 ± 1.00^{a}	3.51 ± 0.11^{a}	2.85 ± 0.01^{ab}	0.81 ± 0.01^{a}	6.36 ± 0.09^{a}	2410.33 ± 189.92^{a}	1471.00 ± 90.51^{abc}	6107.50 ± 231.22^{bc}	31.13 ± 1.30^{bc}
	A-1	37.94 ± 0.86^{a}	3.48 ± 0.12^{a}	2.82 ± 0.04^{bc}	0.82 ± 0.00^{a}	5.91 ± 0.11^{bc}	2402.87 ± 135.37^{a}	1648.50 ± 64.35^{ab}	6557.00 ± 243.24^{b}	34.72 ± 1.70^{ab}
	A-1.5	38.34 ± 0.93^{a}	3.45 ± 0.13^{a}	2.80 ± 0.02^{bc}	0.84 ± 0.01^{a}	5.72 ± 0.06^{c}	2530.30 ± 229.22^{a}	1746.00 ± 97.58^{a}	7401.00 ± 288.50^{a}	37.83 ± 2.23^{a}
Sambucus nigra L.	S-0.5	37.32 ± 1.55^{a}	3.47 ± 0.10^{a}	2.86 ± 0.05^{ab}	0.80 ± 0.01^{a}	6.41 ± 0.11^{a}	2111.52 ± 53.80^{ab}	1255.50 ± 156.27^{cd}	5492.50 ± 255.27^{de}	28.15 ± 2.42^{c}
	S-1	38.13 ± 0.83^{a}	3.42 ± 0.12^{a}	2.78 ± 0.04^{bc}	0.82 ± 0.02^{a}	6.06 ± 0.13^{b}	2302.96 ± 97.80^{a}	1362.00 ± 93.34^{bcd}	5849.50 ± 98.29^{cd}	32.49 ± 1.17^{abc}
	S-1.5	38.38 ± 0.74^{a}	3.39 ± 0.13^{a}	2.74 ± 0.03^{c}	0.82 ± 0.04^{a}	5.82 ± 0.04^{c}	2604.85 ± 262.74^{a}	1625.50 ± 235.47^{ab}	6252.00 ± 240.42^{bc}	35.60 ± 0.72^{ab}
Sig		ns	ns	*	ns	**	*	*	**	**

Sig: significant; ns: not significant; *: $p < 0.05$; **: $p < 0.01$; P: control ice cream; A-0.5: ice cream with 0.5% *A. melanocarpa* extract; A-1: ice cream with 1% *A. melanocarpa* extract; A-1.5: ice cream with 1.5% *A. melanocarpa* extract; S-0.5: ice cream with 0.5% *S. nigra* L. extract; S-1: ice cream with 1% *S. nigra* L. extract; S-1.5: ice cream with 1.5% *S. nigra* L. extract.

levels reduce the relative content of milk solids in the formulation. Moreover, polyphenols or fiber-like compounds found in fruit extracts may interact with milk proteins, altering their structural properties or interfering with protein determination methods, which could result in underestimated protein levels during analysis (Ozdal et al., 2013; Jakobek, 2015). However, the exact mechanisms underlying the observed decrease in protein content have not yet been fully elucidated, indicating the need for further indepth investigations. Erkaya et al. (2012) reported that the addition of Cape gooseberry and Gabbi et al. (2018) reported that the addition of processed ginger caused a decrease in the protein content of ice cream.

The pH values of the samples to which 0.5% extract was added were similar to the control sample. As the extract ratio increased, pH values decreased. Veberic et al. (2009) determined the organic acids in *S. nigra* L. as 3.50 g/kg FW citric acid, 1.10 g/kg FW malic acid, 0.33 g/kg FW shikimic acid, and 0.17 g/kg FW fumaric acid. It is thought that the decrease in pH value in samples containing extract is due to the organic acids contained in these fruits. These results are similar to the results given by Erkaya et al. (2012), Singo and Beswa (2019), Zor and Sengul (2022), who detected a decrease in the pH values of ice cream samples to which extract was added.

The viscosity values of the ice cream samples ranged between 1759.16 ± 321.44 cP (control sample) and 2604.85 ± 262.74 cP (S-1.5). It was observed that the viscosity values of all ice cream samples to which extract was added were similar among themselves and significantly higher ($p < 0.05$) than the control sample (1759.16 ± 321.44 cP). These differences may be attributed to the gummy components found in the fruit extracts. *S. nigra* L. fruits contain sugar at an average of 87.62 ± 2.48 g/kg FW (Veberic et al., 2009), while *A. melanocarpa* contains sugar in the range of 12.20–14.10 °Brix (Hwang and Do Thi, 2016). As the dry matter ratio of the ice cream samples increased, the viscosity values also increased. This situation is similar to the results of Kavaz et al. (2016), who found that viscosity values in ice cream samples with different amounts of Besni grape increased depending on the Besni grape concentration. The researchers reported that this may be due to the increased total dry matter content, the high water-binding capacity of the fiber, and the gummy components present in Besni grape. Singo and Beswa (2019) found a decrease in viscosity along with a decrease in dry matter rates in ice cream samples to which roselle extract was added.

The shortest first dripping time (1110.00 ± 124.45 s) was observed in the control sample, and the longest first dripping time (1746.00 ± 97.58 s) was found in sample A-1.5. The complete melting time of ice cream samples varied between 5170.50 ± 137.89 s (control sample) and 7401.00 ± 288.50 s (A-1.5 sample). Just like the highest first dripping time, the highest complete melting time was also detected in the A-1.5 sample. It was observed that, as the extract concentration increased, both the complete melting time and the first dripping time increased. Various researchers have reported that the first dripping time increases as the dry matter content and fruit content in ice cream increases (Güven and Karaca, 2002; Erkaya et al., 2012). According to the literature, ice creams with high overrun values tend to melt more slowly because air is an excellent insulator (Çakmakçı et al., 2016). It was observed that the overrun values of the ice cream samples increased along with the first dripping times and the complete melting time.

In ice cream production, air is incorporated into the mixture to increase volume (Syed et al., 2018). The air inside ice cream affects physical properties such as hardness and melting and provides a light texture. The quality of ice cream is affected by the amount of air it contains. It is stated in the literature that, for a quality ice cream, the overrun should be between 15% and 50% (Şen, 2016). Overrun values of ice cream samples varied between 22.92 ± 3.90% (control sample) and 37.83 ± 2.23% (A-1.5). Overrun values increased as the extract concentration increased. It is thought that the differences in the overrun rates of ice cream samples are due to their composition. Additionally, as the overrun values of the ice cream samples increased, their viscosity values also increased. In this respect, our results differed from studies that reported a decrease in viscosity as overrun values increased in ice cream samples containing roselle extract and kumquat paste (Çakmakçı et al., 2016; Singo and Beswa, 2019). This difference may be related to the dry matter values. The dry matter ratios decreased in ice cream samples containing roselle extract and kumquat paste.

3.2. Antioxidant activity and TPC, TFC, and TAC analysis results

The results of DPPH• radical scavenging activity IC_{50}, ABTS•$^{+}$ radical scavenging activity IC_{50}, TPC, TFC, and TAC analyses of ice cream samples and *A. melanocarpa* and *S. nigra* L. extracts are summarized in Table 2.

Antioxidants have become compounds of scientific interest due to their many benefits, such as antiinflammatory and antiaging effects, by preventing the known harmful effects of free radicals on human metabolism. In the production of some foods, antioxidants can be added to enrich the products (Zehiroglu and Ozturk Sarikaya, 2019). Today, there are many bioanalytical methods for measuring antioxidant effects. Among these, DPPH• and ABTS•$^{+}$ radical scavenging activity analyses are widely applied to measure the total antioxidant activity of various substances spectrophotometrically (Gulcin and Alwasel, 2023).

Table 2. DPPH•, ABTS•$^{+}$, TPC, TFC, and anthocyanin content results of ice cream samples.

Sample		DPPH• IC_{50} (µg/mL)	ABTS•$^{+}$ IC_{50} (µg/mL)	TPC (mg GA/kg)	TFC (mg QE/kg)	TAC (mg/kg)
Control sample	P	54,527.96 ± 1850.68^{a}	47,120.90 ± 492.63^{a}	54.52 ± 15.49^{e}	21.49 ± 6.06^{e}	1.21 ± 0.17^{d}
Aronia melanocarpa	A-0.5	26,547.33 ± 1218.19^{cd}	21,931.90 ± 2656.61^{c}	591.21 ± 42.22^{bc}	323.41 ± 16.65^{c}	156.43 ± 22.21^{b}
	A-1	21,905.91 ± 1355.16^{e}	15,389.51 ± 1415.18^{d}	663.03 ± 27.16^{b}	377.86 ± 24.65^{b}	242.65 ± 33.64^{a}
	A-1.5	15,862.92 ± 969.35^{f}	9681.23 ± 52.16^{e}	749.33 ± 36.92^{a}	434.70 ± 18.13^{a}	290.97 ± 12.10^{a}
Sambucus nigra L.	S-0.5	33,367.73 ± 492.96^{b}	26,023.86 ± 2251.69^{b}	466.51 ± 27.17^{d}	252.43 ± 8.65^{d}	108.97 ± 6.55^{c}
	S-1	29,228.23 ± 1257.33^{c}	19,585.04 ± 1879.91^{c}	535.75 ± 15.83^{cd}	339.00 ± 34.88^{bc}	188.64 ± 17.47^{b}
	S-1.5	23,764.10 ± 2656.23^{de}	14,217.39 ± 1667.55^{d}	597.51 ± 30.98^{bc}	390.15 ± 25.04^{ab}	252.19 ± 24.16^{a}
	Sig	**	**	**	**	**
Extracts	*Aronia melanocarpa*	986.00 ± 87.50^{a}	133.39 ± 3.06^{b}	8712.37 ± 326.15^{a}	5403.90 ± 167.95^{a}	4526.99 ± 519.12^{a}
	Sambucus nigra L.	1320.65 ± 143.82^{a}	329.43 ± 27.22^{a}	6708.89 ± 91.04^{b}	4418.44 ± 517.55^{a}	3244.99 ± 100.21^{a}
	Sig	ns	**	**	ns	ns

Sig: significant; ns: not significant; **: $p < 0.01$; P: control ice cream; A-0.5: ice cream with 0.5% *A. melanocarpa* extract; A-1: ice cream with 1% *A. melanocarpa* extract; A-1.5: ice cream with 1.5% *A. melanocarpa* extract; S-0.5: ice cream with 0.5% *S. nigra* L. extract; S-1: ice cream with 1% *S. nigra* L. extract; S-1.5: ice cream with 1.5% *S. nigra* L. extract.

According to the statistical analysis results, the difference between the DPPH• radical scavenging activity IC_{50} values of the extracts was insignificant ($p > 0,05$), but they were significantly different from each other in terms of ABTS•$^{+}$ radical scavenging activity IC_{50} values ($p < 0.01$). ABTS•$^{+}$ radical scavenging activity IC_{50} values of *A. melanocarpa* and *S. nigra* L. were determined as 133.39 ± 3.06 µg/mL and 329.43 ± 27.22 µg/mL. Our results are consistent with those of Viskelis et al. (2010), who reported that the radical scavenging activity of *S. nigra* fruits was lower than that of *A. melanocarpa* varieties. Differences in antioxidant levels of extracts may also be related to differences in fruit type and growing conditions. Additionally, processing fruits into products such as juice, powders, and fruit tea may lead to changes in their antioxidant properties (Tolić et al., 2015). The antioxidant capacity of milk and dairy products is due to components of milk such as α-tocopherols, conjugated linoleic acid, β-carotene, vitamins (D3 and A), mineral substances, and phospholipids (Stobiecka et al., 2022).

It was determined that the addition of extract had a significant ($p < 0.01$) effect on the DPPH• radical scavenging activity IC_{50} and ABTS•$^{+}$ radical scavenging activity IC_{50} values of the ice cream samples. DPPH• radical scavenging activity IC_{50} of ice cream samples was determined as the lowest 15,862.92 ± 969.35 µg/mL (A-1.5) and the highest 54,527.96 ± 1850.68 µg/mL (control sample). The lowest ABTS•$^{+}$ radical scavenging activity IC_{50} value was determined as 9681.23 ± 52.16 µg/mL in the A-1.5 sample, and the highest was 47,120.90 ± 492.63 µg/mL in the control sample. The addition of extract positively affected the DPPH• radical scavenging activity IC_{50} and ABTS•$^{+}$ radical scavenging activity IC_{50} values of the ice cream samples. The decrease in absorbance indicates the DPPH• and ABTS•$^{+}$ radical scavenging ability of the samples. As the extract ratio increased, DPPH• radical scavenging activity IC_{50} and ABTS•$^{+}$ radical scavenging activity IC_{50} values decreased. While increases were observed in the TPC, TFC, and TAC values of the ice cream samples, decreases were observed in the DPPH• radical scavenging activity IC_{50} and ABTS•$^{+}$ radical scavenging activity IC_{50} values, and the results are compatible with each other. When we look at the literature, DPPH• radical scavenging activity of different ice cream samples are 91.06–1819.29 µg/mL (Zor and Sengul, 2022) and 23.16–43.96% (Akca and Akpinar, 2021); ABTS•$^{+}$ radical scavenging activity values are reported as 14.12–150.72 µg/mL (Zor and Sengul, 2022). The differences may be due to the fruit ratio used in ice cream production, fruit type, storage time, or the treatment applied to the fruit (Çakmakçı et al., 2016; Zor and Sengul, 2022).

Phenolic compounds are plant secondary metabolites that constitute one of the most common groups of substances in plants, with more than 8000 phenolic structures currently known (Oladeji and Adelowo, 2017). A growing number of studies reveal that these compounds are effective in preventing or delaying various diseases (Zor, 2024). It was observed that the extracts were significantly ($p < 0.01$) different from each other in terms of TPC values. According to Viskelis et al. (2010), the TPC value detected in *A. melanocarpa* (3290.7 mg/100 g) is higher than that detected in *S. nigra* L. (1048 mg/100 g), and our study shows similar characteristics in this respect. The values reported in that study are higher than ours, which may be due to the extraction process applied to the fruits in our study (Kumar et al., 2022). The difference between the TFC and TAC values of the extracts was found to be insignificant.

Extract addition had an effect on the TFC and TAC values of ice cream samples at the $p < 0.01$ level. The addition of extract increased the TPC, TFC, and TAC values of the ice cream samples. *A. melanocarpa* extract was more effective on the TPC, TFC, and TAC values of ice cream samples than *S. nigra* L. extract at the same rate. The lowest TPC, TFC, and TAC values were 54.52 ± 15.49 mg GA/kg, 21.49 ± 6.06 mg QE/kg, 1.21 ± 0.17 mg/kg, respectively, in the control sample; the highest values were detected in the A-1.5 sample as 749.33 ± 36.92 mg GA/kg, 434.70 ± 18.13 mg QE/kg, and 290.97 ± 12.10 mg/kg. *A. melanocarpa* and *S. nigra* L. are rich sources of biologically active substances and have strong antioxidant activities (Viskelis et al., 2010). The fruits of *A. melanocarpa* have been reported by many researchers to be among the species with the highest amounts of anthocyanins and phenolics (Viskelis et al., 2010). In other studies, TPC values detected in ice cream samples were 15.91–82.87 µg GAE/mg (Zor and Sengul, 2022), 64.82–659.63 µg GAE/mg extract (Çakmakçı et al., 2016), and 4.13–30.25 mg GAE/g (Ademosun, 2021); TFC values were 48.18–1148.18 µg QE/mg extract (Çakmakçı et al., 2016) and 0.98–19.11 mg QE/g (Ademosun, 2021); anthocyanin values have been reported as 0.00–2.56 mg/100 g (Zor and Sengul, 2022).

3.3. Antidiabetic and anti-Alzheimer activity analysis results

Antidiabetic activity results of ice cream samples are given in Table 3. Extract addition increased the α-amylase inhibitory activity and α-glucosidase inhibitory activity of ice cream samples at the $p < 0.05$ level. The lowest α-amylase inhibitory activity (1.08% ± 0.18%) and the lowest α-glucosidase inhibitory activity (0.48% ± 0.15%) were in the control sample. The highest α-amylase inhibitory activity (23.08% ± 8.53%) and the highest α-glucosidase inhibitory activity (10.41% ± 3.41%) were observed in the A-1.5 sample (1.5% *A. melanocarpa* extract-added sample). In general, *A. melanocarpa* can be considered a good source of antioxidants that are effective in combating oxidative stress caused by hyperglycemia (Banjari et al.,

Table 3. Antidiabetic and anti-Alzheimer activity results of ice cream samples.

Sample		α-amylase inhibitory activity (%)	α-glucosidase inhibitory activity (%)	AChE inhibitory activity (%)
Control sample	P	1.08 ± 0.18^{c}	0.48 ± 0.15^{c}	4.25 ± 2.24^{c}
Aronia melanocarpa	A-0.5	4.95 ± 2.73^{c}	1.50 ± 0.35^{bc}	8.54 ± 0.45^{b}
	A-1	11.81 ± 3.71^{abc}	4.28 ± 1.15^{bc}	11.60 ± 1.60^{ab}
	A-1.5	23.08 ± 8.53^{a}	10.41 ± 3.41^{a}	14.70 ± 0.73^{a}
Sambucus nigra L.	S-0.5	2.67 ± 0.46^{c}	1.04 ± 0.19^{c}	8.91 ± 2.64^{b}
	S-1	7.90 ± 5.32^{bc}	3.19 ± 2.03^{bc}	11.52 ± 0.10^{ab}
	S-1.5	17.42 ± 5.84^{ab}	6.64 ± 3.97^{ab}	12.19 ± 0.04^{ab}
Sig		*	*	**

Sig: significant; *: $p < 0.05$; **: $p < 0.01$; P: control ice cream; A-0.5: ice cream with 0.5% *A. melanocarpa* extract; A-1: ice cream with 1% *A. melanocarpa* extract; A-1.5: ice cream with 1.5% *A. melanocarpa* extract; S-0.5: ice cream with 0.5% *S. nigra* L. extract; S-1: ice cream with 1% *S. nigra* L. extract; S-1.5: ice cream with 1.5% *S. nigra* L. extract.

2017). It has been reported that *A. melanocarpa* extract and juice significantly reduce blood sugar in diabetic rats and may be beneficial in the prevention and control of diabetes and diabetes-related complications (Mu et al., 2020).

Mohammadian et al. (2020) found the α-glucosidase inhibitory activity to be 70.5–86.4% in ice cream samples produced with the addition of pistachio shell extract. These values are higher than our results and are thought to be related to the type of fruit and extract concentration used. Baskaran and Srinu (2021) determined α-amylase inhibitory activity in the range of 0.00–18.00% and α-glucosidase inhibitory activity in the range of 0.00–25.00%, and these results seem to be compatible with ours.

Anti-Alzheimer activity results of ice cream samples are given in Table 3. The addition of the extract to ice cream samples was effective in AChE inhibitory activity at $p < 0.01$ level. The lowest AChE inhibitory activity (4.25 ± 2.24%) was found in the control sample, while the highest AChE inhibitory activity (14.70 ± 0.73%) was observed in sample A-1.5 (1.5% *A. melanocarpa* extract-added sample). As the extract concentration increased in ice cream samples, an increase in AChE inhibitory activities was observed. It has been suggested that fruits, including berries, may prevent cognitive impairments associated with Alzheimer's disease due to their broad-spectrum biological activities (Bilawal et al., 2021). Taqui et al. (2022) reported that plants have anti-Alzheimer's activity through the flavonoids, alkaloids, terpenoids, coumarins, and polyphenols they contain. Similarly, in the present study, it was determined that the ice cream samples to which 1.5% *A. melanocarpa* extract with the highest AChE inhibitory activity was added also had the highest values in terms of phenolic compounds, flavonoids, and anthocyanin contents with antioxidant properties. In addition, it was proved by Ji et al. (2022), who examined the AChE inhibition activity of milk-derived opioid peptides, that the peptides naturally found in milk have AChE inhibitory activity. The researchers found that βb-casomorphin-5 and βb-casomorphin-7 peptides exhibited 34% and 43% AChE inhibition at concentrations of 1 mg/mL, respectively. However, AChE inhibitory activities of various herbal sources have also been reported in the literature, including *Morus* spp. (Temviriyanukul et al., 2020) and *Euterpe* sp. (ALNasser et al., 2022).

3.4. Microbiological analysis results

Microbiological analysis results of ice cream samples are given in Table 4. Coliform bacteria counts of ice cream samples were determined to be < 1 log CFU/g. Yeast and mold counts ranged from 2.17 ± 0.17 log CFU/g to 4.28 ± 0.63 log CFU/g, and total aerobic mesophilic bacteria counts ranged from 3.54 ± 1.24 log CFU/g to 4.84 ± 0.76 log CFU/g. According to the statistical analysis results, it was seen that the addition of *A. melanocarpa* and *S. nigra* L. extracts did not have an effect on the counts of coliform bacteria, yeast and mold, and total aerobic mesophilic bacteria ($p > 0.05$). The results obtained are similar to those reported by Ahmed and El-Zubeir (2015) and higher than those reported by Espinoza et al. (2020). The microbial load of ice cream may vary depending on factors such as raw materials, the surface of the equipment, the environment of the room in which it is made, and the storage period. There are no provisions in TS 4265 (Turkish standard for milk-based ice cream) (Turkish Standards Institution, 2013) regarding the counts of coliform bacteria, yeast and mold, and total aerobic mesophilic bacteria.

Table 4. Microbiological analysis results of ice cream samples.

Sample		Coliform bacteria (log CFU/g)	Yeast and mold (log CFU/g)	Total aerobic mesophilic bacteria (log CFU/g)
Control sample	P	< 1	2.17 ± 0.17[a]	3.54 ± 1.24[a]
Aronia melanocarpa	A-0.5	< 1	3.55 ± 0.30[a]	4.47 ± 2.00[a]
	A-1	< 1	3.27 ± 1.24[a]	4.43 ± 0.04[a]
	A-1.5	< 1	2.78 ± 2.50[a]	4.84 ± 0.76[a]
Sambucus nigra L.	S-0.5	< 1	3.29 ± 0.39[a]	3.81 ± 2.57[a]
	S-1	< 1	2.35 ± 0.33[a]	5.22 ± 0.75[a]
	S-1.5	< 1	4.28 ± 0.63[a]	4.21 ± 2.45[a]
Sig		-	ns	ns

Sig: significant; ns: not significant; P: control ice cream; A-0.5: ice cream with 0.5% *A. melanocarpa* extract; A-1: ice cream with 1% *A. melanocarpa* extract; A-1.5: ice cream with 1.5% *A. melanocarpa* extract; S-0.5: ice cream with 0.5% *S. nigra* L. extract; S-1: ice cream with 1% *S. nigra* L. extract; S-1.5: ice cream with 1.5% *S. nigra* L. extract.

3.5. Sensory evaluation

Sensory analysis results of ice cream samples are given in Table 5 and Figure 1. According to the statistical analysis results, it was observed that *A. melanocarpa* and *S. nigra* L. extracts added at different rates did not have a significant effect on the color, smell, taste, or general acceptability of the ice cream samples ($p > 0.05$), but had an effect on the texture properties at the level of $p < 0.01$. While the least liked sample in terms of texture was the control sample, the most liked samples were those with 1.5% *A. melanocarpa* and *S. nigra* L. extract added. As the extract concentration increased, texture scores also increased.

3.6. Principal component analysis (PCA)

The PCA results of the physicochemical, microbiological, functional, and sensory properties of ice cream samples enriched with *A. melanocarpa* and *S. nigra* L. extracts are presented in Figure 2. According to the PCA results, the first two principal components (72.4% and 12%) accounted for 84.4% of the variance. According to the dendrogram graph, the ice cream samples were basically divided into two groups as the control group and extract-added samples, and the extract-added samples were grouped as sample A-1.5 and other samples (Figures 2A, 2B, and 2D). This highlights the significant differences between the samples in terms of analyses. As can be seen from the loading scatter plot graph (Figure 2C), protein, fat, pH, $ABTS^{\bullet+}$, and DPPH• were clustered in the upper left plot, while first dripping time, complete melting time, α-amylase, α-glucosidase, total aerobic mesophilic bacteria, color, taste, smell, and overall acceptability parameters were clustered in the upper right plot. All other analyses clustered in the lower right plot of the graph. This indicates that there are positive correlations between the analyses clustered together. When the biplot graph is analyzed, the fact that the parameters clustered in the upper left plot and the control group samples are together indicates higher values for these parameters (pH, protein, fat, DPPH• IC_{50} and $ABTS^{\bullet+}$ IC_{50}). However, it is observed that the A-1.5 sample is superior in terms of the analyses (the first dripping time, complete melting time, overrun, TPC, TFC, TAC, α-amylase, α-glucosidase, AChE, total aerobic mesophilic bacteria, color, smell, taste) on the same axis. The stronger radical scavenging activity of *A. melanocarpa* compared to other fruit species is due to its strong antioxidant capacity associated with its high polyphenol content. Proanthocyanins account for 40%, anthocyanins 24%, hydroxycinnamic acids 18%, and epicatechin 11% of the in vitro antioxidant capacity in *A. melanocarpa* (Jurendić and Ščetar, 2021). Platonova et al. (2021) reported that *A. melanocarpa* extracts reduce the risk of insulin resistance by regulating systems involved in glucose metabolism in the body, including α-glucosidase, DPP-IV, Gys1, and Gsk3β components, in addition to PI3K/protein kinase B and insulin/IGF-1 pathways. However, *S. nigra* L. fruit and extracts are reported to be rich in antioxidant compounds such as anthocyanins (including 3-O-sambubioside and cyanidin 3-O-glucoside), catechin, quercetin 3-rutinoside, chlorogenic acid, and gallic acid (Liu et al., 2022). In addition, it has been reported that *S. nigra* L. extracts exhibit antidiabetic activity as a result of α-amylase and α-glucosidase inhibition, provided by increased glucose and oleic acid absorption in skeletal muscle cells through anthocyanins and procyanidins (Ho et al., 2017). In this context, it can be said that the use of both *A. melanocarpa* and *S. nigra* L. extracts in ice cream production contributes positively to antioxidant and antidiabetic activities through their bioactive compounds and also affects various quality criteria. Therefore, especially in the treatment of metabolic diseases, products containing these fruit extracts may provide a new approach as an alternative treatment.

Table 5. Sensory evaluation results of ice cream samples.

Sample		Color	Smell	Taste	Texture	Overall acceptability
Control Sample	P	2.74 ± 0.97^{a}	2.44 ± 0.45^{a}	2.61 ± 0.29^{a}	1.86 ± 0.28^{d}	2.29 ± 0.21^{a}
Aronia melanocarpa	A-0.5	2.93 ± 1.05^{a}	2.88 ± 0.41^{a}	2.69 ± 0.62^{a}	2.30 ± 0.11^{bcd}	2.66 ± 0.13^{a}
	A-1	2.62 ± 0.37^{a}	2.23 ± 0.23^{a}	2.62 ± 0.56^{a}	2.78 ± 0.17^{ab}	3.98 ± 0.26^{a}
	A-1.5	3.44 ± 0.18^{a}	3.23 ± 0.08^{a}	3.58 ± 0.04^{a}	3.19 ± 0.23^{a}	3.37 ± 0.12^{a}
Sambucus nigra L.	S-0.5	2.35 ± 0.29^{a}	2.29 ± 0.37^{a}	3.01 ± 0.30^{a}	2.18 ± 0.10^{cd}	2.46 ± 0.33^{a}
	S-1	2.62 ± 0.37^{a}	2.70 ± 0.30^{a}	2.55 ± 0.43^{a}	2.63 ± 0.18^{bc}	2.47 ± 0.54^{a}
	S-1.5	2.33 ± 0.33^{a}	2.66 ± 0.52^{a}	2.49 ± 0.25^{a}	3.26 ± 0.30^{a}	2.89 ± 1.09^{a}
Sig.		ns	ns	ns	**	ns

Sig: significant; ns: not significant; **: $p < 0.01$; P: control ice cream; A-0.5: ice cream with 0.5% *A. melanocarpa* extract; A-1: ice cream with 1% *A. melanocarpa* extract; A-1.5: ice cream with 1.5% *A. melanocarpa* extract; S-0.5: ice cream with 0.5% *S. nigra* L. extract; S-1: ice cream with 1% *S. nigra* L. extract; S-1.5: ice cream with 1.5% *S. nigra* L. extract.

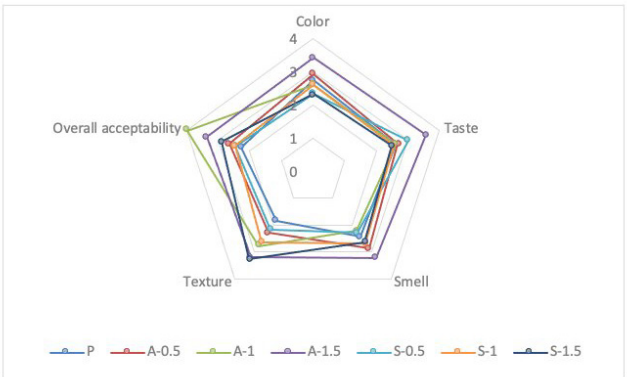

Figure 1. Sensory evaluation results of ice cream samples.

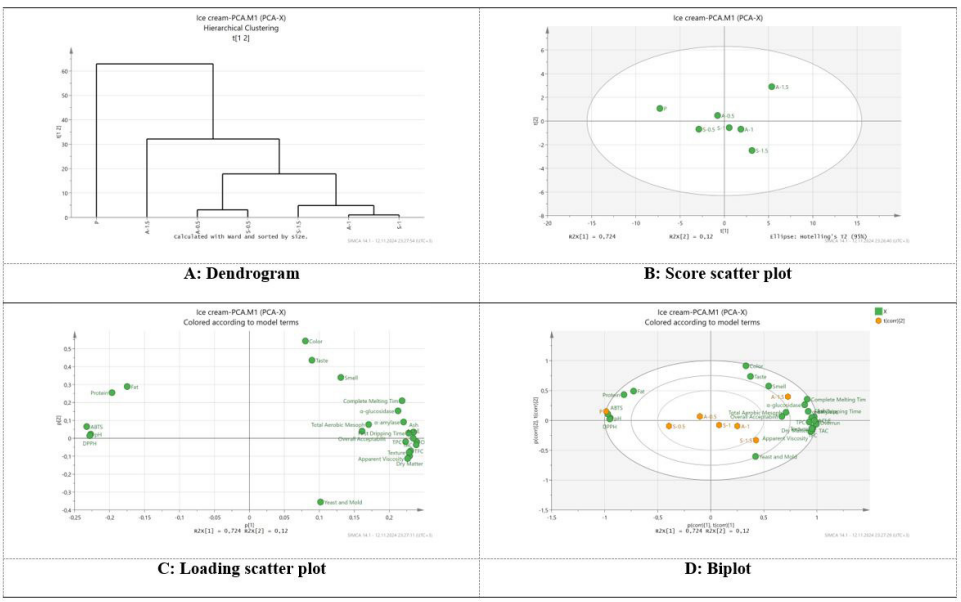

Figure 2. Principal component analysis (PCA) results of ice cream samples.

4. Conclusion

The results obtained showed that adding different amounts of *A. melanocarpa* and *S. nigra* L. extracts to ice cream mixtures significantly affected some physicochemical and sensory properties of the ice cream samples. Additionally, DPPH• IC_{50}, $ABTS^{•+}$ IC_{50}, TPC, TFC, TAC, antidiabetic, and anti-Alzheimer properties were affected by the addition of the extract. The addition of the extract positively affected the DPPH• IC_{50} and $ABTS^{•+}$ IC_{50} values of the ice cream samples and also increased the TPC, TFC, and TAC values. *A. melanocarpa* extract was more effective on DPPH• IC_{50}, $ABTS^{•+}$ IC_{50}, TPC, TFC, and TAC values of the ice cream samples than *S. nigra* L. extract at the same rate. This study showed that *A. melanocarpa* and *S. nigra* L. extracts can be used to obtain functional ice cream without negatively affecting its sensory properties.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Murat Emre TERZİOĞLU: Conceptualization, methodology, validation, formal analysis, investigation, resources, writing – original draft, writing – review and editing, visualization, supervision.

Emine MACİT: Investigation, resources, writing – original draft, writing – review and editing.

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