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Physicochemical, Nutritional, and Antioxidant Properties of Ice Cream Enriched with Red Beetroot (*Beta vulgaris* **L.) at Varying Sucrose Levels**

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Introduction

Ice cream, a universally cherished dessert, is often scrutinized for its high sugar and fat content, contributing to its limited nutritional value. Recent trends in food science have focused on enhancing the nutritional profile of such indulgent foods by incorporating functional ingredients. One promising candidate for this purpose is RB (*Beta vulgaris* L.), renowned for its rich composition of bioactive compounds, including phenolics and antioxidants (Sakr et al., 2023).

Beetroot's potential to improve health outcomes has been well-documented. Its consumption is linked to various benefits such as reduced blood pressure, improved vascular function, and enhanced exercise performance due to its high nitrate content and potent antioxidant properties. These attributes make RB ideal for enriching foods traditionally viewed as less healthy (Clifford et al., 2015).

RB can be consumed raw, juiced, baked, or boiled. Red beets are delicious when roasted, pickled, added to salads, or made into soup, a popular dish in many Eastern and Central European countries. Unlike fruits, the primary sugar in RB is sucrose. (Babarykin et al., 2019).

Incorporating RB into ice cream not only aims to boost its nutritional value but also to exploit its natural colorant properties. Studies have shown that natural colorants from fruits and vegetables can enhance the visual appeal of foods while providing health benefits (Chen et al., 2021).

Additionally, the phenolic compounds in RB may improve the antioxidant capacity of ice cream, potentially offering a functional dessert option (Georgiev et al., 2010).

Previous research on functional ice creams has explored various additives, including fruits, nuts, and other plant-based ingredients, to improve their health benefits. For instance, the inclusion pomegranate peel extract and seed oil has enhanced the antioxidant activity and phenolic content of ice creams (Çam et al., 2013). Similarly, studies involving other fruit extracts have demonstrated significant improvements in the nutritional profiles of ice creams without compromising sensory qualities (Cakmakçı et al. 2016; Çakmakçı et al. 2015; Erkaya et al. 2012; Gürpınar et al. 2022; Kavaz Yuksel 2015).

Conventional ice cream formulations are considered to have high concentrations of sugar and fat, which are the compounds that provide the caloric content in the ice cream. However, increasing concerns about health and nutrition have given an impulse to the market of reducedfat and low-calorie products, as the intake of such products decreases the risk of obesity and cardiovascular diseases (Samakradhamrongthai et al., 2021). Targeting these demands, the food industry has been seeking alternative ingredients without significant modifications in conventional food characteristics, such as texture, flavor, and aroma (Santos and Silva, 2012).

This research aims to develop a functional ice cream with dietary benefits, enriched with antioxidants, reduced sucrose content, and an appealing color and aroma. The goal is to create a product suitable for diabetics, maintaining the original ice cream flavor while offering attractive sensory qualities, and making it appealing to children. The incorporation of RB's antioxidant and fiber content is intended to enhance the nutritional value, producing a healthier food option.

Materials and Methods

Materials

In this research, the ultra high temperature (UHT) milk and skim milk powder (Pınar Dairy and Products Inc., İzmir, Türkiye), stabilizer (salep), emulsifier (mono- and diglycerides of fatty acids), powdered sucrose (Konya Sugar Industry and Trade Inc., Türkiye), and cream (35% fat, Mis, Ak Gida Company, Türkiye) were obtained from Gümüşhane. The red beet used in production was freshly obtained from a local grocer in Gümüşhane.

Preparation of Mixes and Production of Ice Creams

Before the ice cream production, preliminary sensory trials were conducted by faculty members and students of the Department of Food Engineering at Gümüşhane University to determine RB and sucrose ratio. The RB was added in different production methods at raw, pasteurized, prematuration mix, and post-maturation mix stages at concentrations of 5%, 7.5%, 10%, 12.5%, and 15%. At the end of the trial, a 15% ratio and pasteurization together with the mix were selected. Based on the sensory analysis results, four types of ice cream were produced in this study: The first three batches were produced with 15% RB and addition of 5%, (RB5) 10% (RB10) and 15% (RB15) sucrose, respectively. Ice cream produced without the addition of RB containing 15% sucrose was considered as control.

All mixtures were prepared to contain 0.22% emulsifier, 0.77% stabilizer, and 5% fat. The mixture was heated to 80°C, after which raw RB, peeled and processed with a hand chopper, was added. It was homogenized with an electric mixer and pasteurized for 10 minutes. The mixture was then rapidly cooled to 25-30°C in ice baths and transferred to approximately 5-liter sterile plastic containers with lids, where it was matured at 4°C for 24 hours. After maturation, the mixture was churned into ice cream at -5°C (Uğur Refrigeration Machines Inc., Nazilli, Turkey), hardened for one day at -22°C, and stored at - 18°C Fig 1a and 1b.

Physicochemical Parameters

The dry matter and ash content of ice cream samples were determined gravimetrically. Fat content was measured by the Gerber method, pH values with a pH meter (HANNA HI2202-02), and titration acidity (lactic acid %) using 0.1 N NaOH and phenolphthalein. Protein content was determined by the Kjeldahl method (Kurt et al., 2015).

For the analysis of overrun, the change in the volume of the mix during the ice cream production is measured. It is calculated by using the ice cream weight in a certain volume and the mix weight in the same volume, using the formula below (Daw and Hartel, 2015).

$$
0 \text{verrun}\% = \frac{\text{WM}-\text{WIC}}{\text{WIC}} \times 100 \tag{1}
$$

WM : Weight of mix (g) WIC : Weight of ice-cream(g)

Fort the first dripping and complete melting times, 50 g ice cream samples were placed on a wire mesh (0.9 mm) and 250 mL beakers were placed beneath and left to melt for 60 minutes at room temperature (Zor et al. 2022). The first dripping and complete melting times of the ice cream samples were recorded in seconds (Güven and Karaca, 2002).

The viscosity of ice cream samples was measured with J. P Selecta, s.a. (Spain) device at 20 rpm. The averages of the readings were taken and the results were given as cP (Kurt et al., 2015).

Color was measured with a Konica Minolta colorimeter (Chroma Meter, CR-400, Japan), and *L**, *a**, and *b** values were averaged from different points on each sample (Çelik et al., 2009). According to the CIELAB color scale, *L** values show brightness on the (Y) axis ($0 =$ black, $100 =$ white) (darkness/lightness), while the *a** value shows red intensities between $+60$ and 0 and green intensities between 0 and -60 on the (X) axis (+a red, $-a$ green), and the b^* value shows yellow intensities in the range of $+60$ and 0 and blue intensities in the range of 0 and −60 on the (Z) axis (+b yellow, -b blue).

Figure 1. Ice cream made with the addition of RB

Determination of Sugar Content

A sulfonated polystyrene-divinyl benzene cation exchange HPLC column was utilized, characterized by a particle size of 10 μ m, a length of 30 cm, and an internal diameter of 6.5 mm in order to determine sugar content. A non-sterile hydrophilic syringe filter with a pore size of 0.45 µm and a centrifuge with a centripetal acceleration capacity of 1400 g were used.

For the analysis, 2.5 g of the ice cream sample was weighed, and 20 mL of pure water was added. After homogenization, the mixture was transferred to a volumetric flask and made up to 50 mL. It was then incubated in a water bath for 15 minutes, filtered first through filter paper and then through a 0.45 µm filter.

Standard sugar solutions were injected into the device, and retention times were determined. Standard solutions were regularly injected after the analysis samples using the mixture of standards. Sugar concentrations were determined using the external standard method, based on peak areas and peak heights. The relationship between dilution factor and mass or volume values was considered during calculation. The mass concentration of the sugars was calculated as follows (Anonim, 2001).

$$
W=A_1\times V_1\times m_1\times 100/A_2\times V_2 \times m_0 \tag{2}
$$

In this equation, A_1 is the peak area or peak height of the given sugar compound in the sample solution, expressed as units of area, length or integration, A_2 is the peak height of the given sugar compound in the standard solution expressed as unit of area, length or integration, V_1 is total volume of the sample solution in mL, V_2 is total volume of the standard solution in mL, m_1 is mass amount of the sugar in grams in the total volume of the standard (V_2) and m_0 is sample weight in g.

The Extraction of Bioactive Compounds from RB

A 25 grams of RB was weighed and added 10 mL of distilled water and 5 mL of ethanol. The mixture was placed in an ultrasonic water bath (Bandelin, Sonorex) for 20 minutes. The mixture wasthen filtered through Whatman no:1 filter paper and a 0.45 µm filter, and the extract was collected in vials. This extract was used to determine TPC, and DPPH free radical scavenging activity, using the same procedure applied to ice cream samples.

Total Phenolic Content

The extract $(300 \mu L)$ was mixed with 3.4 mL deionized water, 0.5 mL methanol, and 200 μ L folin– ciocalteureagent, then vortexed and incubated for 10 minutes. After adding $600 \mu L$ of 10% Na₂CO₃ and vortexing again, the mixture was incubated in the dark for 120 min. Absorbance was measured at 760 nm, with results expressed as mg Gallic Acid Equivalent (GAE) mg/kg (Kasangana et al., 2015).

DPPH Radical Scavenging Activity

A 100 µL of the extract was mixed with 3000 µL DPPH solution, vortexed, and incubated for 30 minutes. Absorbance was measured at 517 nm, with results expressed as inhibition capacity percentage (Ahmed et al., 2015).

Statistical Analysis

ANOVA was employed to analyze the statistical significance between group means for all data. The obtained results were subjected to variance analysis in the SPSS 22 (2013) program. Duncan Multiple Comparison Test was applied to the means that were significant as a result of the test. All experiments were performed in at least duplicate, and the results were expressed as mean ± standard deviation.

Results

Characteristics of Raw Materials Used in Ice Cream Production

Some physicochemical properties of raw materials (milk, cream and RB) used in ice cream production were given in Table 1.

Physicochemical Parameters

Table 2 presents the pH, LA, dry matter, and ash content of various ice cream samples. The pH remained consistent across samples (6.46-6.50), while LA was highest in the control (0.21% lactic acid) and lower in RB samples (0.13- 0.14%). These consistency contrasts with findings in the literature, where the addition of various fruits to dairy products often leads to different effects on pH due to the specific fruit's composition. For instance, studies by Dölek (2012) and Aliyev (2006) showed that adding blueberry extract to ice cream and kefir resulted in pH reduction, attributed to the lower pH of the fruit itself. Similarly, in another study, the addition of molasses also led to decreased pH values in ice cream due to the lower pH of molasses compared to the ice cream mix (Temiz and Yeşilsu 2010). Other studies have observed pH variations with different additives, such as microbial transglutaminase, which had negligible effects on pH (Göncü, 2012). These discrepancies highlight that the pH impact of fruit additions can vary significantly based on the fruit's inherent properties and the composition of the ice cream base.

C:15% sucrose added plain ice cream; RB5: 5% sucrose + 15% RB added ice cream, RB10: 10% sucrose + 15% RB added ice cream; RB15: 15% sucrose + 15% RB added ice cream; ** The results are statistically significant at p<0.01; * The results are statistically significant at p<0.05

This finding contrasts with other studies where the addition of fruit extracts, such as pomegranate peel phenolics and pomegranate seed oil, increased the total acidity of ice creams to a range of 0.15-0.26%, with higher concentrations leading to higher acidity (Çam et al., 2013). Patel et al. (2006) reported that ice creams with added whey protein and milk protein had LA values between 0.19% and 0.22%, with increased protein content leading to higher acidity. These differences highlight that the impact of fruit and other additives on the acidity of ice cream can vary significantly based on their inherent properties and concentrations used.

The control had the highest dry matter (33.70%), followed by RB15, RB5, and RB10, respectively, indicating that dry matter decreased with RB addition but increased at the highest sugar level. This data aligns with literature stating that varying sugar levels significantly impact dry matter content in ice cream. For instance, studies have shown that increased sugar content enhances dry matter, crucial for ice cream texture and stability (Guinard et al., 1997). However, the addition of RB decreased in dry matter contents, with the lowest content observed in RB10. This was possibly due to the lower dry matter contribution of RB compared to sugar. In contrast to this situation, it was reported that pomegranate peel and seed oil did not show a significant difference in dry matter values depending on the amount of additive and ranged between 35.84% and 38.18% (Çam et al., 2013). These variations highlighted the complexity of ingredient interactions in ice cream formulations, where the type and amount of additives like sugar and fruit components can significantly influence the final product's dry matter content.

The ash content varied slightly, being highest in RB5 (0.95%) and lowest in RB15 (0.82%), with the control at 0.91%. This finding is in agreement with findings in the literature, such as the study by Bajwa et al. (2003), where the addition of strawberry pulp increased the ash content in ice creams from 0.616% in the control to 0.881% in samples with 25% strawberry pulp. Similarly, Arslaner and Salık (2017)) observed increased ash content in ice creams with dried mulberry powder and walnut paste. These comparisons indicate that the addition of certain ingredients can affect the mineral content (ash) of ice cream, with variations depending on the specific additives and their proportions.

Table 3 reveals first dropping time, complete melting time and viscosity values of ice creams**.** The controlsample (C) had the highest overrun (39.18%), indicating better air incorporation, whereas RB samples (RB5, RB10, RB15) show lower overrun (23.08-27.82%). As the sugar content increases, viscosity decreases, leading to an increase in overrun. This occurs because the reduced viscosity enables higher mixing speeds, facilitating greater air incorporation

into the ice cream (Nateghi and Paidari, 2022). Our results are consistent with the findings of Güven and Karaca (2002), who reported that overrun increases with higher sugar content in yogurt ice creams. Similarly, Singh et al. (2018) observed a decrease in overrun with increased fruit content, which aligns with our observation that adding RB reduces the overrun. Iqbal et al. (2022) also found that increasing kiwi fruit puree decreased overrun, highlighting the impact of fruit content on ice cream's overrun. Additionally, Şimşek and Gün (2021) noted that the dry matter content varies with the ingredients used and processing conditions, further explaining the variations in overrun observed in our study. Our results are consistent with these findings, demonstrating that RB addition decreased the overrun due to its impact on the ice cream's dry matter and composition.

The first dropping time was significantly longer for RB5 and RB10 (2475 and 2970 s) compared to the control (595 s), indicating improved melting resistance, while RB15 had a shorter time (1170 s). As seen in Table 3, the complete melting times for RB-added ice cream samples were significantly longer than for the control sample. Complete melting time followed a similar pattern, with RB5 and RB10 taking longer to melt (7845 and 6105 s) compared to the control (2700 s), indicating better structural integrity, while RB15 melts slightly faster (5820 s). This supports the statement that RB addition delays the first dropping time. The delayed melting in RB-added samples may be attributed to an initial increase in waterholding capacity due to RB and sugar, followed by a decrease as sugar content increases. This observation is consistent with Aykan (2001), who found that adding maltodextrin and oligofructose, both with high waterholding capacities, delayed the first melting rate compared to control samples. This aligns with Kuşçu (2015), who found that full melting times for ice creams with varying stevia levels ranged from 3810 to 4650 seconds, with the longest in the 100% stevia sample. The delayed melting in RB-added samples is likely due to increased water-holding capacity and sugar content effects, similar to stevia's impact. Rossa et al. (2012) found higher fat content and microbial transglutaminase as well as slow melting. Our study supports these findings, showing that RB addition prolongs full melting time due to its impact on the ice cream's composition.

Viscosity at 20 rpm and 50 rpm was highest for RB5, suggesting a thicker consistency, while the control and other RB samples had lower viscosity, indicating a smoother texture. Overall, RB improved melting resistance and thickness but reduced overrun in ice cream. The viscosities of the RB-enhanced ice cream samples at 20 rpm and 50 rpm were higher than the control sample (20 rpm: 9508.61, 50 rpm: 5090.46).

Samples	Overrun $(\%)$	First dropping time (s)	Full melting time (s)	Viscosity cP.	
				20 rpm	50 rpm
C	39.18 ± 1.10^a	595.00 \pm 5.77 ^d	$2700.00\pm0.00^{\circ}$	9508.61 \pm 331 ^{bc}	5090.46 ± 89 °
R _{B5}	24.81 ± 2.83 ^{bc}	$2475.00\pm75.49^{\circ}$	7845.00±79202 ^a	11729.58 ± 20082 ^a	6425.60 ± 10371 ^a
RB10	23.08 ± 2.97 °	$2970.00\pm31176^{\circ}$	$6105.00\pm26095^{\rm b}$	9575.30 ± 31.87^b	5308.59 ± 16492^b
RB15	$27.82\pm4.04^{\circ}$	$1170.00\pm32680^{\circ}$	$5820.00\pm41856^{\circ}$	9227.69 ± 63.69 °	5285.85 ± 3.84^b
$C = 1.50$ 11.11 11.71					

Table 3. The physical analysis results of ice cream samples and variance analysis results of these data

C:15% sucrose added plain ice cream; RB5: 5% sucrose + 15% RB added ice cream, RB10: 10% sucrose + 15% RB added ice cream; RB15: 15% sucrose + 15% RB added ice cream; ** The results are statistically significant at p<0.01; * The results are statistically significant at p<0.05

This observation supported the statement that increasing sugar ratios reduced viscosity in RB-enhanced samples. This can be due to sugar content of RB. Similar findings were observed when different concentrations of sugar and white mulberry juice was added to ice cream as a sugar substitute (Nateghi and Paidari 2022). The authors stated that the hydroxyl groups in the juice form stronger hydrogen bonds with water molecules compared to sucrose, thereby increasing viscosity by restricting the mobility of free water. Marshall et al. (2003) emphasized the significant influence of pasteurization, homogenization, and maturation on viscosity. El‐Nagar et al. (2002) found increased viscosity with higher inulin content in yogurt ice creams, similar to the impact of RB in our study. Kuşçu (2015) discovered that 100% stevia resulted in the lowest viscosity, while 100% sugar resulted in the highest, demonstrating that increasing stevia reduces viscosity due to its lower volume and inherent viscosity compared to sugar. Our findings align with these studies, showing that RB and sugar ratios significantly affect ice cream viscosity.

Figure 2 illustrates the color parameters of different ice cream samples. The control sample (C) had the highest lightness (87.6). In contrast, the samples with RB (RB5, RB10, RB15) had significantly lower *L** values (around 50), making them darker. The red/green value (*a**) was negative for the control (-2.35), indicating *a** slight greenish hue, whereas the RB samples had positive *a** values (ranging from 26.13 to 28.01), reflecting a strong red hue. The yellow/blue value (*b**) was highest in the control (7.81), suggesting *a** more yellowish color, and decreased in the RB samples, with RB15 having the lowest *b** value (8.94), indicating a reduced yellow component. This data indicated that adding RB significantly darkened the ice cream, enhanced the red hue, and slightly reduced the yellowish tint.

In terms of color, the *L** value, indicating lightness, decreased in RB-added samples, while increased sugar content did not significantly affect color (Figure 2). These findings align with Abdullah Kurt and Atalar (2018), who reported decreased lightness (*L**) and yellowness (*b**), and increased redness (*a**) in ice creams with added quince seed powder. Kavaz Yuksel (2015) similarly found reduced *L** and *b** values and increased *a** values with more hackberry fruit. Roland et al. (1999) observed increased *L** and *b** values but decreased *a** values over time with stored red beet juice concentrate, and higher fat content increased all three values. Hwang et al. (2009) reported that adding grape wine to ice cream reduced *L** and increased *a**, with the highest *b** at moderate wine concentrations. Overall, our study supports these observations, showing that RB addition affects ice cream's

viscosity and color properties, with higher sugar content reducing viscosity and altering color metrics, increasing redness and blueness while reducing lightness and yellowness.

Total Phenolic Content and the Scavenging Activity of the DPPH

The adding RB to ice cream significantly enhanced its antioxidant properties $(p<0.05)$ (Table 4). The control sample (C) had the lowest total phenolic content (TPC) at 329.69 mg GAE/kg and the lowest DPPH inhibition at 13.66%, reflecting minimal antioxidant capacity. In contrast, the RB samples (RB5, RB10, RB15) showed substantially higher TPC and DPPH values. RB5 had the highest TPC at 558.55 mg GAE/kg, followed by RB10 (494.08 mg GAE/kg) and RB15 (483.50 mg GAE/kg). Regarding DPPH inhibition, RB10 exhibited the greatest antioxidant activity at 27.98%, with RB5 and RB15 both at 25.16%. These results highlighted that RB significantly increased the antioxidant properties of ice cream, making it potentially more beneficial for health. The high TPC content in samples containing RB is attributed to the substantial TPC levels in RB (258.47±35.95). The decrease in TPC content as sugar levels increase in these samples is related to the reduced proportion of RB. A similar trend was observed in a study by Nateghi and Paidari (2022) where increasing sugar content in ice creams containing white mulberry juice led to a decrease in TPC levels.

These results consistent with Hwang et al. (2009) who found increased antioxidant capacity using grape sediment. Dölek (2012) demonstrated higher phenolic content in ice creams with blueberry extract, especially gellanencapsulated. Hassan and Barakat (2018) observed increased phenolic content and DPPH values with added pumpkin and carrot pulp. Our study aligns with these findings, showing that RB addition enhances TPC, indicating that incorporating fruit and vegetable extracts boosts phenolic content and antioxidant capacity in ice creams.

Sugar Content

Due to the high-calorie production of sugar in ice cream, today there is a great demand for low-calorie ice cream . Figure 3 shows the sugar content of different ice cream samples, highlighting the distribution of fructose, glucose, and sucrose. The fructose content in the RB-added samples was statistically higher than in the control sample. There was no significant difference between the RB5 and RB15 samples, but RB15 differed from RB10. The highest fructose content was observed in the RB5 sample, with fructose levels decreasing as the sugar ratio increased. This was due to the reduced amount of red beets and the

decreased proportion of fruit-derived sugar in the ice cream as the sugar content increased. Regarding glucose content, the control sample and RB15 were statistically similar but differed from RB5 and RB10, with the highest glucose content in RB5 and the lowest in the control sample. For sucrose content, the control and RB15 samples were statistically similar but differed from RB5 and RB10. This aligns with the study's objective, showing that higher added sugar increased sucrose levels. The control and RB15 samples had similar sucrose content due to a consistent 15% sucrose ratio. Temiz and Yeşilsu (2010) reported that total sugar, invert sugar, and sucrose levels increased in molasses-added ice creams with higher molasses ratios, which is consistent with our findings.

Conclusion

The study demonstrated that incorporating RB into ice cream significantly alters its physicochemical properties. The RB addition notably reduced the overrun and resulted in longer first dropping and full melting times. Despite increased sucrose content generally reducing viscosity, RB's presence counteracted this, leading to higher viscosities. Color analysis showed that RB addition darkened the ice cream and shifted its hue towards red, with higher sugar levels intensifying these effects. Additionally, RB-enriched samples exhibited the highest TPC, underscoring the antioxidant enhancement provided by RB. These findings collectively highlight the potential of RB to improve both the stability and nutritional profile of ice cream.

 $C:15\%$ sucrose added plain ice cream; RB5: 5% sucrose + 15% RB added ice cream, RB10: 10% sucrose + 15% RB added ice cream; RB15: 15% sucrose + 15% RB added ice cream; ** The results are statistically significant at p<0.01; * The results are statistically significant at p<0.05

Figure 3. Sugar content of ice-cream samples

Declarations

Author Contribution Statement

Firdevs HACIBEKTAŞOĞLU: Data collection, investigation, formal analysis, and writing the original draft

Engin GÜNDOĞDU: Supervision, conceptualization, methodology, review and editing, writing the original draft

Conflict of Interest

The authors declare no conflict of interest.

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