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Symbiotic ice-cream production using *Lactiplantibacillus plantarum* and oleaster (*Elaeagnus angustifolia* L.) flour

Lactiplantibacillus plantarum ve iğde (Elaeagnus angustifolia L.) unu kullanılarak simbiyotik dondurma üretimi

Beyza Nur SUREN[1], Sedanur SALMAN[2], Emel KAYA[3], Yagmur BUYUKKAL[4], Gozde KUTLU[5*], Fatih TORNUK[6]

[1,2,3,4]Yildiz Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Food Engineering, Davutpasa Campus, Istanbul, Türkiye.
[5]Ankara Medipol University, Faculty of Fine Arts, Design and Architecture, Department of Gastronomy and Culinary Arts, Ankara, Türkiye.
[6]Sivas Cumhuriyet University, Faculty of Health Sciences, Department of Nutrition and Dietetics, 58140, Sivas, Türkiye.

[1]https://orcid.org/0009-0005-4452-9592; [2]https://orcid.org/0009-0005-2409-6136; [3]https://orcid.org/0009-0002-4505-5059; [4]https://orcid.org/0009-0003-5651-9311; [5]https://orcid.org/0000-0001-7111-1726; [6]https://orcid.org/0000-0002-7313-0207

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Address for Correspondence:
Gözde KUTLU
e-mail:
gozcelk@gmail.com

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ABSTRACT

The aim of this study was to produce a functional and low-calorie ice cream by incorporating oleaster flour (OF) into the ice cream mix formulation at different ratios as milk powder and/or sugar substitute. The ice cream was also probiotificated by supplementation of *Lactiplantibacillus plantarum* NRIC 1838, thus preparing symbiotic ice-creams. For this purpose, 8 different ice-cream formulations were designed and their physicochemical, bioactive, microbiological, thermal and sensory properties were analyzed. The results showed that dry matter content, pH values and total phenolic content of ice creams were in the range of 43.78-46.59 %, 5.65-6.38, and 0.25-0.94 mg GAE g^{-1}, respectively. Addition of OF made the samples darker, as indicated by lower *L** values. Additionally, the control samples had the highest brightness while the darkness of the ice-creams increased as the amount of OF in the formulations. Furthermore, the highest *a** and *b** values were determined in the ice cream sample supplemented with probiotic and OF while the lowest value was determined in the reference ice-creams enriched with probiotics. The cell counts of the ice cream mixes and samples were higher than 7 log CFU g^{-1}. The incorporation of OF, skimmed milk powder, and sugar in the different proportions and combinations led to a reduction in thermodynamic stability of ice-creams compared the control samples. The amount of OF was the most effective ingredient in the overall sensory acceptance of the produced samples. These results suggest that symbiotic ice-creams enriched with OF and *Lactiplantibacillus plantarum* NRIC 1838 exhibited good quality and sensory characteristics.

Key Words: Oleaster flour, *Lactiplantibacillus plantarum* NRIC 1838, symbiotic ice-creams, characterization.

ÖZ

Bu çalışmanın amacı, süt tozu ve/veya şeker yerine farklı oranlarda iğde ununun (İU) dondurma karışımına dahil edilerek fonksiyonel ve düşük kalorili bir dondurma üretimini sağlamaktır. Bu kapsamda üretilen dondurmalar *Lactiplantibacillus plantarum* NRIC 1838 kullanılarak probiyotik hale getirilmiş ve simbiyotik dondurmalar hazırlanmıştır. Bu amaçla, 8 farklı dondurma formülasyonu oluşturulmuş ve bu dondurmaların fizikokimyasal, biyoaktif, mikrobiyolojik, termal ve duyusal özellikleri analiz edilmiştir. Sonuçlar, dondurmaların kuru madde içeriği, pH değerleri ve toplam fenolik içeriklerinin sırasıyla % 43.78-46.59, 5.65-6.38 ve 0.25-0.94 mg GAE g^{-1} olduğunu göstermiştir. Ayrıca, kontrol örneklerinin en yüksek parlaklık değerlerine sahip olduğu ve formülasyonlardaki İU miktarı arttıkça dondurma rengindeki koyuluk miktarının da arttığı tespit edilmiştir. Yanı sıra, probiyotik ve İU ile takviye edilmiş dondurma örneklerinde en yüksek *a** ve *b** değerleri belirlenirken, en düşük değer probiyotikle zenginleştirilmiş kontrol dondurmalarında belirlenmiştir. Dondurma karışımlarının ve örneklerinin hücre sayıları 7 log CFU/g'dan yüksektir. Farklı oranlarda ve kombinasyonlarda İU, yağsız süt tozu ve şekerin dahil edilmesi, kontrol örnekleriyle karşılaştırıldığında dondurmalardaki termodinamik stabiliteyi azaltmıştır. İU, üretilen örneklerin genel duyusal kabulünde en etkili bileşen olmuştur. Bu sonuçlar, İU ve *L. plantarum* NRIC 1838 ile zenginleştirilmiş simbiyotik dondurmaların iyi kalite ve duyusal özelliklere sahip olduğunu göstermektedir.

Anahtar Kelimeler: İğde unu, *Lactiplantibacillus plantarum* NRIC 1838, simbiyotik dondurma, karakterizasyon

Introduction

In recent years, probiotic dairy products have become a significant part of the functional food market in accordance with consumer demands. The term probiotic is defined by FAO/WHO (2002) as "a living microorganism, most of which belong to the genera of *Lactobacillus* and *Bifidobacterium*, that when taken in sufficient quantities, provides a positive effect on the health of the host organism" (Acu et al., 2021; Akman et al., 2023; Bagdat et al., 2024a,b; Di Criscio et al., 2010; El-Sayed et al., 2014). Furthermore, prebiotics are described as indigestible food components that benefit the host by promoting the growth and/or activity of probiotics in the colon. One approach to the management of intestinal microflora is the use of symbiotics, a combination of probiotics and prebiotics. The simultaneous presence of probiotics and prebiotics in the product allows better colonization ability in the colon compared to the use of prebiotics or probiotics alone (Acu et al., 2021; El-Sayed et al., 2014; Elkot et al., 2020).

Elaeagnus angustifolia L. (oleaster, Russian olive), a member of the family "*Elaeagnaceae*", comprises a group of flowering shrubs and has a wide geographical distribution in Asia, Central Asia, the Caucasus, and Europe. Fresh or dried reddish-brownish fruits ripen in September and are used different medicinal purposes such as anti-diarrheal, anti-inflammatory, anti-pyretic, diuretic, and tonic (Yavuz et al., 2022). The oleaster fruit consists of 50% oleaster powder, 15% crust, and 35% seed (Yavuz, 2019). It is rich in minerals, vitamins and dietary fibers, as well as exhibiting high bioactive properties. Oleaster flour (OF) is obtained by grinding of the dried oleaster fruits (Yavuz et al., 2022). The protein content of oleaster powder was determined to be 3.88%, dietary fiber content 20.10%, moisture content 21.96%, and ash content 1.85%. The TPC was measured at 3957.06±20.81 mg GAE kg^{-1}, while the DPPH inhibition percentage was found to be 6.48% (Yavuz, 2019). Due to its unique properties (*e.g.* floury structure, specific taste and chemical composition), it can be included as a functional ingredient in various food formulations such as cakes, chocolate, bakery products, ice-cream, infant food and yogurt (Sahan et al., 2013; Çakmakcı et al., 2014; Yavuz et al., 2021; Yavuz et al., 2022).

Functional ice cream can be defined as dairy products that contain basic ingredients such as air, emulsifiers, flavoring agents, milk fat, milk solids-not-fat, stabilizers and water as well as functional ingredients such as probiotics, prebiotics, bioactive peptides, antioxidants, various essential fatty acids and dietary fibers (Genovese et al., 2022). The relative high fat and sugar content of ice cream led to the high consumption of these nutrients, which raises the risk of obesity, especially pediatric obesity and health-related issues (Akalın et al., 2008; Drewnowski, & Greenwood, 1983). For this reason, there has been an increase demand in food industry related to the ice-cream including high-fiber content with low-calorie (Arslaner, & Salık, 2020). Different functional symbiotic ice-cream formulations were developed using different combinations ((Litesse ultra, Tagatose and polydextrose/ *Bifidobacterium bifidum, Bifidobacterium longum, and Lacticaseibacillus paracasei* (formerly *Lactobacillus paracasei*)) (Acu et al., 2021); inulin/*Lacticaseibacillus casei* (formerly *Lactobacillus casei*) and *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus*) (Criscio et al., 2010); inulin, lactulose and Fructooligosaccharides/ *Bifidobaterium bifidum*, *L. casei, L. plantarum* (El-Sayed et al., 2014); black rice powder/ *Lactobacillus acidophilus* LA-5 (Elkot et al., 2022*);* inulin/ *Lactobacillus brevis* PML1 (Falah et al., 2021); inulin and *L. acidophilus* (Pandiyan et al., 2012); fructooligosaccharides/ *L. casei, L. plantarum* (Sabet-Sarvestani et al., 2021); banana flour/ *L. casei* TISTR 1463, *L. acidophilus* TISTR 1338 (Phuapaiboon, 2016). Moreover, Çakmakçı et al (2014) compared the quality, color, and sensory characteristics of ice cream using OF and oleaster crust at ratios of 1-3 (w:w) and investigated the contribution of oleaster to the nutritional and functional properties of ice cream. Although OF, as a dietary fiber source, has been used incorporated into several baked products such as white and gluten-free bread, cookie, gluten-free cake, biscuit, sponge cake and breakfast cereals (Yavuz et al., 2022; Şahin, 2023; Sahan et al., 2013; Lavini et al., 2021; Zangeneh et al., 2021; Tatari et al., 2022), to the best of our knowledge, no scientific data have been published on the use of OF as both a dietary fiber source and sugar substitute in ice cream production with the supplementation of probiotics. Therefore, the aim of the present work was to develop a new symbiotic combination using OF as prebiotics and *L. plantarum* NRIC 1838 as probiotics to produce functional ice-creams, and to investigate their physicochemical, microbiological, bioactive, rheological, and sensorial properties.

Materials and Methods

Materials

Pasteurized cow's milk (3.1 % milkfat; Mis, Ak Gida Company, Türkiye), pasteurized cream (35% milkfat; Mis, Ak Gida Company, Türkiye), powdered soybean lecithin (E322, Tito, İzmir, Türkiye), and sugar (Ismen Food Company, İstanbul, Türkiye) were purchased from supermarkets in Istanbul, Türkiye. Skimmed milk powder and pure salep were obtained from Aktar Diyari (Istanbul, Türkiye) while mature oleaster fruit (*Elaeagnus angustifolia* L., 20.1 % dietary fiber content) was kindly supplied from Ziya Organik Tarim (Istanbul, Türkiye). De Man Rogosa Sharp (MRS) agar (Merck, Germany) and MRS broth (Merck, Germany) were supplied.

Preparation of oleaster flour (OF)

Firstly, the oleaster fruits were meticulously cleaned with distilled water and scrubbing to eliminate any possible contaminants. Subsequently, the inner parts of the fruits were separated from the skins, dried in a hot air oven (Memmert UF-110, Germany) at 45-50 °C for 24 h, and then powdered using a grinder (Tefal 8100.31 coffee grinder, France) (Çakmakçı et al., 2015)

Preparation of probiotic inoculum

For this aim, *Lactiplantibacillus plantarum* strain NRIC 1838 was inoculated on MRS agar and left for incubation at 35 °C for 48 h. Then, grown cultures were collected with a sterile inoculation loop and transferred to sterile MRS broth and incubated at 37 °C for 24 h. Following a subsequent incubation at the same conditions, bacterial cells were collected by centrifugation at 9000 rpm, at 4 °C for 10 min (Centrifuge Multifuge X3 FR, Thermo Scientific). Finally, sterile peptone water was added into the collected cells and probiotic inoculum solution was prepared.

Ice cream production

In this study, 8 different ice cream formulations were prepared, as listed in Table 1. The ice cream mix samples were prepared following the flow chart given in Figure 1 (Sabet-Sarvestani, 2020).

Table 1. The recipes for different ice-cream mixes

Sample codes	Milk (%)	Cream (%)	Milk powder (%)	Sugar (%)	OF (%)	Lecithin (%)	Sahlep (%)	*L. plantarum*
C	65	7	7	20	0	0.5	0.5	-
CP	65	7	7	20	0	0.5	0.5	+
OF	65	7	7	0	20	0.5	0.5	-
OFP	65	7	7	0	20	0.5	0.5	+
OFS	65	7	7	10	10	0.5	0.5	-
OFSP	65	7	7	10	10	0.5	0.5	+
SPOF	65	7	17	0	10	0.5	0.5	-
SPOFP	65	7	17	0	10	0.5	0.5	+

C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

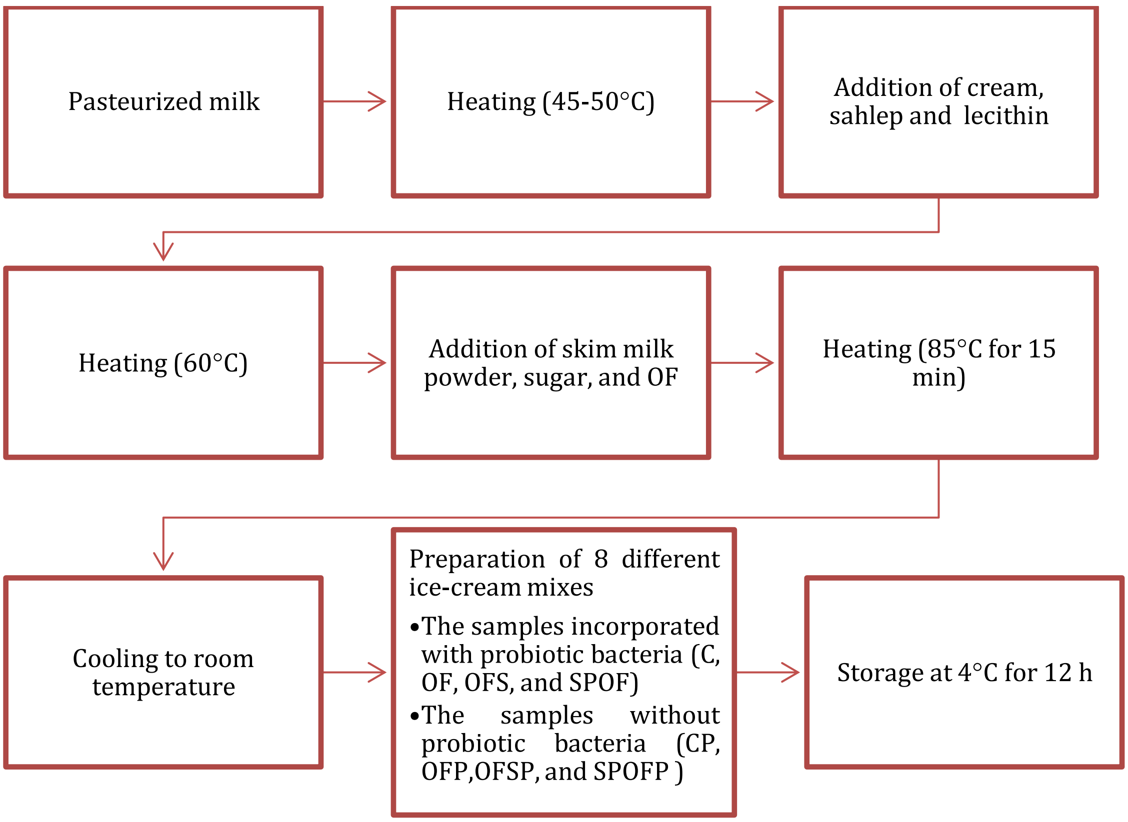

Figure 1. Process flowchart for the preparation of ice-cream mix.

C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

To begin with, milk was heated 45-50 °C and sahlep and lecithin were incorporated into it during medium heating (50ºC) and mixing. Lecithin is used in ice cream to act as an emulsifier, improve texture and creaminess, control crystallization, and facilitate homogenization. After addition of skim milk powder, sugar and/or OF at 60 °C, the mixture was pasteurized at 85°C for 15 min. Finally, the ice cream mixes were rapidly cooled to room temperature and probiotic inoculums were added at a targeted initial level of ~10^7 kob mL^{-1}, which was determined by serial dilution and plating methods to ensure accurate microbial counts. Following the aging of ice cream mixes at 4°C for 12 h, ice cream was prepared using the ice cream machine (Delonghi, Il Gelataio, ICK5000, China) at a constant rotation speed for 15 min. Then, the samples were placed in polypropylene food containers and stored at – 18 °C for 24 h. The samples were analyzed after the storage period (Sagdic et al., 2012). To facilitate a clearer understanding of the prepared ice cream formulations, they were schematized, and the analyses performed were presented in a graphical abstract format in Figure 2.

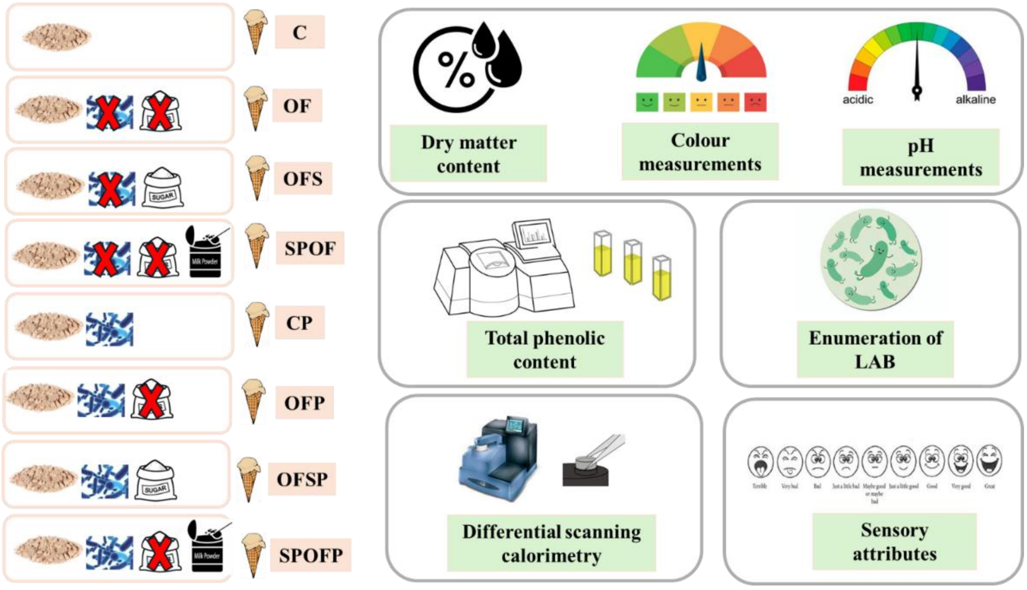

Figure 2. An overview of ice-cream formulations and analysis.
C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

Physico-chemical analysis

The official procedure was followed for the determination of dry matter content of the ice-cream samples (AOAC International, 2000). pH values of the samples were measured employing a Mettler-Toledo pHmeter (Model: S220 SevenCompact™ pH/Ion meter) (Karaman et al., 2014). Furthermore, the color measurements were performed using a portable colorimeter (CR-400, Minolta Camera Co., Osaka, Japan) after melting the samples to ensure a uniform surface, minimize reflection and refraction effects, and provide consistent sample states for accurate and comparable color readings.The a^*, b^* and L^* values measured in the colorimeter represented red-greenness, blue-yellowness and lightness-darkness, respectively (Yavuz et al., 2022; Kutlu et al., 2024). Following formulas were used to calculate the Δa^*, Δb^*, ΔL^* and ΔE^* (total color difference) values.

$$\Delta E^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \qquad (1)$$
$$\Delta a^* = a_1^* - a_0^* \qquad (2)$$
$$\Delta b^* = b_1^* - b_0^* \qquad (3)$$
$$\Delta L^* = L_1^* - L_0^* \qquad (4)$$

Total phenolic content (TPC)

In order to determine the TPC content of the ice cream samples, the methodology proposed by Karaman et al. (2014) was followed with minor modifications. Firstly, ice-cream samples (10 g) were well-mixed with 5 mL of hexane and 50 mL of 80% methanol. This mixture was shaken and kept in dark at room temperature for 24 h. Then, the samples were transferred into centrifuge tubes and centrifuged at 9000 rpm for 10 min at 4 °C. Afterwards, the oil layers on the samples were removed with a syringe and centrifugation was performed again. Up till the oil was totally separated, the mixture was filtered through filter paper. After mixing the 0.5 mL sample and 2.5 mL Folin-Ciocalteau reagent for 3 min, 2 mL of sodium bicarbonate (7.5%) was added. These mixtures were kept in the dark at room temperature for 30 min and absorbance values were read at 760 nm wavelength. The results were given as mg gallic acid (GAE) equivalents per g of ice-cream sample (mg GAE g^{-1} sample) (Erol et al., 2023; Kutlu, 2024).

Enumeration of LABs

Enumaration of LABs in both ice-cream mixes and ice-creams was performed based on the protocol described by Sabet-Sarvestani et al. (2020). Briefly, 10 grams of sample was mixed with 90 mL of sterile peptone water using a stomacher. Afterwards, six decimal serial dilutions (10^1–10^6 CFU g^{-1}) were prepared and 0.1 mL of appropriate serial dilutions (10^4, 10^5 and 10^6 CFU g^{-1}) were spread plated onto MRS agar. Next, the plates were incubated at 37 °C for 48 h under aerobic conditions to facilitate the growth of LAB because *Lactiplantibacillus plantarum* is aerotolerant. Colonies were counted and the findings were given as log CFU g^{-1}.

Differential scanning calorimetry

Thermal properties of ice-cream samples were determined using a differential scanning calorimeter (DSC, Q100, TA Instruments Inc., New Castle, DE, USA) according to the applied method by Ertugay et al. (2020) with minor modifications. For this aim, 10 mg of sample was heated under nitrogen gas flow at a rate of 20 mL min^{-1}, with a heating rate of 5°C min^{-1}, between -20 °C and 20 °C, after being placed in hermetically sealed aluminum pans before loading into the instrument. Onset, midpoint, offset temperatures and enthalpy values of eight different ice cream samples were determined from the DSC thermograms.

Sensory attributes

A total of 20 panelists consisting of faculty members and undergraduate students from Yildiz Technical University Department of Food Engineering were selected as panelists in sensory evaluation. In the sensory analysis ice cream quality parameters (color, consistency, taste and aroma, odor, and overall acceptability) was evaluated by the panelists using a hedonic scale from 0 to 9 (0: very bad; 9: very good) (Sagdic et al., 2012).

Statistical evaluation

SAS software package, version 8.2 (SAS Institute Inc., Cary, NC) was employed for statistical evaluation of the obtained data using one-way analysis of variance (ANOVA). The statistical differences were evaluated using the Duncan's multiple range test at 95% of significance level.

Results and Discussion

Dry matter content

The type of milk used to prepare the mix has a significant impact on the characteristics of the product, and the physical properties of the ice cream mix fabricated by various processing techniques can change both the texture and appearance of the finished product (Elkot et al., 2022). In this study, the dry matter of ice-cream samples was OFSP (46.59 %) > OFP (45.57 %) > SPOF (45.44 %) > OFS (45.05 %) > C (44.82 %) > SPOFP (44.54 %) > CP (44.10 %) > OF (43.78 %) (Table 2). The results showed that the supplementation of probiotics into the ice-creams had no significant effect on the dry matter content ($p>0.05$). When sugar was substituted with OF in the formulation, a slight increase in the dry matter content was determined, which can be due to the higher water content of oleaster flour compared to the sugar. This finding suggested that while the type of ingredient affects the dry matter, the presence of probiotics alone does not significantly alter this parameter. Similar findings were reported for ice-creams incorporated with tahini (Bayrakçı, 2018). Contrary, lower dry matter content values were reported for ice-creams incorporated with Kavılca fibre (Ertugay et al., 2020), ice-creams including OF (Çakmakçı et al., 2014), and the ice-cream samples enriched with black rice powder and *Lactobacillus acidophilus* LA-5 (Elkot et al., 2022). The variations between the dry matter content of the ice creams in the literature were due to the differences in the mix recipes of the ice creams. This study highlighted that ingredient substitutions and additions, such as OF and probiotics, could affect the dry matter content but did not impact all formulations in the same way.

Table 2. Some physico-chemical (dry matter, pH, and color values) properties of ice-cream samples.

Sample codes	Dry matter (%)	pH	*L**	*a**	*b**	*ΔE**
C	44.82 ± 0.62^{ba}	6.37 ± 0.01^{a}	86.33 ± 0.31^{a}	-1.53 ± 0.17^{b}	6.08 ± 0.43^{bc}	0.00
OF	43.78 ± 0.37^{b}	5.65 ± 0.01^{e}	67.45 ± 2.53^{c}	7.40 ± 0.57^{a}	21.68 ± 3.21^{bac}	26.07
OFS	45.05 ± 0.10^{ba}	5.93 ± 0.00^{b}	72.38 ± 2.80^{bc}	5.63 ± 1.11^{a}	18.83 ± 1.40^{ba}	20.21
SPOF	45.44 ± 0.47^{ba}	5.96 ± 0.01^{b}	68.32 ± 0.53^{c}	6.43 ± 0.12^{a}	15.30 ± 0.41^{bac}	21.74
CP	44.10 ± 0.44^{b}	6.38 ± 0.01^{a}	78.93 ± 1.93^{ba}	-2.47 ± 0.21^{b}	4.50 ± 1.24^{bac}	0.00
OFP	45.57 ± 0.67^{ba}	5.70 ± 0.01^{d}	66.19 ± 7.19^{c}	8.15 ± 3.75^{a}	2.33 ± 2.42^{a}	16.73
OFSP	46.59 ± 0.28^{a}	5.89 ± 0.01^{c}	70.03 ± 0.68^{cb}	4.80 ± 1.18^{a}	16.77 ± 1.19^{bac}	16.81
SPOFP	44.54 ± 0.10^{b}	5.95 ± 0.01^{b}	71.71 ± 5.91^{bc}	4.99 ± 1.79^{a}	21.65 ± 1.29^{a}	20.05

C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

[a,b,c,d]: Means with different letters in the same column are significantly different (p<0.05).

pH

The pH values of the ice creams affect both the viability of probiotic bacteria and the sensory properties of the product. In this study, the following order in the pH values was determined among the ice cream samples, CP (6.38) > C (6.37) > SPOF (5.96) > SPOFP (5.95) > OFS (5.93) > OFSP (5.89) > OFP (5.70) > OF (5.65) (Table 2). Similar results were reported for ice-creams incorporated with OF (Çakmakçı et al., 2014), ice-creams enriched with free/encapsulated bacteria (*L. plantarum, L. casei* and *B. bifidum*) (El-Sayed et al., 2014), ice-creams enriched with cornelian cherry and *Bifidobacterium lactis* (Haghani et al., 2021). However, higher pH values were reported for ice-creams incorporated with Kavılca fibre (Ertugay et al., 2020) and ice-cream mixes incorporated with *Lactobacillus johnsonii* La1 (Alamprese et al., 2002). It is also noteworthy that OFSP, and OFP had higher pH values in comparison to SPOFP. This phenomenon may be due to decreased LAB activity as the sugar content in the formulation increased, because higher sugar concentrations can sometimes inhibit LAB metabolism.as previously reported by Falah et al. (2021). Moreover, OFP had the lowest pH value among the probiotic incorporated ice-cream samples, indicating the combination of *L. plantarum* and OF in ice-cream samples supplied a synergistic influence to enhance metabolism and activity of probiotic cells (Kemsawasd and Chaikham, 2020). This result indicated that the incorporation of *L. plantarum* with OF into the ice cream formulation was effective in maintaining a more acidic environment, which might favor the survival and growth of probiotics. Furthermore, the pH value of non-probiotic ice-creams were lower than their reference samples. This might be ascribed to the fact that the incorporation of phenolic compounds (*e.g.* hydroxycinnamic acid derivatives, hydroxylbenzoic acid derivatives) (Karkar and Şahin, 2022) found in OF and milk proteins led to the pH reduction in comporison with their reference sample, impacting the overall acidity of the ice cream (Shazly et al., 2022).

Color properties

One of the most important quality factors influencing consumers' food choices is color, and visual quality includes the appearance of a product (Yavuz et al., 2022). *L** is regarded as a convenient unit of measurement for brightness, the feature that allows each color to be equated to a member of the grey scale between white and black. Among the tested samples, C was found to have the highest *L** value (86.33) (Table 2), followed by CP (78.93), OFS (72.38), SPOFP (71.71), OFSP (70.03), SPOF (68.32), OF (67.45), and OFP (66.19), respectively. From the experimental data, it was possible to observe that the *L** values of the ice cream samples decreased with the increase in the proportion of OF, indicating that the addition of OF affects the lightness of the ice cream. This was in agreement with previous reports informing that the *L** values of probiotic ice-creams decreased with a rise in level of cornelian cherry peel (Haghani et al., 2021). Likewise, Çakmakçı et al. (2014) reported that the brightness values decreased from 90.02 to 77.18 with the addition of 3% OF; this decrease may be due to the presence of brown pigments in OF. Similar *L** values also reported for ice cream mix prepared with fructooligosaccharides/ *L. plantarum* and *L. casei* (73.33-83.00) (Sabet-Sarvestani et al., 2021). Besides, the *a** values (+*a*-redness, − *a*-green) exhibited the following trend: OFP (8.15) > OF (7.40) > SPOF (6.43) > OFS (5.63) > OF (7.40) > SPOFP (4.99) > OFSP (4.80) > C (-1.53) > CP (-2.47) (Table 2). While *a** values were negative in control samples (C and CP), they were positive in enriched ice creams. The concentrations, probiotic supplemantion and types of ingredients used in formulations affected the *a** values. Increase the incorporation ratio of OF in formulation led to the increase in *a** values. The increase in *a** values with higher proportions of OF suggests that OF contributes to a more pronounced red hue in the ice cream. Likewise, Haghani et al. (2021) reported an increase in the *a** values of ice-cream samples from 0.36 to 27.6 upon supplementation of 9% of cornelian cherry peel into the probiotic ice-cream samples. The *a** values reported for the ice-creams incorporated with OF (-2.77- 2.04) (Çakmakçı et al., 2014). Moreover, the *b** values of ice-cream samples were found in the following order: OF (21.68) > SPOFP (21.65) > OFS (18.83) > OFS (18.83) > OFSP (16.77) > SPOF (15.30) > SPOF (15.30) > C (6.08) > CP (4.50) > OFP (2.33) (Table 2). The *b** values reported for the ice-creams incorporated with OF (7.57- 10.24) (Çakmakçı et al., 2014) and ice cream mix prepared with fructooligosaccharides/ *L. plantarum* and *L. casei* (15.33-22.67) (Sabet-Sarvestani et al., 2021). The higher *b** values in ice creams with OF indicate increased yellowness. The differences in *b** values may be due to the caretonoid content of OF, the fermentation process and the use of OF as a source of prebiotics. Δ*E** values for non-probiotic ice-creams and probiotic ice-creams were in the range of 0-26.07 and 0-20.05 (Table 2), respectively, showing that the color differences can be easily recognizable by eyes due to the *Δ*E*>3 (Atlar et al., 2024; Yavuz et al., 2022). The above results allowed us to confirm that the addition of phenolic compounds could potentially alter the color properties (Sagdic et al., 2012). Overall, we can conclude that supplementation of OF and probiotics led to a reduction in the lightness and yellowness values, but increased redness, so that OF could significantly affect the colour in ice-creams.

TPC

As fruit peels, cereal grains, vegetable seeds, and their pulp had high level dietary fiber content, they are great source of phenolic compounds (Akca and Akpınar, 2021). The data on TPC values presented in Table 3 indicated that TPC values of ice-cream samples ranged from 0.25 (C) to 0.94 (OFP) mg GAE g^{-1} depending on the ice-cream formulation. Both increase in the OF ratio and probiotic supplementation resulted in higher TPC content. In a related work, an increase in the cornelian cherry content led to an increase in TPC content of probiotic ice-cream samples (Haghani et al., 2021). Similar TPC values were reported for the ice-creams supplemented with *L. casei,* dark blue and white *Myrtus communis* pulps (Öztürk et al., 2018); however, higher TPC values were noted for the ice-cream samples enriched with black rice powder and *L. acidophilus* LA-5 (Elkot et al., 2022). Among the ice-cream samples, the lowest TPC was determined in C and CP, which was in accordance with earlier findings (Öztürk et al., 2018). This indicated that the milk used in the ice cream formulation contained trace amounts of TPC or that the Maillard reaction occurred during pasteurization and this was the main reason for measuring TPC values for C and CP (Sagdic et al., 2012). Additionally, it was revealed that TPC results were close to each other in samples including equal amount of OF. A similar report showed that when *B. longum* + *B. bifidum* + *L. paracasei* subsp. *paracasei* mutual culture, raspberry, blackberry ready fruit sauces and raspberry sauce were used for the production of the ice-creams, TPC values were measured between 3.14 and 6.98 mg GAE g^{-1} (Acu, 2014). Overall, these findings highlighted that the incorporation of both OF and probiotics significantly enhanced the phenolic content of the ice cream, which could potentially improve its nutritional and functional properties.

Table 3. Total phenolic content (TPC) and LAB counts of ice-cream samples.

Sample codes	TPC (mg g^{-1} GAE)	Cell numbers (log CFU g^{-1})		Yield (%)
		Mix (N_0)	Ice-cream (N_1)	($logN_1×100×N_0^{-1}$)
C	0.25 ± 0.01^e	-	-	-
OF	0.86 ± 0.03^b	-	-	-
OFS	0.77 ± 0.02^c	-	-	-
SPOF	0.82 ± 0.01^{cb}	-	-	-
CP	0.39 ± 0.01^d	7.32 ± 0.63^a	7.23 ± 0.14^a	98.77
OFP	0.94 ± 0.02^a	7.30± 0.17^a	7.38 ± 0.09^a	101.10
OFSP	0.83 ± 0.02^{cb}	7.37 ± 0.13^a	7.38 ± 0.13^a	100.14
SPOFP	0.93 ± 0.02^a	7.46 ± 0.21^a	7.43 ± 0.18^a	99.60

C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder, N_0 = Cell numbers in the mix (initial amount or concentration, log CFU/g), N_1= Cell numbers in the ice cream (final amount or concentration, log CFU/g). ^{a,b,c,d}: Means with different letters in the same column are significantly different (p<0.05).

Survival of probiotics

It is very crucial both to maintain the survival of probiotics during the extreme conditions in food processing such as freezing (dos Santos Leandro et al., 2013). In this study, probiotics were enumerated at both ice cream mix and ice cream samples in order to reveal the effect of ice cream formulation and processing in probiotic survival, as the results were presented in Table 3. The descending order of cell numbers of ice-cream mixes including probiotic bacteria were SPOFP (7.46 log CFU g^{-1}) > OFSP (7.37 log CFU g^{-1}) > CP (7.32 log CFU g^{-1}) > OF-P (7.30 log CFU g^{-1}). Moreover, cell numbers of ice-cream samples including probiotic bacteria were determined as CP (7.23 log CFU g^{-1}) > OFP = OFSP (7.38 log CFU g⁻

^{1}) > SPOFP (7.43 log CFU g^{-1}) in increasing order (Table 3). Obviously, there was no significant differences on cell numbers of probiotics depending on the different formulations both in ice-cream mixes and samples, showing that aeration, freezing, and formulation had no effect on their viability. Similarly, dos Santos Leandro et al. (2013) reported that there was no significant difference on the probiotic viability (*L. delbrueckii* UFV H2b20) depending on the different formulations (low fat, fat free and high fat) after production of ice-cream. Also, Alamprese et al. (2002) and Alamprese et al. (2005) also noted that various sugar and fat concentrations had no significant effect on the viability of *L. johnsonii* La1 and *L. rhamnosus* GG. Besides, the populations of *L. plantarum* in SPOFP and CP samples were slightly negatively affected from ice-cream processing, which may be due to the toxic effect of oxygen during the aeration, resulting in thermal shock and injury (Haghani et al., 2021; Homayouni et al., 2008). However, the percent survival of probiotics for SPOF and CP samples was still >98.77%, indicating that probiotics were able to adapt to lower temperatures, as previously reported by Kemsawasd and Chaikham, (2020). Similarly, Sauvageot et al. (2008) also reported that insignificant decreases in the number of viable cells after cold storage treatment can be explained by the ability of *Lactobacillus* spp. bacteria to adapt to low temperature and cold shock conditions. Moreover, the percent yield for the ice-cream samples coded as OFP and OFSP exceeded 100%, showing that these formulations protected the viability of probiotics against adverse environmental conditions. Also, these findings showed the postive role of OF on the growth of probiotics. OF contains numerous bioactive compounds (Yavuz et al., 2022) that can protect probiotic cells from oxidative stress and toxicity, thus increase cell viability. In the ice-cream formulations of OFP and OFSP, the symbiotic effect between *L. plantarum* and OF was clearly observed. It is well known that the minimum allowable concentration of probiotic cells that can be added to food products for health benefits is 6 log CFU g^{-1} (Lee and Salminen, 1995). The cell counts of the ice cream mixes and samples were all higher than 7 log CFU g^{-1}, but storage and *in vitro* digestion studies are needed to observe the viability of probiotics in our future studies before they can be expressed as fully probiotic ice cream.

Thermal properties

DSC thermograms (Figure 3) of the ice-cream samples exhibited an endothermic peak, which was linked to the melting of ice. Similar characteristic DSC curves were also reported by Hwang et al. (2008). Onset, midpoint, offset temperatures and enthalpy results were given in Table 4. Among the samples whose thermographs were determined, the enthalpy values of all ice cream samples (except OFS) were found to be lower compared to the control samples. Hwang et al. (2008) reported that the decrease in the final moisture content and the amount of frozen water in the sample were two potential reasons for the decrease in enthalpy values. However, in our study, we couldn't find a relationship between moisture content and entalphy. In a related study (Hwang et al. 2008), different proportions of grape wine lees were incorporated into ice creams and this phenomenon led to a decrease in enthalpy values. Results revealed that midpoint of melting temperature were slightly decreased in OFS, SPOF, OFSP, and SPOFP compared to their control sample, showing that the incorporation of OF, skimmed milk powder and sugar and their combinations led to a reduction in thermodynamic stability of ice-creams. However, contrary results were also reported. For example, Soukoulis et al. (2009) found that the supplementation of four dietary fiber sources (apple, inulin, oat and wheat) in ice creams led to an increase in melting temperatures due to the restriction of the mobility of water molecules and increased thermodynamic stability of the formulations. However, as the molecular weights of the OF supplemented ice-creams were higher compared to the reference sample formulations, the increase in the proportion of OF in the formulation led to shift lower midpoint of melting temperature. For this reason, the thermographs of OF and OFP were not visible in the Figure 3. This was probably due to the high-water retention capacity of OF and thus the decrease in the amount of freezable water. In summary, the DSC results revealed that the incorporation of OF affected the thermal properties and stability of the ice cream, with lower enthalpy values and shifted melting temperatures observed in the formulations with OF.

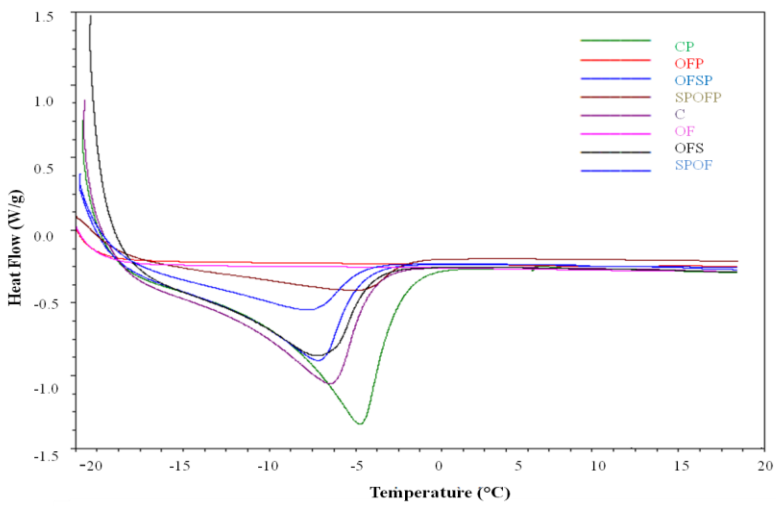

Figure 3. DSC thermographs of ice-cream samples.
C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

Table 4. Thermal properties of ice-cream samples.

Sample codes	**Melting temperature**			***ΔHm***
	Onset (°C)	**Midpoint (°C)**	**Offset (°C)**	**(J g^{-1})**
C	-11.28	-5.06	-0.77	42.57
OF	-	-	-	-
OFS	-13.41	-5.83	-0.98	47.59
SPOF	-12.99	-6.19	-1.41	17.53
CP	-8.61	-3.43	1.22	56.47
OFP	-	-	-	-
OFSP	-11.67	-5.74	-1.05	34.56
SPOFP	-12.76	-3.43	0.83	15.13

C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

Sensory evaluation

Overall acceptability scores of the ice cream samples were given in Figure 4. According to the sensory analysis, the acceptability of ice cream samples received higher scores in formulations in which OF was used as a sugar or milk powder substitute. However, they had lower sensory acceptability in formulations in which it was used as a substitute for both sugar and skimmed milk powder. This phenomenan can be due to decrease in sweetness as a result of supplementing OF instead of sugar in the formulation. Furthermore, the presence of probiotic bacteria in the formulation did not affect the overall acceptability of the ice cream, indicating that this could likely mask the unpleasant flavor of the "probiotic" (Alamprese et al., 2005). These results were in line with the findings of Shazly et al. (2022). The findings revealed that the amount of OF was the most effective parameter in the sensory acceptance of the produced ice creams. Çakmakçı et al. (2014) reported that incorporation of OF in the ratio of 2 and 3% resulted in higher general acceptability in comparison to the reference sample. Overall, symbiotic ice cream with high bioactivity, rich in dietary fiber and sensory acceptable quality were produced with the incorporation of OF and probiotic bacteria in this study.

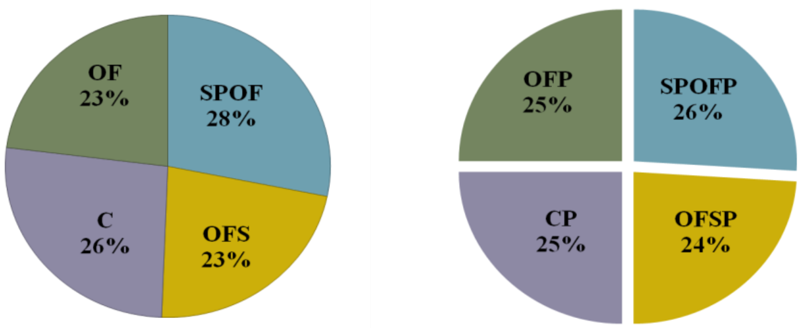

Figure 4. Overall acceptability results of ice-cream samples.
C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

Conclusions

The physicochemical, bioactive, microbiological, thermal, and sensory properties of prebiotic, probiotic, and symbiotic ice-cream samples produced with the supplementation of *L. plantarum* NRIC 1838 and/or OF were evaluated in the current study. The results revealed that the incorporation of probiotics into the ice-creams had no significant effect on the dry matter content while a slight increase in the dry matter content was observed when sugar was used instead of OF in the formulation. Incorporated ice-creams had lower pH value compared to their reference samples, due to the synergistic activity of OF and *L. plantarum* and presence of phenolic compounds in OF. Furthermore, supplementation of OF and/or probiotics led to a reduction in the lightness and yellowness values and provided an increase in TPC. Microbiological analysis revealed that aeration, freezing and formulation had no significant effect on viability, although a slight decrease in probiotic counts was detected after production of the ice creams compared to the mix forms. Enrichment with OF and/or probiotics decreased thermodynamic stability of the formulations. Sensory analysis revealed that the amount of oleaster powder was the most effective parameter in the sensory acceptance of the produced ice creams. Overall, the use of OF in the production of probiotic ice cream enabled the production of low-calorie ice cream, reducing production costs and adding economic value to the oleaster fruit for industrial purposes. As a recommendation, further study can be done for the examination of the changes in cell numbers during *in vitro* digestion and storage as well as *in vitro* antidiabetic activities.

Acknowledgments

Prebiotic and probiotic (symbiotic) ice cream with oleaster flour and production method has been registered by the Turkish Patent and Trademark Agency in Türkiye. Patent Number: TR 2019/23160.

Conflict of Interest Statement

The authors declare no conflict of interest.

Data Availability Statement

The datasets produced throughout the present study can be obtained upon reasonable request from the corresponding author.

Author contributions

Beyza Nur SUREN: Methodology, Writing original draft; Sedanur SALMAN: Methodology, Writing original draft; Emel KAYA: Methodology, Writing original draft; Yagmur BUYUKKAL: Methodology, Writing original draft; Gozde KUTLU: Investigation; Writing original draft; Review & editing, Visualisation; Fatih TORNUK: Review & editing, Supervision, Resources.

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