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# Nutritional profile of probiotic goat yoghurt ice cream enriched with iron fumarate and pomegranate seed powder

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## Abstract

Dairy products can be fortified using various methods to address iron deficiency and improve their functionality. Lactose, a component of milk, inhibits iron absorption; however, when milk is fermented into yoghurt, lactose is converted to lactic acid, which enhances iron absorption. However, due to their physicochemical and structural properties, dairy products are considered suitable food matrices for the transport and integration of bioactive components of fruits or fruit by-products. Pomegranate seeds, considered waste after fruit processing, can be ground and incorporated into various products to exploit their high nutritional content. In this context, within the scope of the present study, yoghurt ice cream was produced using ABT-2 probiotic yoghurt culture (*Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Streptococcus thermophilus*), iron source (iron fumarate, IF), pomegranate seed powder (PSP, at 1 % and 3 % concentrations) and goat milk. The use of IF and PSP in the production of probiotic yoghurt ice cream had a statistically very significant effect ($p<0.01$) on dry matter, pH, titratable acidity, Mg, P, Ca, Fe, Zn, DPPH trolox equivalent, and ABTS radical scavenging activity values, as well as on the numbers of *L. acidophilus* LA-5, *B. animalis* subsp. *lactis* BB-12, and *S. thermophilus*. The addition of PSP was found to increase dry matter and titratable acidity values as well as antioxidant capacity, while decreasing Na, Mg, P, and Ca values. Compared to the control group, the combined use of IF and PSP was shown to increase dry matter, titratable acidity, Na, Mg, P, Ca, and antioxidant capacity. On the other hand, Fe and Zn content was determined only in samples with added IF. In conclusion, this study showed that the use of IF in combination with PSP inhibited iron enrichment, but this combination had positive effects on some properties. These by-product utilization and component enrichment strategies may contribute to the development of innovative and sustainable dairy products.

**Keywords**: probiotic yoghurt ice cream; iron fumarate; pomegranate seed powder; waste recycling; innovative and sustainable products

# Introduction

Probiotic dairy products, classified as functional foods, have attracted attention in recent years owing to their consumption and beneficial effects on health (Turgut and Diler, 2023). Dairy products are known to be highly effective matrices for the uptake of probiotic microorganisms into the body via the gastrointestinal tract (Talearngkul et al., 2023). Goat milk and yoghurt, which act as good carriers of probiotic microorganisms in the body, also have a rich nutritional content and high buffering capacity, which positively affects the growth of probiotic microorganisms (Terzioğlu and Bakirci, 2024). The increasing scientific evidence and consumer awareness regarding the positive effects of probiotics on health have led to the idea of expanding the range of probiotic products by integrating yoghurt into ice cream, in addition to fermented products like traditional yoghurt (Latif et al., 2023; Jang et al., 2024). These innovative approaches increase the potential for functional products in the ice cream industry, while preventing ice cream from being regarded solely as a dessert (Mohammed et al., 2022; Rashwan et al., 2023). In this context, probiotic ice cream retains the taste, flavor, and texture characteristics expected from traditional ice cream, while also possessing functional properties owing to probiotics (Hadjimbei et al., 2022). Similar to yoghurt, the composition, structure, and texture of ice cream allow for the integration of beneficial ingredients, thereby increasing its potential as a health and nutritional component of the daily diet (Genovese et al., 2022; Mohammed et al., 2022). On the other hand, yoghurt, which can be enriched with different probiotic microorganisms when needed and is an important source of calcium, protein, vitamins, and lactic acid bacteria, is a food poor in iron and phenolic compounds (phenolics, anthocyanins, and flavonoids). The lack of these compounds weakens yoghurt's ability to exhibit anti-obesity, anti-diabetes, and anti-cancer properties (Rashwan et al., 2023).

Iron deficiency, which affects approximately 2 billion people worldwide, is one of the most significant nutritional problems. Although red meat is considered a good source of iron, it is not a sufficient solution on its own because of factors such as population, cost, and dietary habits (Hamad and Singh, 2025). This situation demonstrates the importance of iron-fortified foods and supplements in the food industry (Kumari and Chauhan, 2022). Choosing the correct form of iron is crucial for eliminating health problems caused by iron deficiency, particularly anemia, and for improving the effectiveness of iron in foods (Camaschella, 2015; Cardoso et al., 2019). Iron supplements such as iron fumarate, iron sulfate, and iron gluconate are frequently preferred for iron deficiency anemia (Cancelo-Hidalgo et al., 2013). Iron fumarate, which stands out among iron supplements for its effectiveness, is sparingly soluble in water but readily dissolves in the dilute acid of gastric juice during digestion and is considered to have similar bioavailability to iron sulfate (Moretti et al., 2006).

Pomegranate seeds, obtained from the pomegranate (*Punica granatum* L.) fruit and constituting approximately 3-20 % of the fruit weight, are an important byproduct due to their bioactive component profile and are generally considered waste by businesses today (Mahesar et al., 2019; Noreen et al., 2025). However, pomegranate seeds are a concentrated source of pomegranate fatty acids (oleic, linoleic, stearic, punic, and palmitic acids) and phenolic compounds (ellagic, punicalagin, and gallic acids), which exhibit antidiabetic, antioxidant, anticancer, and anti-inflammatory effects (Khemakhem et al., 2021; Cairone et al., 2023; Cheng et al., 2023).

In light of all this information, the literature contains studies using pomegranate seed powder (PSP) in dairy products (Elaltunkara, 2018; Akca and Akpinar, 2021), as well as some studies enriching them with iron sources (Gutiérrez et al., 2016; Ghorbani et al., 2023). On the other hand, no studies have been found that show PSP and iron fumarate (IF) being used together in the production of probiotic goat yoghurt ice cream. In the study, four groups of probiotic ice creams were produced to observe the effect of each component: control, with added IF, with added PSP, and with both added IF and PSP. The main reason for choosing yoghurt ice cream as the product in the present study was to provide the dairy industry with an innovative alternative product by combining traditional yoghurt and ice cream into a single product using an integrated approach. In addition to goat milk, which is increasingly used, the production of probiotic yoghurt ice cream has been targeted by using *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12, which exhibit probiotic properties and help improve the functional properties of the product. On the other hand, the aim was to improve the nutritional and health aspects of the product by using PSP, a byproduct rich in iron mineral (IF) and bioactive components, which are found in limited amounts in dairy products. The physicochemical properties, antioxidant activity, mineral profile, and microbiological counts of the probiotic yoghurt ice cream samples were analyzed to reveal their nutritional profile and health benefits.

# Materials and methods

## Materials

The goat milk used as a raw material in production was sourced from local farms in Erzurum (Türkiye), while the pomegranate, salep, emulsifier, and sugar were purchased from local markets in Erzurum (Türkiye). ABT-2 probiotic yoghurt culture (*Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Streptococcus thermophilus*) was obtained from CHR-Hansen (Istanbul, Türkiye), and iron (II) fumarate (IF, $FeC_4H_2O_4$; ≥97 %) was obtained from Roth (İstanbul, Türkiye).

## Methods

### *Preparation of pomegranate seed powder (PSP)*

The pomegranate seeds were carefully removed from the washed fruit, and then dried at room temperature overnight. The dried seeds were then ground into powder using a grinder (Kiwi KSPG-4820, Türkiye).

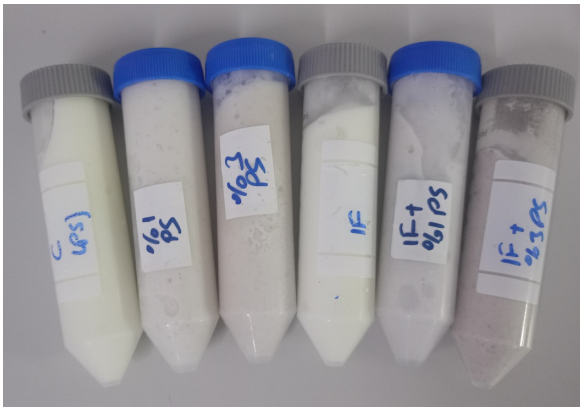


*Figure 1. Probiotic yoghurt ice cream enriched with iron fumarate and pomegranate seed powder*

### *Probiotic yoghurt ice cream production*

Raw goat milk, placed in stainless steel containers, was mixed with 5 % cream at 50 °C, and then pasteurized at 90 °C for 10 minutes. The milk was then cooled to 60 °C and mixed with 2 % milk powder, 0.7 % salep, 0.3 % emulsifier, and 15 % sugar. After all ingredients were thoroughly mixed and the mixtures were cooled to 45 °C, IF (30 mg/kg) and PSP (1 % and 3 %) were added. The proportions of PSP added were adjusted according to preliminary trials conducted based on previous studies on enriching dairy products with various additives (Akalın et al., 2018; Ahmed et al., 2022). The mixtures were then cooled to 37 °C and ABT-2 probiotic culture was added at a rate of 30 g/100 L (w/v) according to the Direct Vat Inoculation. Once the culture addition was complete, the samples were incubated in an oven until the pH reached 4.6. The incubated samples were left to mature at +4 °C for 24 hours. Once the maturation process was complete, the samples were frozen in a batch freezer. The prepared ice cream samples were then stored at -18 °C for one day and subsequently used in all analyses. The probiotic yoghurt ice creams produced are shown in Figure 1.

### *Physicochemical properties*

Dry matter, titratable acidity, and pH analyses were performed on probiotic yoghurt ice cream samples according to the methods described by Uruc et al. (2022).

### *Mineral profile*

10 mL of acid solution (8 mL $HNO_3$ (65 %) and 2 mL $H_2O_2$ (30 %)) and 150 mg of yoghurt ice cream were mixed and subjected to decomposition in a Milestone Connect Ethos Up microwave incineration device (1800 MW (w), 200 °C T1 and 200 °C T2 for 15 min each). Next, a stock solution was prepared using 5 mL of ultrapure water and 10 mL of sample, and both the blank solution and the samples were diluted fivefold with 2 % $HNO_3$ solution. Subsequently, the mineral profile was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7800) (Terzioğlu, 2026). The LOD (Limit of Detection) and LOQ (Limit of Quantification) values of the minerals examined in this study are presented in Table 1.

*Table 1. LOD (Limit of Detection) and LOQ (Limit of Quantification)*

| Minerals | LOD | LOQ |
|---|---|---|
| Na | 3.581 | 11.937 |
| Mg | 1.294 | 4.313 |
| P | 1.817 | 6.057 |
| K | 2.027 | 6.756 |
| Ca | 1.174 | 3.912 |
| Fe | 0.222 | 0.741 |
| Zn | 0.0657 | 2.191 |

### *Antioxidant activity*

ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity analyses of probiotic yoghurt ice cream samples were carried out according to the methods given by Terzioğlu (2026). Extracts were first prepared for the analyses. Accordingly, 3 g of ice cream sample was diluted to 30 mL with methanol, centrifuged (6000 rpm, 15 min), and extracts were obtained. For the analysis of ABTS free radical scavenging activity, an ABTS solution (7 mM) prepared with $K_2S_2O_8$ (2.45 nM) was used, and absorbance measurements were performed at a wavelength of 734 nm. For DPPH free radical scavenging activity analysis, DPPH solution (0.5 mL) was used, and absorbance measurements were performed at a wavelength of 517 nm.

### *Microbiological analyses*

Selective (1.5 g/L bile (bile salts, Sigma-Aldrich) and 0.5 g/L cysteine (L-cysteine, Sigma-Aldrich)) MRS agar (Oxoid Ltd.) was used to count *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12, and petri dishes were incubated at 37 °C for 72±1 hours under anaerobic conditions. For *S. thermophilus* counting, M17 agar (Oxoid Ltd.) was used, and petri dishes were incubated at 37 °C for 24±1 hours under aerobic conditions (Terzioğlu and Bakirci, 2024).

### *Statistical analyses*

The study was conducted using ABT-2 probiotic yoghurt culture (*L. acidophilus* LA-5, *B. animalis* subsp. *lactis* BB-12 and *S. thermophilus*), iron form (IF), two different concentrations of PSP (1 % and 3 %), and goat milk, according to a fully randomized experimental design with a factorial arrangement in two replicates. The obtained data were analyzed in tabular form using the SPSS 25 software package and the Duncan multiple comparison test. The SIMCA-P+ 14.1 program (UMETRICS, Umea, Sweden) was used for principal component analysis to demonstrate the relationships between the samples and the analyses performed.

## Results and discussion

### *Physicochemical properties*

The dry matter, pH, and titratable acidity results of probiotic yoghurt ice cream samples supplemented with IF

and PSP are given in Table 2. Enriching probiotic yoghurt ice creams with IF and PSP resulted in significant differences in physicochemical properties. Indeed, the use of IF and PSP in the production of probiotic yoghurt ice cream had a statistically very significant effect ($p<0.01$) on dry matter, pH, and titratable acidity values. The dry matter content was 29.53 % in the control group, while it decreased to 29.43 % in samples to which only IF was added. However, in the groups with added PSP and in the groups with added PSP + IF, the values increased to between 30.13 % and 31.99 %. On the other hand, while the titration acidity was determined as 1.14 LA % in the control group, it varied between 1.19-1.35 LA % in all other samples. Additionally, while the pH value of the control group was 4.56, it varied between 4.54 and 4.72 in the samples with added IF and PSP. Adding PSP to probiotic yoghurt ice cream samples increased dry matter and titratable acidity values. The addition of IF had a negative effect on the dry matter content.

*Table 2. Physicochemical properties of probiotic yoghurt ice cream samples*

| Samples | Dry matter (%) | pH | Titratable acidity (LA %) |
|---|---|---|---|
| C | 29.53±0.06$^{c}$ | 4.56±0.00$^{d}$ | 1.14±0.01$^{a}$ |
| IF | 29.43±0.16$^{c}$ | 4.69±0.01$^{b}$ | 1.19±0.01$^{d}$ |
| 1 % PSP | 30.64±0.30$^{b}$ | 4.72±0.01$^{a}$ | 1.21±0.01$^{d}$ |
| 3% PSP | 31.99±0.11$^{a}$ | 4.61±0.01$^{c}$ | 1.35±0.01$^{a}$ |
| 1 % PSP + IF | 30.13±0.37$^{b}$ | 4.60±0.01$^{c}$ | 1.26±0.01$^{c}$ |
| 3 % PSP + IF | 31.82±0.29$^{a}$ | 4.54±0.01$^{e}$ | 1.30±0.01$^{b}$ |
| Sign. | ** | ** | ** |

*Sign.: significant; **: p<0.01; C: Control group; IF: Probiotic yoghurt ice cream with added iron fumarate; 1 % PSP: Probiotic yoghurt ice cream with added 1 % pomegranate seed powder; 3 % PSP: Probiotic yoghurt ice cream with added 3 % pomegranate seed powder; 1 % PSP + IF: Probiotic yoghurt ice cream with added iron fumarate and 1 % pomegranate seed powder; 3 % PSP + IF: Probiotic yoghurt ice cream with added iron fumarate and 3 % pomegranate seed powder*

The physicochemical properties of probiotic yoghurt ice cream samples with added IF and PSP were observed to be consistent with studies in the literature. De and Nayak (2017) stated that the addition of Fe to yoghurt reduces its dry matter value. Ali et al. (2016) reported that dry matter and acidity increased in ice cream samples depending on the addition rate of pomegranate seed powder, and ranged between 37.64-41.12 % and 0.19-0.27 %, respectively. On the other hand, there were also differences with the studies in the literature. Indeed, Jasim and Al-Saadi (2020) found that there was no significant difference in pH values in yoghurt samples to which iron sulfate and iron chloride were added compared to the control group. Ahmed and Ayodele (2023) stated that the dry matter content of ice cream samples to which different proportions of pomegranate seed and pomegranate juice extract were added ranged from 38.18-40.50 %, pH values ranged from 5.85-6.00, and acidity values ranged from 0.22-0.29 %. The differences in the results are thought to be influenced by many factors, including the production conditions of probiotic yoghurt ice cream, as well as the culture type, iron form, PSP production method, addition rates, and type of raw material.

## *Mineral profile*

In the present study, the mineral profile of probiotic yoghurt ice cream samples was examined for 7 minerals: Na, Mg, P, K, Ca, Fe, and Zn. Table 3 presents the mineral profile results of probiotic yoghurt ice creams enriched with IF and PSP. The use of IF and PSP in the production of probiotic yoghurt ice cream was found to have a statistically very significant effect ($p<0.01$) on the Mg, P, Ca, Fe, and Zn contents. However, a statistically significant difference was observed at the $p<0.05$ level for Na and K contents. In the production of probiotic yoghurt ice cream, it has been observed that the individual and combined use of IF and PSP results in varying effects in terms of mineral content. Indeed, Fe and Zn content was detected only in the group containing IF, while in all other samples, Fe and Zn content was determined to be <LOD. In this case, it has been shown that the use of IF alone contributes to strategies for increasing the Fe content in dairy products. On the other hand, the absence of any change in Fe content in the PSP and IF + PSP supplemented groups highlights the importance of component interactions in the matrix. Indeed, Edebali (2025) emphasized that the use of pomegranate in yoghurt production creates a weakly acidic environment, and that the conversion of Fe in the readily soluble iron fumarate to free form in this environment affects both the Fe content and the overall mineral profile of the

*Table 3. Mineral profile of probiotic yoghurt ice cream samples (mg/kg)*

| Samples | C | IF | 1 % PSP | 3 % PSP | 1 % PSP + IF | 3 % PSP + IF | Sign. |
|---|---|---|---|---|---|---|---|
| Na | 231.39±7.78$^{a}$ | 141.30±106.12$^{ab}$ | 101.59±2.44$^{bc}$ | 195.93±10.57$^{ab}$ | nd$^{c}$ | 127.22±1.15$^{ab}$ | * |
| Mg | 150.48±1.32$^{a}$ | 144.07±19.71$^{ab}$ | 123.57±6.68$^{b}$ | 125.25±3.25$^{b}$ | 88.52±1.20$^{c}$ | 136.69±1.72$^{ab}$ | ** |
| P | 810.64±197.35$^{a}$ | 395.36±31.93$^{b}$ | 648.63±5.95$^{a}$ | 734.84±63.41$^{a}$ | 295.48±30.52$^{b}$ | 806.81±74.84$^{a}$ | ** |
| K | 1195.91±12.79$^{ab}$ | 1325.18±208.81$^{ab}$ | 1353.77±32.33$^{a}$ | 1125.40±17.39$^{bc}$ | 960.00±0.77$^{c}$ | 1221.56±2.16$^{ab}$ | * |
| Ca | 226.87±2.93$^{a}$ | 172.51±52.42$^{a}$ | 109.02±1.39$^{b}$ | 200.00±5.16$^{a}$ | nd$^{c}$ | 90.82±3.26$^{b}$ | ** |
| Fe | nd$^{b}$ | 1448.51±166.70$^{a}$ | nd$^{b}$ | nd$^{b}$ | nd$^{b}$ | nd$^{b}$ | ** |
| Zn | nd$^{b}$ | 1425.09±225.10$^{a}$ | nd$^{b}$ | nd$^{b}$ | nd$^{b}$ | nd$^{b}$ | ** |

*Sign.: significant; nd: not detected; *: p<0.05; **: p<0.01; C: Control group; IF: Probiotic yoghurt ice cream with added iron fumarate; 1 % PSP: Probiotic yoghurt ice cream with added 1 % pomegranate seed powder; 3 % PSP: Probiotic yoghurt ice cream with added 3 % pomegranate seed powder; 1 % PSP + IF: Probiotic yoghurt ice cream with added iron fumarate and 1 % pomegranate seed powder; 3 % PSP + IF: Probiotic yoghurt ice cream with added iron fumarate and 3 % pomegranate seed powder*

final product. Furthermore, Ashraf et al. (2023) reported that lactoferrin, found in goat milk at levels of approximately 0.2-2.2 mg/mL, binds $Fe^{2+}$ and $Fe^{3+}$ ions.

It is also known that the form of Fe used in enriching dairy products with Fe, and the added rate, are important. Similarly, De and Nayak (2017) reported that the amount of Fe increased depending on the ratio at which ammonium ferrous sulfate was added to yoghurt. Sugiarto et al. (2009) reported that the interaction and binding of Fe to milk proteins is affected by factors such as pH, temperature, ionic strength, and time. They also emphasized that different milk proteins exhibit different binding properties, and that this is particularly related to Fe concentration, pH, and the number of binding sites. It is stated that the acidification and protein hydrolysis that occur during the fermentation process increase the solubility of Zn. It is also emphasized that Fe and Zn have similar dissolution tendencies (Drago and Valencia, 2002; Drago and Valencia, 2008). Furthermore, Brnić et al. (2014) reported that polyphenols in fruits rich in polyphenols, such as pomegranates, have an inhibitory effect on Zn absorption. Therefore, the study suggests that polyphenols in probiotic yoghurt ice creams with added PSP have an effect on Zn.

In all probiotic yoghurt ice cream samples, the major mineral was determined to be mineral K. In probiotic yoghurt ice cream samples containing PSP, a decrease in Na, Mg, P, and Ca levels was observed compared to the control group. On the other hand, Sharma et al. (2018) stated that pomegranate seeds have a rich composition in terms of Mg, Na, and Ca. Dadashi et al. (2013) reported that pomegranate seeds contain 378.00-675.33 mg/kg of Ca, 84.50-211.33 mg/kg of Na, 1327.67-2052.00 mg/kg of Mg, 16.28-31.23 mg/kg of Fe, 2194.00-2766.33 mg/kg of P, and 2024.67-3724.67 mg/kg of K. Rowayshed et al. (2013) revealed that pomegranate seed powder contains 229.20 mg/100 g Ca, 33.03 mg/100 g Na, 10.88 mg/100 g Fe, 434.40 mg/100 g K, 481.10 mg/100 g P and 5.54 mg/100 g Zn. In addition, Ahmed and Ayodele (2023) reported that the Zn, Mg, Fe and Mn contents increased in the mineral profile of ice cream samples they produced using pomegranate seed extract and pomegranate juice.

## *Antioxidant activity*

Table 4 presents the DPPH and ABTS radical scavenging activity analysis results of probiotic yoghurt ice creams enriched with IF and PSP, expressed as % inhibition. Figure 2 presents the results of DPPH and ABTS free radical scavenging activity in terms of trolox equivalent of probiotic yoghurt ice creams. The use of IF and PSP in the production of probiotic yoghurt ice cream was found to have a statistically very significant effect ($p<0.01$) on DPPH trolox equivalent and ABTS radical scavenging activity (% inhibition and trolox equivalent) values. On the other hand, it was determined that the enrichment process of probiotic yoghurt ice cream had a statistically significant effect ($p<0.05$) on DPPH % inhibition. In all yoghurt ice cream samples, the addition of PSP was found to increase antioxidant capacity more than the addition of IF.

***Table 4.*** *Antioxidant capacity of probiotic yoghurt ice cream samples*

| Samples | DPPH Radical Scavenging Activity (%) | ABTS Radical Scavenging Activity (%) |
|---|---|---|
| **C** | 5.93±2.18$^{b}$ | 29.76±1.11$^{c}$ |
| **IF** | 7.76±1.45$^{b}$ | 27.05±2.72$^{c}$ |
| **1 % PSP** | 21.09±6.22$^{a}$ | 46.95±2.47$^{b}$ |
| **3 % PSP** | 22.92±1.14$^{a}$ | 57.68±4.32$^{a}$ |
| **1 % PSP + IF** | 15.08±2.28$^{ab}$ | 37.26±8.77$^{bc}$ |
| **3 % PSP + IF** | 22.55±5.38$^{a}$ | 59.69±1.97$^{a}$ |
| **Sign.** | * | ** |

*Sign.: significant; *: $p<0.05$; **: $p<0.01$; C: Control group; IF: Probiotic yoghurt ice cream with added iron fumarate; 1 % PSP: Probiotic yoghurt ice cream with added 1 % pomegranate seed powder; 3 % PSP: Probiotic yoghurt ice cream with added 3 % pomegranate seed powder; 1 % PSP + IF: Probiotic yoghurt ice cream with added iron fumarate and 1 % pomegranate seed powder; 3 % PSP + IF: Probiotic yoghurt ice cream with added iron fumarate and 3 % pomegranate seed powder*

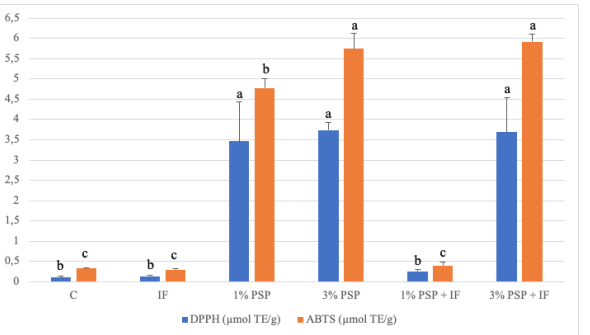



***Figure 2.*** *Antioxidant capacity of probiotic yoghurt ice cream samples*

In addition to the bioactive components naturally present in milk, bioactive peptides formed during fermentation possess a certain level of antioxidant activity. However, the various proteolytic activities of lactic acid bacteria can affect its antioxidant capacity to varying degrees. In fact, the specific proteases possessed target specific peptide bonds (Sah et al., 2014; Kariyawasam et al., 2021). In this context, Donkor et al. (2006) revealed the differences in bioactive peptides produced between yoghurts produced with classical yoghurt culture and yoghurts produced with probiotic culture. Kim et al. (2021) stated that the use of different probiotic microorganisms in yoghurt production increased DPPH and ABTS radical scavenging values compared to commercial starter culture. On the other hand, Terzioğlu and Bakırcı (2023) stated that the type of milk is also important in antioxidant activity and that the use of goat milk as a raw material in yoghurt production exhibits higher antioxidant effects compared to buffalo milk. Darwish et al. (2021) stated that the use of ascorbic acid and folic acid together with free iron (iron sulfate) in yoghurt production increased DPPH $IC_{50}$ values and decreased antioxidant

capacity compared to the control group. On the other hand, pomegranate, with its rich content of phenolic compounds such as punicalagin, punicalinin, pedunculaginin, and ellagic acid, stands out with its high free radical scavenging effect (Dżugan et al., 2018). The density and distribution of these components vary in different parts of the pomegranate, such as the seeds and peel (Benchagra et al., 2021). Singh et al. (2002) reported that methanol extract of pomegranate seeds exhibited high antioxidant activity in DPPH radical scavenging activity analyses, and that this effect was due to the phenolic compounds it contained. Akca and Akpinar (2021) stated that the DPPH value of pomegranate seed powder was 22.35 %, and the DPPH values of the ice creams they produced with pomegranate seed powder ranged between 23.16 % and 32.05 %. Çalişkanlar et al. (2024) found that the DPPH value of yoghurt samples produced using pomegranate seed powder, yoghurt culture, and *Lactobacillus casei* ranged from 63.63-70.62 %, and the TEAC value ranged from 0.5170-0.5446 µM trolox/g. Bakirci et al. (2023) stated that the addition of fruit increased the antioxidant activity of yoghurt in terms of DPPH trolox equivalent.

## Microbiological analyses

Table 5 presents the microbiological analysis results of probiotic yoghurt ice creams enriched with IF and PSP. The use of IF and PSP in the production of probiotic yoghurt ice cream was found to have a statistically significant effect ($p<0.01$) on the numbers of *S. thermophilus*, *L. acidophilus* LA-5, and *B. animalis* subsp. *lactis* BB-12. In the current study, the presence of either *L. acidophilus* LA-5 or *B. animalis* subsp. *lactis* BB-12 at a level above 6 log cfu/g conferres probiotic properties to the product. Accordingly, when probiotic properties are examined within the scope of individual samples, it is observed that each sample possesses probiotic properties. In this context, while only *L. acidophilus* LA-5 is effective in samples coded IF and 1 % PSP + IF, this effect is provided by both probiotic microorganisms in the other samples. It was determined that PSP promoted the growth of microorganisms in probiotic yoghurt ice cream samples. IF, on the other hand, has been shown to negatively affect microorganism growth.

Ghorbani et al. (2023) reported that in frozen samples produced using free and encapsulated iron with the *Lactobacillus casei* probiotic strain, the *L. casei* count remained above the minimum threshold value of $10^6$ CFU/g after a 90-day storage period. Darwish et al. (2021) found that the use of free Fe form and ascorbic acid in yoghurt promoted the growth of *S. thermophilus* and *L. acidophilus*, but resulted in a decrease in *B. bifidum* numbers. Jalal Aghdasian et al. (2022) reported that the addition of Fe as a potential substrate to yoghurt samples increased the numbers of *L. acidophilus* and *B. lactis*. Talearngkul et al. (2023) emphasized that the pre-digestive survival levels of *L. acidophilus* LA-5, *Lacticaseibacillus rhamnosus* and *B. animalis* subsp. *lactis* BB-12 in the probiotic yoghurt ice cream they produced were higher than $10^6$ CFU/g, suggesting that yoghurt ice cream could provide a suitable matrix for supplementing probiotic microorganisms and could be a potential functional food. Al-Moghazy et al. (2023) reported that pomegranate peel extracts showed antimicrobial activity against *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enteric Typhimurium* pathogenic microorganisms in cheeses, but did not inhibit *B. lactis* BB-12 and *Lactobacillus plantarum*. Saatloo et al. (2023) stated that phenolic compounds, water-soluble vitamins, and carbohydrates exhibit a prebiotic effect during cold storage processes, supporting the growth of probiotic bacteria, while Wong et al. (2021) emphasized that pomegranate seeds, along with pomegranate itself, possess a significant composition in terms of anthocyanins, phenols, tannins, vitamins, carbohydrates, minerals, and fatty acids.

*Table 5. Microbiological analyses of probiotic yoghurt ice cream samples (log CFU/g)*

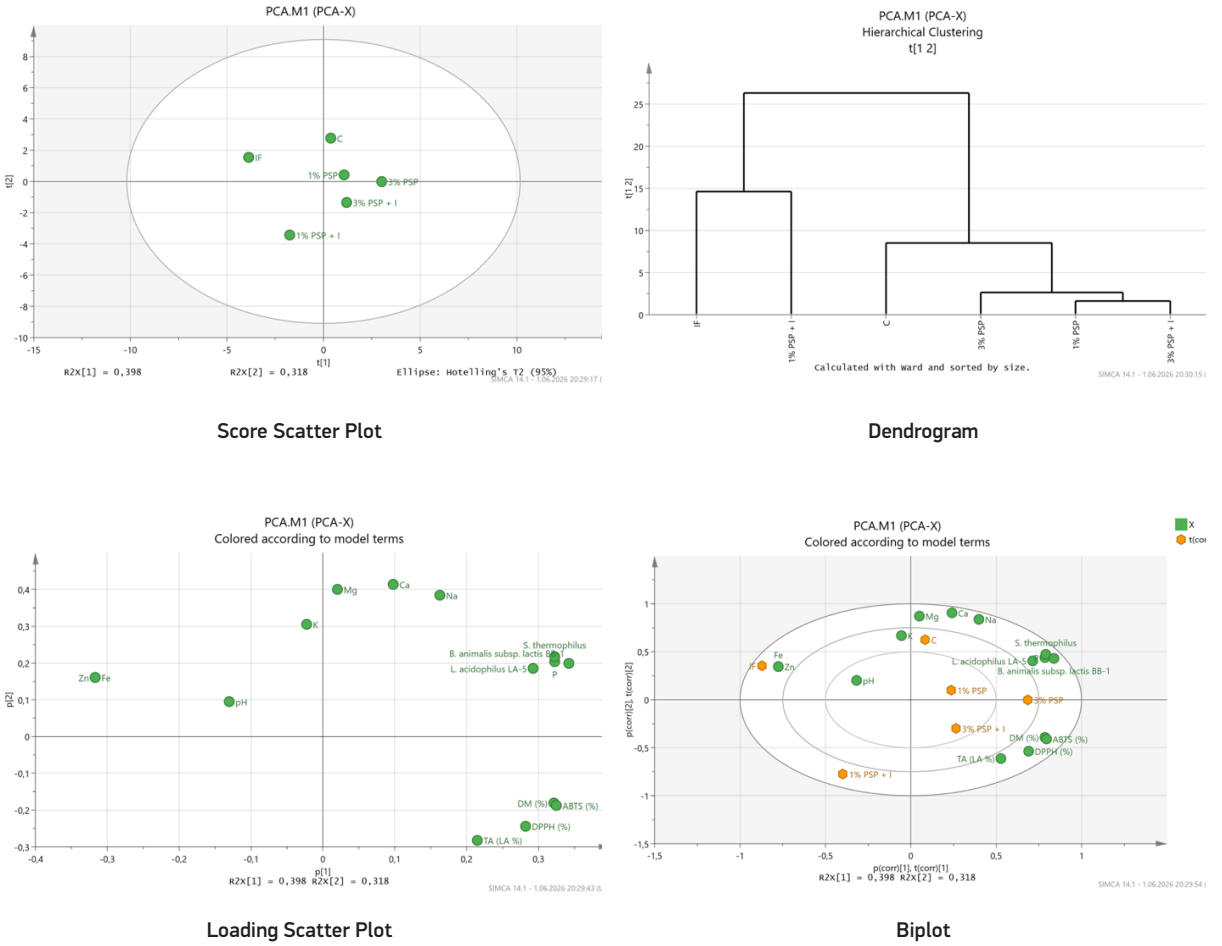
| Samples | *S. thermophilus* | *L. acidophilus* LA-5 | *B. animalis* subsp. *lactis* BB-12 |
|---|---|---|---|
| C | 6.89±0.05$^{a}$ | 6.67±0.04$^{a}$ | 6.34±0.11$^{a}$ |
| IF | 6.25±0.07$^{c}$ | 6.08±0.05$^{b}$ | 5.80±0.14$^{bc}$ |
| 1 % PSP | 6.89±0.02$^{a}$ | 6.73±0.01$^{a}$ | 6.47±0.01$^{a}$ |
| 3 % PSP | 6.96±0.02$^{a}$ | 6.81±0.03$^{a}$ | 6.54±0.04$^{a}$ |
| 1 % PSP + IF | 6.28±0.11$^{bc}$ | 6.19±0.06$^{b}$ | 5.77±0.10$^{c}$ |
| 3 % PSP + IF | 6.42±0.06$^{b}$ | 6.11±0.10$^{b}$ | 6.02±0.09$^{b}$ |
| Sign. | ** | ** | ** |

*Sign.: significant; **: p<0.01; C: Control group; IF: Probiotic yoghurt ice cream with added iron fumarate; 1 % PSP: Probiotic yoghurt ice cream with added 1 % pomegranate seed powder; 3 % PSP: Probiotic yoghurt ice cream with added 3 % pomegranate seed powder; 1 % PSP + IF: Probiotic yoghurt ice cream with added iron fumarate and 1 % pomegranate seed powder; 3 % PSP + IF: Probiotic yoghurt ice cream with added iron fumarate and 3 % pomegranate seed powder*

## PCA analysis

PCA was applied to evaluate the physicochemical properties, mineral profile, antioxidant activity, and microbiological characteristics of probiotic yoghurt ice creams with added IF and PSP, and to interpret the differences between the samples. Score scatter plot, dendrogram, loading scatter plot and biplot plots are presented in Figure 3.

As can be seen from the graphs, the first two principal components (PC1=39.8 % and PC2=31.8 %) explained 71.26 % of the variance. Probiotic yoghurt ice cream samples were divided into two main groups: IF and 1 % PSP + IF and other groups (Score Scatter Plot and Dendrogram). This indicates that samples grouped together exhibit similar characteristics, while separate groups show significant differences. When the Loading Scatter Plot is examined, Na, Ca, Mg, P, *S. thermophilus*, *L. acidophilus* LA-5, and *B. animalis* subsp. *lactis* BB-12 are clustered together in the upper right corner of the plot, indicating a positive correlation between them. In the lower right corner of the plot, dry matter, titratable acidity, DPPH, and ABTS free radical scavenging activity are clustered together. On the other hand, Fe, Zn, K, and pH are clustered




**Score Scatter Plot** **Dendrogram**

**Loading Scatter Plot** **Biplot**

*Figure 3. Principal component analysis*

together to the left of the plot. While the properties clustered together here exhibit a positive correlation, it can be said that Fe and Zn, in particular, are more distinctly positioned and are effective variables in differentiating the samples. The Biplot graph also confirmed that the IF group was superior in terms of Fe and Zn, while the 3 % PSP group was shown to support microbial growth. In the production of probiotic yoghurt ice cream, the use of IF and PSP was observed to exhibit different profiles in terms of physicochemical properties, mineral profile, antioxidant activity, and microbiological counts. In conclusion, it can be said that the use of these additives, individually or in combination, as well as their proportions, have an effect on the properties of the final product.

## Conclusions

In the present study, various nutritional profiles and quality characteristics of yoghurt ice cream samples produced using goat milk, probiotic yoghurt culture, PSP, and IF were examined. According to the findings of this study, integrating probiotic microorganisms with a popular product like ice cream creates a new area of utilization for these microorganisms. Furthermore, it has been determined that food-derived by-products such as pomegranate seeds, which have increased in recent years, can be used as an economically valuable resource in the dairy industry. It has shown that the use of PSP in probiotic yoghurt ice cream supports microbial activity through its prebiotic effect and also makes positive contributions to antioxidant capacity due to its phenolic compound content. On the other hand, while it was determined that the use of IF alone in probiotic yoghurt ice cream contributed to strategies for increasing iron content, the combined use of IF and PSP did not lead to an increase in iron content. In this context, strategies for enriching dairy products with iron should consider the iron source, the rate of addition, and the effects of other components.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' contribution

Murat Emre Terzioğlu: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization. Ezgi Edebali: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization. Zeynep Çağla Tekgül: Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization.

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## Nutritivni profil probiotičkog sladoleda od kozjeg jogurta obogaćenog željezo-fumaratom i prahom sjemenki nara

### Sažetak

Mliječni proizvodi mogu se obogaćivati različitim metodama kako bi se ublažio nedostatak željeza i unaprijedila njihova funkcionalna svojstva. Laktoza, sastavni dio mlijeka, inhibira apsorpciju željeza; međutim, tijekom fermentacije mlijeka u jogurt laktoza se pretvara u mliječnu kiselinu, čime se povećava apsorpcija željeza. Nadalje, zbog svojih fizikalno-kemijskih i strukturnih svojstava, mliječni se proizvodi smatraju prikladnim prehrambenim matricama za prijenos i inkorporaciju bioaktivnih sastavnica voća ili nusproizvoda prerade voća. Sjemenke nara, koje se nakon prerade ploda smatraju otpadom, mogu se samljeti i inkorporirati u različite proizvode kako bi se iskoristio njihov visok nutritivni potencijal. U tom je kontekstu, u okviru ovog istraživanja, proizveden sladoled od jogurta primjenom probiotičke jogurtne kulture ABT-2 (*Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 i *Streptococcus thermophilus*), izvora željeza (željezo-fumarat, IF), praha sjemenki nara (PSP, u koncentracijama od 1 % i 3 %) te kozjeg mlijeka. Primjena IF-a i PSP-a u proizvodnji probiotičkog sladoleda od jogurta imala je statistički vrlo značajan utjecaj ($p<0,01$) na udio suhe tvari, pH-vrijednost, titracijsku kiselost, sadržaj Mg, P, Ca, Fe i Zn, vrijednosti DPPH-ekvivalenta Troloxa i sposobnost uklanjanja ABTS-radikala, kao i na brojnost bakterija *L. acidophilus* LA-5, *B. animalis* subsp. *lactis* BB-12 i *S. thermophilus*. Utvrđeno je da dodatak PSP-a povećava udio suhe tvari i titracijsku kiselost te antioksidacijski kapacitet, dok istodobno smanjuje sadržaj Na, Mg, P i Ca. U usporedbi s kontrolnom skupinom, primjena IF-a i PSP-a u kombinaciji rezultirala je povećanjem udjela suhe tvari, titracijske kiselosti, sadržaja Na, Mg, P i Ca te antioksidacijskog kapaciteta. S druge strane, sadržaj Fe i Zn utvrđen je isključivo u uzorcima kojima je dodan IF. Zaključno, ovo je istraživanje pokazalo da je primjena IF-a u kombinaciji s PSP-om inhibirala obogaćivanje željezom, no navedena je kombinacija imala pozitivan učinak na neka svojstva proizvoda. Strategije iskorištavanja nusproizvoda i obogaćivanja prehrambenih proizvoda funkcionalnim sastojcima mogu pridonijeti razvoju inovativnih i održivih mliječnih proizvoda.

**Ključne riječi**: probiotički sladoled od jogurta; željezo-fumarat; prah sjemenki nara; recikliranje otpada; inovativni i održivi proizvodi

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