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The quality characteristics and shelf life of probiotic ice cream produced with Saruç and *Saccharomyces boulardii*

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Abstract

Ten types of classic and probiotic (*Saccharomyces boulardii*) ice cream were produced using different mixture combinations containing 10 or 20% Saruç, a traditional Turkish snack containing dried Cimin grape, Kemah walnut, and 0.5 or 1.0% grape seed. Physical, chemical, and microbiological analyses conducted during the storage period (days 1, 15, 30 and 60 at -20 ± 1°C) were used to assess the probiotic shelf life and quality properties of the ice cream samples. The desired therapeutic effects of *S. boulardii* levels in probiotic products were from 10^6 to 10^8 CFU/g during storage. The results revealed that Saruç, grape seed, and *S. boulardii* had a significant effect on the quality properties of ice cream samples. Titration acidity, total dry matter, fat, protein, ash, glucose, fructose, total sugar, viscosity, first dripping time, complete melting time, *a** value, and calorie (kcal) values increased with increasing concentration of Saruç. However, pH, sucrose, melting rate, *L**, and *b** values decreased. Grape seed increased titration acidity, protein, fat, ash, viscosity, first dripping time, complete melting time, and calorie values in all samples, but decreased the pH values. The addition of Saruç and grape seed positively affected the growth and viability of *S. boulardii* at levels from 10^6 to 10^8 CFU/g during storage. Taking all the quality parameters into consideration, it was shown that with the addition of Saruç, grape seed, and *S. boulardii*, new functional ice creams with high nutritional value could be produced.

Introduction

Functional foods can be defined as foods that provide basic nutrition and also positively affect the health of the consumer (Hashemi *et al.,* 2015). In recent years, studies that associated the use of food additives with increasing health problems, and the consumer's preference for "natural", "organic", and "synthetic-additive-free" healthy foods have led researchers to study bioactive components and their use in functional food production (Arslaner and Salik, 2019). Various phytochemicals, vitamins, minerals, bioactive peptides, soluble, dietary fibres, probiotics, prebiotics (Tsevdou *et al.,* 2019), whey and whey products (Tsuchiya *et al.,* 2017), and fatty acid fractions (Ullah *et al.,* 2017) are widely used for enhancing the nutritional and functional properties of ice cream. In addition, foods that include components such as fruits, wild fruits, vegetables, medicinal-aromatic plants and spices, bee products, and various sugar substitutes can also be used (Hwang *et al.,* 2009; Limsuwan *et al.,* 2014; Hashemi *et al.,* 2015; Çakmakçi *et al.,* 2015; Kavaz *et al.,* 2016; Arslaner and Salik, 2017; Vital *et al.,* 2018; Arslaner *et al.,* 2019).

Grapes and grape products (such as pulp, waste, and seed extracts) are a good alternative in the development of functional foods due to their various components (polyphenols, antioxidant components, and dietary fibres) (Nascimento *et al.,* 2018, Vital *et al.,* 2018). Saruç is a traditional snack of the Erzincan region, Turkey which is prepared by combining dried Cimin grapes with walnut (Arslaner *et al.,* 2011; Kalkan *et al.,* 2012), and it has a very high nutritional value. Very few studies have been conducted on this traditional product even though it has superior quality properties and high geographical indication potential (Arslaner *et al.,* 2011; Kalkan *et al.,* 2012; Arslaner and Salik, 2018). Erzincan Cimin grape, one of the components of Saruç, was awarded a geographical indication by the Turkish Patent and Trademark Office in 2001, and became the first registered grape of Turkey (Arslaner and Salik, 2018). Berries of this grape are round and oval, purplish and dark black colour, cloudy and thick-skinned, crispy, slightly aromatic, and have a tannin structure (Kalkan *et al.,* 2012). Grapes and grape seeds play a major role in human nutrition and health in terms of nutritional components and bioactive components (Vital *et al.,* 2018). Walnut is considered to be a natural functional food with high economic value due to its nutritional and medicinal benefits because of its

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association with minerals, polyphenolic compounds, proteins, sterols, polyunsaturated fatty acids, and tocopherols (Abdallah *et al.,* 2015).

Saccharomyces boulardii is a patented probiotic yeast isolated from the lychee fruit by a French biologist, Henri Boulard, in 1920. Lychee is a tropical fruit variety in the Indochina region (Tomicic *et al.,* 2016). *S. boulardii* yeast is not a microorganism inherent to the intestinal microflora and has been shown to be resistant to gastric acid, bile salts, and pancreatic fluids. In addition, the fact that its optimum temperature requirement is 37°C and it has the ability to inhibit the development of many pathogenic microorganisms indicate probiotic specificity (McFarland and Bernasconi, 1993; Tomicic *et al.,* 2016). *S. boulardii*, which is the only yeast that can be used as a probiotic and a biotherapeutic, is a microorganism that can be used in fermented milk and dairy products (Pandiyan *et al.,* 2012; Karaolis *et al.,* 2013; Niamah *et al.,* 2018).

In the present work, functional ice cream formulations with high nutritional value, health, and traditional properties were developed to meet consumer health demands and produce a different taste and flavour in ice cream. In the production of ice creams, a mixture of Saruç (Cimin grape and Kemah walnut) and grape seed was used, which have been reported to possess a high antioxidant content with various functional properties. In addition, *S. boulardii* was used as a probiotic to increase functional properties. The samples were analysed for various quality properties, and the effect of the addition of Saruç and grape seed on the viability of *S. boulardii* and the physicochemical properties of ice cream were also determined.

Materials and methods

Materials

Cimin grape was supplied from a local farm in the Üzümlü district, and Kemah walnut was supplied from a local farm in the Kemah district in Erzincan, Turkey; and dried by a conventional method in the village of Bayırbağ (Erzincan, Turkey). Harvested grapes that had reached maturity were dried following berry separation, selection, washing, cutting, and seed removal. Drying was carried out in the final week of September, under average weather conditions with day and night temperatures of 27.7 and 10.8°C respectively, and an average relative humidity of 45.6%. For grape berries with an initial water activity of 0.95, drying was continued until it was ≤ 0.50 on white drying cloths in a clean, dust-free, and well-ventilated environment. Grape seeds were also dried to be used in ice cream production. Dried Cimin grapes, walnuts, and grape seeds were stored at -80°C (Hettich, Switzerland) until the production of ice cream. Cow milk (3% fat, 3% protein, 7.5% non-fat dry matter, pH 6.7), emulsifier (fresh egg yolk 0.5%) and sugar were obtained from local markets. Sahlep (powdered orchid tubers) was supplied from Altındal Sahlep World (Burdur, Turkey), skimmed milk powder (1.25% fat, 36.0% protein, 95.7% non-fat dry matter, pH 6.75) was supplied by Pınar Dairy Products Co. (Istanbul, Turkey), and cream (60.0% fat, pH 5.1) was obtained from Çizmelioğlu dairy factory (Erzurum, Turkey). *Saccharomyces boulardii* CNCM I-745 was supplied by BIOCODEX Ltd. Co., Istanbul, Turkey.

Preparation of the Saruç mixture

Preparation of the Saruç mixture was essentially performed by the traditional Saruç production method of Erzincan (Arslaner and Salik, 2018). Frozen grapes and walnut were milled in Waring laboratory blender and mixed in a ratio of 60:40 grape:walnut. Saruç mix was transferred into sterile jars and pasteurised in an autoclave at 90°C for 5 min, then quickly cooled and stored at 4°C. Dried ground grape seeds were added to the ice cream mix before pasteurisation.

Preparation of the probiotic culture

Ten percent of the total milk allocated for the production of probiotic ice cream was transferred into five sterile glass bottles in equal amounts, then inoculated with a culture of lyophilised *S. boulardii* CNCM I-745 and incubated at 37°C for 8 to10 h. Fermented milk (pH 6.46) containing 10^8 CFU/mL of live probiotic yeast stored at 4°C was added to the ice cream mix before the freezing process.

Production of the ice cream samples

Ice cream production was carried out in the Dairy Technology Laboratory, Faculty of Engineering, Bayburt University (Figure 1). The ice cream mix recipe was used in accordance with the fatty ice cream definition in the Turkish Food Codex (2017). Ice cream samples were produced in 10 different combinations;

1. C = Control (no Saruç, grape seed or probiotic),
2. C1 = 10% (w/w) Saruç added,
3. C2 = 20% (w/w) Saruç added,
4. C1G = 10% (w/w) Saruç and 0.5% (w/w) grape seed added,
5. C2G = 20% (w/w) Saruç and 1% (w/w) grape seed added,
6. PC = Probiotic control (no Saruç or grape seed),
7. P1 = 10% (w/w) Saruç and probiotic added,
8. P2 = 20% (w/w) Saruç and probiotic added,
9. P1G = 10% (w/w) Saruç, 0.5% (w/w) grape seed and probiotic added, and

10. P2G = 20% (w/w) Saruç, 1% (w/w) grape seed and probiotic added.

After the addition of Saruç, matured classic and probiotic ice cream mixtures were frozen in the freezing machine (-5°C; L/30-3, SEVEL Cooling Inc. Co., Izmir, Turkey), and stored at -20°C for 60 d and sampled at storage days 1, 15, 30, and 60 (Figure 1). All measurements were duplicated.

Physical and chemical analysis

Dry matter, ash, protein (micro-Kjeldahl method), fat, pH, titratable acidity (% tartaric acid in grape and grape seed, total acidity in walnut), and water activity analyses (Novasina/LabMaster-aw) of grape, grape seed, and walnut were performed according to Cemeroglu (2010). Dry matter, ash, protein, titratable acidity, and fat contents of ice cream samples were determined based on the Association of Official Analytical Chemists (AOAC, 2005), and pH was measured with a pH meter (Mettler Toledo AG 8603 Schwerzenbach, Switzerland) at 20°C. Overrun and melting analyses of ice cream samples were performed according to Cotrell *et al.* (1979). Total calories were calculated according to Eq. 1 using conversion factors based on the Commission Regulation No 1169/2011, as defined by Arbuckle (1986):

$$\text{Carbohydrate}\,\% \times 3.87 + \text{Fat}\,\% \times 8.79 + \text{Protein}\,\% \times 4.27 \quad \text{(Eq. 1)}$$

Determination of the sugar profile

Sugar profile analyses were performed for dried Cimin grape, Saruç, and ice cream samples based on DIN 10758 (DIN, 1997) with a minor modification. A HPLC (LC-10A Series, Shimadzu, Kyoto, Japan) and refractive-index detector (RID-10A) were used. Analytes were separated by using an Inertsil NH_2 Column (4.6 × 250 mm, id 5 µm, GL Sciences, Japan) with acetonitrile-water as the mobile phase (80:20, v/v; 2 mL/min flow rate) for gradient elution. The injection volume was 20 µL and the column oven temperature was 40°C. Monosaccharides and disaccharides were identified by comparing their retention times with sugar standards (glucose, fructose, sucrose, and lactose), and external standard calibration curves were used for quantification.

Colour measurement

The colour parameters of Saruç, dried Cimin

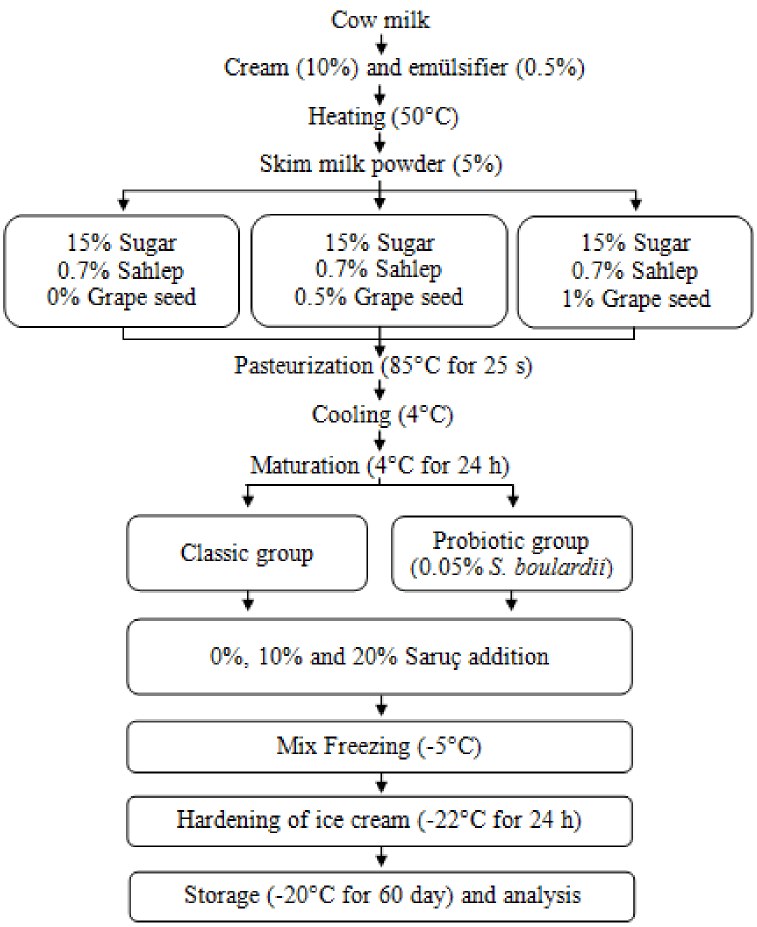

Figure 1. Production steps of ice cream samples.

grape, walnut, grape seed, and ice cream samples were obtained by measuring *L** (brightness, 0 = black, 100 = white), *a** (+ = redness, − = greenness), and *b** (+ = yellowness, − = blueness) values using a Chroma Meter (CR-300, Konica Minolta, Osaka, Japan). The hue angle (*H*°) and chroma (*C**) values were calculated using the formulas given by Chunthaworn *et al.* (2012). *H*° values are 0, 90, 180, 270 and 360°, and they refer to red, yellow, green, blue, and red, respectively (Chunthaworn *et al.,* 2012).

Microbiological analyses

The appropriate dilutions were selected to determine the number of coliform bacteria (Violet Red Bile Agar, Merck), *Staphylococcus aureus* (Baird Parker Agar, Merck), and *Escherichia coli* (Chromocult® Tryptone Bile X Glucuronide Agar, Merck) (Harrigan, 1998). To determine yeast and mould counts in the classic group ice cream samples and *S. boulardii* CNCM I-745 counts in the probiotic group samples, Dichloran Rose Bengal Chloramphenicol Agar (Merck, Darmstadt, Germany) was used per the method of Karaolis *et al.* (2013). The viability rate of *S. boulardii* CNCM I-745 was calculated using Eq. 2 as suggested by Shin *et al.* (2000):

$$\% \text{ viability} = (\text{cfu at 8 weeks of storage} / \text{initial cfu}) \times 100 \quad \text{(Eq. 2)}$$

Statistical analyses

To conduct the experiments with ten ice cream samples, four storage periods and two replications of a completely randomised factorial design was used. The effect of using Saruç, grape seed, and *S. boulardii* on each parameter was estimated by multiple analysis of variance (ANOVA) using SPSS ver. 22 statistical software. Statistically different groups ($p < 0.05$) were determined by Duncan's multiple range test.

Results and discussion

Physical and chemical properties

The results of the analysis of some physicochemical and microbiological properties of dried grape, walnut, Saruç mixture, and grape seed are given in Table 1. It is seen that the Saruç mixture had a rich nutritional composition (6.13% protein, 22.18% fat, 19.77% glucose, and 18.59% fructose). Dry matter, ash, and protein values in the Saruç mixture are similar to those reported by Kalkan *et al.* (2012). In traditional products such as Saruç, the chemical composition of the product may vary depending on the nature of the materials used.

Table 2 shows that the addition of Saruç, grape seed, and probiotic yeast (*S. boulardii*) significantly affected the physicochemical properties of the ice cream samples ($p < 0.01$). Increasing concentrations

Table 1. Properties of materials used in the production of ice cream samples.

Property	Result			
	Dry grape	Walnut	Saruç mixture	Grape seed
pH	3.29 ± 0.01	6.12 ± 0.04	3.87 ± 0.01	5.59 ± 0.01
Titration acidity (%)	2.94 ± 0.03[a]	3.90 ± 0.0[b]	1.79 ± 0.03[a]	0.67 ± 0.01[a]
Dry matter (%)	86.49 ± 0.18	96.68 ± 0.07	87.65 ± 0.51	91.49 ± 0.17
Ash (%)	3.13 ± 0.02	2.15 ± 0.06	2.13 ± 0.14	2.77 ± 0.05
Protein (%)	1.70 ± 0.09	14.00 ± 0.01	6.13 ± 0.10	7.98 ± 0.08
Fat (%)	-	66.91 ± 0.24	22.18 ± 0.20	15.93 ± 0.01
Water activity (a_w)	0.492 ± 0.01	0.477 ± 0.00	0.624 ± 0.00	0.516 ± 0.00
Glucose (%)	35.67 ± 0.62	-	19.77 ± 0.42	-
Fructose (%)	34.73 ± 0.15	-	18.59 ± 0.01	-
Sucrose (%)	0.00 ± 0.00	-	0.00 ± 0.00	-
*L**	20.81 ± 0.74	62.62 ± 1.70	18.42 ± 0.51	33.52 ± 1.15
*a**	1.48 ± 0.68	2.02 ± 0.52	7.26 ± 1.12	10.02 ± 0.16
*b**	-0.35 ± 0.26	21.51 ± 0.46	-0.25 ± 0.14	14.63 ± 0.37
H°	346.70 ± 0.64	84.64 ± 0.09	358.03 ± 0.13	55.57 ± 0.57
*C**	1.52 ± 0.01	21.61 ± 0.04	7.26 ± 0.06	17.74 ± 0.13
Coliform (log CFU/g)	-	-	< 1	-
Yeast-mould (log CFU/g)	-	-	< 1	-

[a]tartaric acid; [b]total acidity.

of Saruç and grape seed increased the total dry matter, fat, protein, and ash values in all ice cream samples. The dry matter values of the ice cream samples varied between 34.64 and 52.16%. Similar results have been reported by Kavaz *et al.* (2016) for ice cream samples containing dried Besni grape. Among the samples, the lowest fat value (8.09%) was determined in the C sample whereas the highest fat value (13.23%) was found in the P2G sample. Dry matter and fat ratios were found to be compatible with the values specified for the product groups by the Turkish Food Codex (2017). Based on Turkish Food Codex, it was seen that the ice cream samples containing 20% Saruç corresponded to the full-fat ice cream class and the other samples were included in the fat ice cream class. The fat values found in the present work were lower than those reported by Góral *et al.* (2018) for ice cream samples produced with the addition of coconut milk. On the other hand, these results are similar to those reported by Pandiyan *et al.* (2012) for symbiotic ice cream samples produced with the addition of *Lactobacillus acidophilus* and *S. boulardii*. The protein values of the samples varied between 4.18 and 5.05%. The lowest ash values (0.91 - 0.94%) were found in the C and PC samples whereas the highest ash value (1.27%) was found in the C2G sample. Similar results were reported by Kavaz *et al.* (2016). The protein, fat, and ash values obtained in the present work were higher than those reported by Nascimento *et al.* (2018) and Vital *et al.* (2018) for ice cream samples produced with the addition of industrial grape and grape juice waste, respectively.

The addition of Saruç and grape seed increased the titratable acidity values whereas the pH values decreased ($p < 0.01$) (Table 2). The lowest titratable acidity value (0.12%) was found in the C sample, while the highest titratable acidity value (0.69%) was found in the C2 and C2G samples. Similarly, the highest and lowest pH values were found in the C (6.68) and C2G (5.00) samples, respectively. This was probably due to the tartaric acid (1.79%) and other organic acids found in the composition of Saruç, which constitute a significant part of the total mix (Table 1). In the present work, it was determined that titratable acidity and pH values of the control group (C and PC), and the ice cream samples produced with the addition of 10% Saruç (C1, C1G, P1, and P1G) are similar with published literature. The titratable acidity values of the samples containing 20% Saruç (C2, C2G, P2, and P2G) were generally higher than those reported in the literature except for fermented ice creams (such as yogurt and kefir ice creams). Titratable acidity values obtained in the present work are similar to those reported by Çakmakçi *et al.* (2015) and Nascimento *et al.* (2018) for ice cream samples produced with the addition of kumquat fruit and grape waste. Hwang *et al.* (2009) reported that the pH value of the ice cream samples produced by adding grape wine lees (GWL) changed between 6.32 and 7.14. However, the pH values of all samples in the present work, except for the control group, were lower than those reported by Hwang *et al.* (2009) and Kavaz *et al.* (2016)

The effect of *S. boulardii* on the chemical composition of the ice cream samples (titratable acidity, pH, protein, and etc.) significantly differed between the samples ($p < 0.01$). This may be due to the proportional change in the components that make up the total dry matter as a result of *S. boulardii*'s use of various sugars as carbon sources (Lazo-Velez *et al.,* 2018). In published literature, it has been stated that yeasts ferment simple sugars to form carbon dioxide, water, and ethanol. In addition, it has also been stated that yeasts can convert peptides, amino acids, and sugars into aromatic / flavour compounds such as higher alcohols, organic acids, aldehydes, ketones, esters, terpenes, and lactones (Rajkowska and Kunicka-Styczynska, 2009; Tranquilino-Rodriguez *et al.,* 2017). The differences in chemical compositions between probiotic and classic group samples can be associated with the addition of fermented milk (10% of total milk) used to activate the probiotic yeast in the mix after the ripening step.

Glucose, fructose, sucrose, lactose, and total sugar values of the ice cream samples were 0.21 to 9.32%, 0.29 to 8.10%, 4.29 to 15.15%, 6.11 to 6.89% and 20.75 to 29.67%, respectively (Table 2). Increasing concentrations of Saruç increased the glucose, fructose, and total sugar values in all ice cream samples but decreased the sucrose ratio ($p < 0.01$). This may be due to the high sugar content (35.67% glucose + 34.73% fructose) of the dried grape that form the composition of Saruç (Table 1). The effect of grape seed on the sugar profile significantly differed between the samples ($p < 0.01$). It was found that *S. boulardii* significantly affected the sugar profile ($p < 0.01$). The addition of probiotic yeast increased glucose and fructose, but decreased sucrose and total sugar values. The addition of Saruç and grape seed did not affect the lactose ratio; however, the lactose ratio was generally higher in probiotic samples as compared to the classic group. Previous studies have reported that *S. boulardii* assimilated sugars including D-glucose, D-galactose, Methyl-α-D-glucopyranoside, D-maltose, D-sucrose, D-raffinose (Tranquilino-Rodriguez *et al.,* 2017), D-cellobiose, D-trehalose (Rajkowska and Kunicka-Styczynska, 2009; Tranquilino-Rodriguez *et al.,* 2017), and that assimilation

Table 2. Physicochemical and quality properties of ice cream samples.

Chemical property

Sample	Dry matter (%)	Fat (%)	Protein (%)	Ash (%)	Titration acidity (%)	pH	Sugar (%)					Calorie (kcal/100 g)
							Glucose	Fructose	Sucrose	Lactose	Total sugar	
C	35.83 ± 0.30^{f}	8.09 ± 0.07^{e}	4.18 ± 0.13^{g}	0.94 ± 0.00^{e}	0.12 ± 0.01^{i}	6.68 ± 0.00^{a}	0.21 ± 0.02^{g}	0.29 ± 0.07^{g}	15.15 ± 0.87^{a}	6.89 ± 0.56^{b}	22.55 ± 1.19^{e}	176.2^{i}
C1	43.38 ± 0.80^{de}	10.48 ± 0.19^{d}	4.52 ± 0.04^{f}	1.21 ± 0.00^{b}	0.38 ± 0.00^{g}	5.56 ± 0.03^{c}	2.84 ± 0.12^{f}	2.46 ± 0.04^{e}	12.66 ± 0.24^{b}	6.86 ± 0.04^{b}	24.82 ± 0.30^{e}	207.4^{f}
C2	51.76 ± 0.64^{a}	12.91 ± 0.19^{b}	4.97 ± 0.08^{ab}	1.24 ± 0.01^{ab}	0.69 ± 0.01^{a}	5.01 ± 0.02^{h}	7.35 ± 0.17^{b}	6.43 ± 0.43^{b}	9.12 ± 0.72^{d}	6.78 ± 0.39^{b}	29.67 ± 0.63^{a}	249.5^{a}
C1G	45.27 ± 0.17^{c}	10.54 ± 0.03^{cd}	4.77 ± 0.08^{cd}	1.25 ± 0.00^{ab}	0.46 ± 0.01^{d}	5.44 ± 0.01^{e}	3.26 ± 0.11^{e}	2.88 ± 0.06^{e}	12.38 ± 0.16^{b}	6.70 ± 0.24^{b}	25.22 ± 0.51^{c}	210.6^{e}
C2G	52.16 ± 0.77^{a}	12.98 ± 0.16^{b}	5.05 ± 0.03^{a}	1.27 ± 0.00^{a}	0.69 ± 0.00^{a}	5.00 ± 0.00^{h}	6.10 ± 0.16^{c}	5.61 ± 0.07^{c}	9.95 ± 0.18^{c}	6.53 ± 0.09^{b}	28,19 ± 0.20^{b}	245.0^{c}
PC	34.64 ± 0.20^{g}	8.19 ± 0.05^{e}	4.22 ± 0.07^{g}	0.91 ± 0.01^{e}	0.16 ± 0.01^{h}	6.46 ± 0.01^{b}	2.67 ± 0.15^{f}	1.26 ± 0.16^{f}	9.41 ± 0.30^{cd}	7.41 ± 0.10^{a}	20.75 ± 0.32^{f}	170.3^{j}
P1	42.83 ± 0.96^{e}	10.51 ± 0.05^{cd}	4.60 ± 0.04^{ef}	1.04 ± 0.01^{d}	0.45 ± 0.01^{e}	5.48 ± 0.01^{d}	5.94 ± 0.47^{c}	4.71 ± 0.57^{d}	5.77 ± 0.67^{f}	7.35 ± 0.20^{a}	23.77 ± 1.56^{d}	204.4^{h}
P2	45.23 ± 0.35^{c}	12.88 ± 0.10^{b}	4.79 ± 0.05^{cd}	1.12 ± 0.02^{c}	0.60 ± 0.00^{c}	5.19 ± 0.01^{f}	7.36 ± 0.04^{b}	5.74 ± 0.40^{c}	4.63 ± 0.06^{g}	7.53 ± 0.08^{a}	25.26 ± 0.26^{c}	231.4^{d}
P1G	44.09 ± 0.20^{d}	10.72 ± 0.24^{c}	4.69 ± 0.12^{de}	1.16 ± 0.02^{c}	0.42 ± 0.00^{f}	5.48 ± 0.01^{d}	5.56 ± 0.30^{d}	4.66 ± 0.48^{d}	6.59 ± 0.18^{e}	6.73 ± 0.06^{b}	23.54 ± 0.05^{de}	205.0^{g}
P2G	49.96 ± 0.84^{b}	13.23 ± 0.22^{a}	4.89 ± 0.11^{bc}	1.21 ± 0.03^{b}	0.65 ± 0.01^{b}	5.08 ± 0.01^{g}	9.32 ± 0.07^{a}	8.10 ± 0.08^{a}	4.29 ± 0.36^{g}	6.11 ± 0.08^{c}	27.83 ± 0.34^{b}	244.9^{b}

Physical property

Sample	Overrun (%)	First dripping time (s)	Complete melting time (s)	Melting rates (%)			Colour parameters				
				30 min	45 min	60 min	*L**	*a**	*b**	*H°*	*C**
C	13.38 ± 0.58^{e}	986 ± 98^{cd}	5169 ± 65^{d}	74.64 ± 0.56^{a}	84.08 ± 3.93^{a}	84.86 ± 3.86^{a}	87.88 ± 0.90^{a}	-4.10 ± 0.32^{g}	13.01 ± 0.62^{a}	107.31 ± 0.07^{a}	13.63 ± 0.12^{a}
C1	20.26 ± 0.62^{a}	1138 ± 89^{b}	16336 ± 322^{b}	18.97 ± 5.64^{c}	31.86 ± 1.96^{d}	36.56 ± 1.51^{d}	61.96 ± 0.80^{c}	4.32 ± 0.19^{e}	3.26 ± 0.22^{e}	36.53 ± 0.16^{d}	5.38 ± 0.03^{h}
C2	14.63 ± 0.39^{de}	NM^{e}	NM^{e}	NM^{d}	NM^{e}	NM^{e}	45.11 ± 0.87^{fg}	6.97 ± 0.35^{a}	1.09 ± 0.42^{h}	8.89 ± 0.46^{i}	7.06 ± 0.08^{e}
C1G	10.10 ± 0.13^{f}	1893 ± 60^{a}	18019 ± 99^{a}	0.00 ± 0.00^{d}	25.91 ± 8.44^{d}	30.68 ± 7.31^{d}	54.61 ± 0.18^{d}	5.21 ± 0.29^{d}	2.84 ± 0.32^{ef}	28.64 ± 0.15^{f}	5.94 ± 0.07^{g}
C2G	17.10 ± 0.14^{cd}	NM^{e}	NM^{e}	NM^{d}	NM^{e}	NM^{e}	33.92 ± 0.59^{h}	6.34 ± 0.32^{b}	1.65 ± 0.42^{g}	14.58 ± 0.25^{h}	6.56 ± 0.06^{f}
PC	19.62 ± 0.17^{ab}	931 ± 65^{d}	5018 ± 145^{d}	81.49 ± 4.60^{a}	84.85 ± 3.77^{a}	85.48 ± 3.30^{a}	81.92 ± 0.74^{b}	-1.55 ± 0.67^{f}	8.64 ± 0.79^{b}	100.16 ± 0.62^{b}	8.78 ± 0.19^{b}
P1	17.28 ± 0.96^{bc}	1043 ± 43^{bc}	14228 ± 168^{c}	23.25 ± 2.78^{c}	44.76 ± 8.74^{c}	47.53 ± 6.61^{c}	43.73 ± 0.53^{g}	5.77 ± 0.21^{c}	3.92 ± 0.24^{d}	34.17 ± 0.20^{e}	6.98 ± 0.01^{e}
P2	16.53 ± 0.75^{cd}	NM^{e}	NM^{e}	NM^{d}	NM^{e}	NM^{e}	46.89 ± 0.05^{f}	7.16 ± 0.25^{a}	2.39 ± 0.52^{f}	18.45 ± 0.29^{g}	7.56 ± 0.02^{d}
P1G	13.88 ± 0.13^{e}	1101 ± 153^{b}	16528 ± 260^{b}	56.38 ± 5.53^{b}	67.44 ± 3.75^{b}	69.48 ± 2.78^{b}	52.31 ± 0.74^{e}	5.37 ± 0.24^{d}	5.11 ± 0.47^{c}	43.56 ± 0.35^{c}	7.42 ± 0.01^{d}
P2G	10.53 ± 0.11^{f}	NM^{e}	NM^{e}	NM^{d}	NM^{e}	NM^{e}	47.05 ± 0.20^{f}	7.19 ± 0.17^{a}	4.00 ± 0.01^{d}	29.10 ± 0.64^{f}	8.24 ± 0.02^{c}

C = control (without Saruç, grape seed and probiotic); C1 = 10% (w/w) Saruç added; C2 = 20% (w/w) Saruç added; C1G = 10% (w/w) Saruç and 0.5% (w/w) grape seed added; C2G = 20% (w/w) Saruç and 1% (w/w) grape seed added; PC = Probiotic control (without Saruç and grape seed); P1 = 10% (w/w) Saruç and probiotic added; P2 = 20% (w/w) Saruç and probiotic added; P1G = 10% (w/w) Saruç, 0.5% (w/w) grape seed and probiotic added; P2G = 20% (w/w) Saruç, 1% (w/w) grape seed and probiotic added; NM = no melting. Means with different superscript small letters in the same column are significantly different from each other ($p < 0.01$).

of sugars varied depending on the strain. Based on the results obtained in the present work, the effect of *S. boulardii* on the sugar profile can be explained by the ability of this yeast to assimilate sugars. Figure 2 shows that the *S. boulardii* CNCM I-745 strain used in the production of probiotic group ice creams used sucrose as the carbon source. Hashemi *et al.* (2015) found that the lowest sucrose value was 13.29% whereas the highest sucrose value was 19.29% in a low-calorie probiotic, prebiotic and symbiotic functional ice cream samples produced using inulin and lactulose.

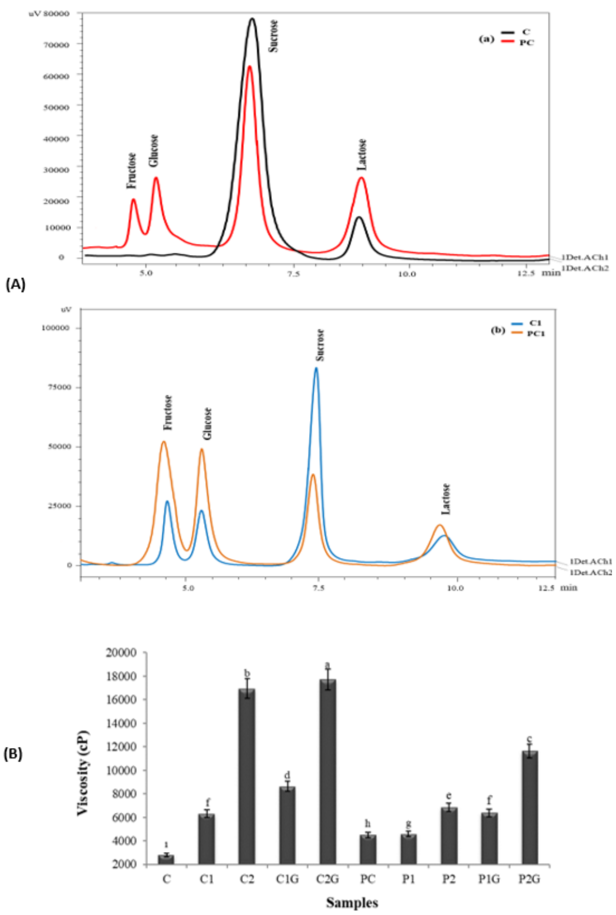

Figure 2. (A) The sugar chromatograms of the control group (a), and with 10% Saruç, (b) ice cream samples; and (B) Viscosity values of ice cream mixes. Different letters above the bars indicate significant differences ($p < 0.01$) by using the Duncan multiple comparison test. C = control (without Saruç, grape seed and probiotic); C1 = 10% (w/w) Saruç added; C2 = 20% (w/w) Saruç added; C1G = 10% (w/w) Saruç and 0.5% (w/w) grape seed added; C2G = 20% (w/w) Saruç and 1% (w/w) grape seed added; PC = probiotic control (without Saruç and grape seed); P1 = 10% (w/w) Saruç and probiotic added; P2 = 20% (w/w) Saruç and probiotic added; P1G = 10% (w/w) Saruç, 0.5% (w/w) grape seed and probiotic added; P2G = 20% (w/w) Saruç, 1% (w/w) grape seed and probiotic added.

The calorific value of ice cream samples varied between 170.3 kcal/100 g and 249.5 kcal/100 g ($p < 0.01$). It was determined that the calorific value in the samples increased with increasing concentration of Saruç. This was an expected result considering the fat, protein, and sugar contents of Saruç. The use of probiotic yeast significantly reduced the calorific value ($p < 0.01$). This was associated with a decrease in total sugar content as a result of *S. boulardii*'s use of sugar as a source of carbon (Rajkowska and Kunicka-Styczynska, 2009; Tranquilino-Rodriguez *et al.,* 2017). The calorific values calculated here were generally higher than those reported in published literature (Limsuwan *et al.,* 2014; Arslaner and Salik, 2017).

The ice cream mixture should have a certain viscosity value in terms of various quality properties (Goff and Hartel, 2013). Many factors such as the fat ratio, the use of stabiliser, pasteurisation, homogenisation, and the ripening process contribute to the viscosity of the ice cream mixture (Arbuckle, 1986). Also in fruit ice-cream, the composition of the fruit used in production (the constituents of dry matter such as protein and fat, the presence of stabilising components such as fibre, pectin, and water), and the method adopted for adding the fruit to the mix (directly or in heat-treated form, before or after ripening) affect the flow characteristics of the mix. The viscosity values of the ice cream samples at 50 rpm varied in a range from 2790 to 17730 cP (Figure 2). The increase in the concentrations of Saruç and grape seed increased the viscosity in all ice cream samples ($p < 0.01$). The lowest viscosity value was determined in the C sample whereas the highest value was determined in the C2G sample. This increase can be associated with the high dry matter content of Saruç (87.65%), given that Saruç constitutes a significant portion of the total mix and the fibre in its composition has a high water-binding capacity. In addition, due to the addition of Saruç, the increase in glucose, fructose, and total sugar in ice cream samples might have increased the viscosity. The viscosity-increasing effect of grape seed can be associated with the high fibre content and various stabilising components in its composition. Kavaz et al. (2016) found that viscosity increased with increasing concentrations of dried Besni grape in ice cream samples. Similarly, Hwang *et al.* (2009) reported increased viscosity in samples as a result of the addition of grape wine lees (GWL) in increasing concentrations. It was determined that *S. boulardii* had a significant ($p < 0.01$) effect on the viscosity values in ice cream samples and decreased viscosity in samples other than the control groups (C and PC).

As shown in Table 2, the addition of Saruç, grape seed, and probiotic yeast (*S. boulardii*) significantly affected the overrun, first dripping time, complete melting time and melting rate values of the ice cream samples ($p < 0.01$). The overrun is directly related to the yield of ice cream, as well as the structure, texture, and flavour properties of the product (Sofjan and Hartel, 2004). Therefore, in a quality ice cream, the incorporation of air should be adequate to provide the desired overrun rate. When excessive air is whipped into a mix during freezing it can yield a snowy, foamy, and unsavoury product whereas inadequate air can lead to a tough structure and a heavy product (Goff and Hartel, 2013). The effect of the addition of Saruç, grape seed, and probiotic yeast on overrun values significantly differed between the samples ($p < 0.01$). The lowest overrun (10.10%) was found in the C1G whereas the highest value (20.26%) was found in the C1 sample. This can be associated with the different ice cream formulations, the high dry content of the samples, the composition, and properties of Saruç, and the technical properties associated with the ice cream machine. The overruns determined in the present work were lower than those reported by Hwang *et al.* (2009) and Tsevdou *et al.* (2019). On the other hand, similar results have been reported by Yeon *et al.* (2017) in ice cream samples produced with the addition of fermented pepper powder.

Melting properties are affected by many factors including the amount of air in the ice cream and the structure of the ice crystals (Muse and Hartel, 2004). The first dripping and complete melting times of the samples ranged from 931 to 1893 s and 5018 to 18019 s, respectively (Table 2). The lowest first dripping and complete melting times were found in the PC sample whereas the highest values were found in the C1G sample. There was no melting detected in samples containing 20% Saruç and 1% grape seed ($p > 0.01$). At the 30, 45, and 60th min, the lowest melting rate was observed in the C1G sample whereas the highest melting rate was observed in the PC sample. The addition of 10% Saruç and 0.5% grape seed increased the first dripping and complete melting time in all samples. The addition of 10% Saruç decreased the melting rates at the 30, 45, and 60th min. The effect of adding 0.5% grape seed differed between the samples. This can be attributed to the increased protein and fat ratios in the composition of ice creams due to the increase in the total dry matter as a result of adding Saruç to the mix. Thus, the reduction of the water content in its free form as a result of the emulsion of oil and water present in the composition of samples containing Saruç may have had an effect on the melting properties of the ice cream samples. Furthermore, the water-soluble or insoluble fibre found in the composition of Saruç may have bound some of the water or affected the emulsion system of the ice creams prolonged the melting process.

It was found that *S. boulardii* had a significant ($p < 0.01$) effect on the melting properties of ice cream samples. It was found that the addition of *S. boulardii* reduced the first dripping and complete melting time of the samples, and increased the melting rate at the 45 and 60th min in all the samples except for the control groups (C and PC). The fact that melting in probiotic ice cream samples started earlier in comparison to the classic product group and the consequent higher melting rate can be attributed of the change in the sugar profile (sugar form, amount, and solubility) as a result of the metabolic activity of S. boulardii.

Colour properties of the ice cream samples

In ice cream, colour is the first parameter that captures the consumers' attention before taste and aroma. Therefore, the colour parameters (L^*, a^*, b^*, $H°$, and C^*) were analysed to determine the effect of the addition of Saruç at different concentrations on the ice cream samples (Table 2). The effects of Saruç, grape seed, and probiotic yeast on colour values were statistically significant ($p < 0.01$). The value of the colour parameter L^* decreased significantly ($p < 0.01$) in all samples except for the control groups (C and PC). Similar results have been reported by Hwang *et al.* (2009), Çakmakçi *et al*. (2015), Vital *et al.* (2018), and Tsevdou *et al.* (2019). The lowest L^* value (33.92) was observed in C2G, whereas the highest L^* value (87.88) was observed in the control C sample. The lowest and the highest a^* values were -4.10 and 7.19 determined in the C and P2G samples, respectively. The b^* value of the samples varied between 1.09 and 13.01. The $H°$ values of the C and PC samples represent a colour in the yellow-green region since they were between 90 and 180°. With the addition of Saruç, this region shifted to the red-yellow region between 0 and 90°. It is seen that with increasing Saruç concentration, $H°$ approached 0° and the hue became darker. Chroma (C^*), which indicates the degree of saturation, purity, or density of the visual colour (Chunthaworn *et al.,* 2012) in the samples, varied over a range from 5.38 to 13.63. It was associated with anthocyanin and dark purple colour pigments composition of Saruç. The effects of grape seed and probiotic yeast on colour values differed between the samples.

Microbiological properties

To determine the microbiological quality of ice cream samples as a baseline, no coliform bacteria (< 1 logCFU/g), *E. coli* (< 1 logCFU/g), *S. aureus* (< 2 log CFU/g), and yeast-mould (< 1 log CFU/g) were detected as a result of the analyses carried out on the 1st day of the storage. The results showed that Saruç was effectively pasteurised and there were no contaminants in the samples. Yeast-mould and coliform bacteria can be found in Saruç production with traditional methods since the pasteurisation process is not applied (Arslaner and Salik, 2018).

Table 3 shows that the lowest *S. boulardii* count was determined in the probiotic control sample (PC = 6.50 log cfu/g) on the 30th day of storage, whereas the highest *S. boulardii* count was determined in the probiotic sample (P1G = 6.80 log CFU/g) containing 10% Saruç and 0.5% grape seed on the 1st day of storage ($p < 0.01$). Although there was generally a decrease in *S. boulardii* counts with an increasing concentration of Saruç, it was observed that the addition of Saruç increased probiotic yeast counts as compared to the control group ($p < 0.01$). The effect of grape seed on *S. boulardii* counts differed among the samples ($p < 0.01$). On the 30, 45, and 60th days of storage, the differences in *S. boulardii* counts were not statistically significant except for the P2G sample ($p > 0.01$). *S. boulardii* counts did not fall below 10^6 CFU/g during the 60-day storage in all ice cream samples and it was determined that the *S. boulardii* counts were at levels that can provide the desired therapeutic effects in probiotic products (10^6 to 10^8 CFU/g) (Kailasapathy *et al.,* 2008). The lowest viability rate (96.62%) for *S. boulardii* was found in the P1G sample, whereas the highest viability rate (103.06%) was found in the P2G sample ($p < 0.01$). Saruç used in two different ratios (10 and 20%) for production increased the viability of *S. boulardii* as compared to that in the control group (PC) ($p < 0.01$). Although the effect of grape seed on the viability of *S. boulardii* varied among the samples, it was found that the use of grape seed increased the viability rate by 1.0%. This was attributed to the rich composition of the P2G sample.

In synbiotic ice cream samples produced using *L. acidophilus* and *S. boulardii*, Pandiyan *et al.* (2012) determined the *S. boulardii* counts to be in the range from 6.37 to 7.26 log CFU/g during 15-days of storage. Niamah et al. (2018) reported that S. boulardii counts varied between 5.25 and 8.55 log CFU/g during 21 d of storage in probiotic ice cream samples produced by adding 5% (33×10^9 CFU/g) *S. boulardii* in different forms. As a result of the literature research, it was found that *S. boulardii* preserves its viability better in ice cream as compared to in yogurt. In some studies on yogurt, it has been reported that *S. boulardii* counts fell below 10^6 CFU/g by the end of the storage (Karaolis *et al.,* 2013).

Conclusion

In the present work, the combination of Saruç, grape seed, and *S. boulardii* which have high nutritional values and various functional properties were used in the production of functional ice cream. To the best of the authors' knowledge, this is the first study on the use of Saruç, grape seed, and *S. boulardii* in enriched fruit ice cream. To conclude, it was determined that the use of Saruç, grape seed, and *S. boulardii* did not have a negative effect on the physical, chemical, and microbiological characteristics of the ice cream samples. The addition of Saruç and grape seed increased the nutritional value of all ice cream samples and positively affected the development and viability of *S. boulardii* in probiotic samples. *S. boulardii* maintained the viability during 60 d of storage and live cell counts were at levels (10^6 to 10^8 CFU/g) that can provide the desired

Table 3. *S. boulardii* count and viability ratios in ice cream samples during storage period of 60 days.

Sample	Mix	Storage (day)				Viability ratio (%)
		1	15	30	60	
PC	6.74 ± 0.01^{d}	6.79 ± 0.10^{abA}	6.52 ± 0.01^{cB}	6.50 ± 0.03^{cB}	6.58 ± 0.01^{bB}	96.91^{d}
P1	6.85 ± 0.00^{c}	6.72 ± 0.02^{bA}	6.68 ± 0.07^{aA}	6.69 ± 0.05^{aA}	6.71 ± 0.02^{aA}	99.85^{b}
P2	6.92 ± 0.01^{b}	6.78 ± 0.06^{abA}	6.62 ± 0.11^{abB}	6.57 ± 0.07^{bB}	6.58 ± 0.01^{bB}	97.05^{c}
P1G	6.92 ± 0.01^{b}	6.80 ± 0.10^{aA}	6.62 ± 0.04^{abB}	6.56 ± 0.05^{bB}	6.57 ± 0.08^{bB}	96.62^{e}
P2G	7.00 ± 0.00^{a}	6.54 ± 0.04^{cC}	6.60 ± 0.01^{bB}	6.59 ± 0.01^{bB}	6.74 ± 0.03^{aA}	103.06^{a}

PC = probiotic control (without Saruç and grape seed); P1 = 10% (w/w) Saruç and probiotic added; P2 = 20% (w/w) Saruç and probiotic added; P1G = 10% (w/w) Saruç, 0.5% (w/w) grape seed and probiotic added; P2G = 20% (w/w) Saruç, 1% (w/w) grape seed and probiotic added. Means with different superscript small letters in the same column are significantly different from each other ($p < 0.01$). Means with different superscript capital letters in the same row are significantly different from each other ($p < 0.01$).

therapeutic effects in probiotic products. *S. boulardii* used sucrose as source of a carbon in the ice cream samples and significantly changed their sugar profile. Results also showed that *S. boulardii* can be used as a probiotic in the development of plain and fruity functional ice cream. In ice cream production, the most appropriate Saruç ratio was determined to be 10% in terms of nutritional value, taste, and some quality characteristics. The use of grape seed did not cause any negativity, and it was further concluded that it can be used in ice cream production at a ratio of 1%. Taking all the quality parameters into consideration, it was shown that new functional ice cream with high nutritional value can be produced with the addition of Saruç, grape seed, and *S. boulardii*. It is suggested that future studies should focus on the prolongation of the storage period (75, 90 and 105 d) in fruit ice creams containing *S. boulardii*, and on the determination of the storage in which live cell counts fall below 10^6 log CFU/g in these ice creams.

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