

# Physical properties and bacterial viability of functional ice cream enriched with kefir

Nazlı KANCA<sup>1,a,✉</sup>, Bülent BAŞ<sup>2,b</sup>, Rabia ALBAYRAK DELIALİOĞLU<sup>3,c</sup>, Ayşe GÜRSOY<sup>1,d</sup>

<sup>1</sup>Ankara University, Faculty of Agriculture, Department of Dairy Technology, Ankara, Türkiye; <sup>2</sup>Ankara University, Faculty of Veterinary Medicine, Department of Microbiology, Ankara, Türkiye; <sup>3</sup>Ankara University, Faculty of Agriculture, Biometry and Genetics Unit, Ankara, Türkiye

<sup>a</sup>ORCID: 0000-0002-4219-8903; <sup>b</sup>ORCID: 0000-0001-9992-8738; <sup>c</sup>ORCID: 0000-0002-1969-4319; <sup>d</sup>ORCID: 0000-0001-6077-6087

## ARTICLE INFO

### Article History

Received : 13.10.2021

Accepted : 11.05.2022

DOI: 10.33988/auvfd.1008552

### Keywords

Kefir

*Lactobacillus* spp.

*Lactococcus* spp.

Probiotic ice cream

### ✉Corresponding author

nazli.turkmen@ankara.edu.tr

## ABSTRACT

In this study, different ratios (A-0%, B-25%, C-50%, D-75%) of kefir were used in the ice cream mix in order to obtain functional ice cream enriched with probiotic bacteria. There was no difference between the chemical and physical properties of the samples ( $P<0.05$ ), except for acidity and overrun values ( $P>0.05$ ). Kefir containing samples showed probiotic properties during 90-day storage when the probiotic bacterial counts were considered. In terms of texture and flavor properties, sample D had the lowest scores, while B and C had similar scores compared to sample A in sensory evaluation. As a result, B and C were identified as probiotic products with acceptable properties during 90-day storage.

**How to cite this article:** Kanca N, Baş B, Albayrak Delialioğlu R, Gürsoy A (2023): Physical properties and bacterial viability of functional ice cream enriched with kefir. Ankara Univ Vet Fak Derg, 70 (3), 293-301. DOI: 10.33988/auvfd.1008552.

## Introduction

Ice cream is a product that is produced worldwide and consumed by individuals of all ages. It is a highly nutritious product due to the ingredients used. The composition of ice cream can be changed easily in comparison to the other dairy products, giving it a special place among other functional products that are increasing in production and consumption (38).

Enrichment of ice cream by probiotics and/or prebiotics has been the subject of majority of research. In these studies, the production is usually carried out by adding pure probiotic culture and/or prebiotic compounds directly to the ice cream mix (4, 18, 30).

Probiotics are microorganisms that have a positive effect on the intestinal system when consumed in a certain amount (17). Probiotics in the intestinal microflora improve the immune system, protect the body against various diseases and they also have anticarcinogenic and

serum cholesterol-lowering effects (28). Lactic acid bacteria are the most commonly used bacteria group for enriching foods with probiotic microorganisms. Prebiotics, on the other hand, are substances that directly enter the intestinal system when taken into the body and stimulate the growth of probiotic bacteria (17). They were reported to have positive effects on the digestion of sugars, protective effects against heart disease risk and inhibiting effects on pathogenic microorganisms (28). Prebiotic substances are generally classified as inulin, oligofructoses and fructooligosaccharides (27).

Enriching ice cream with probiotics is a more appropriate way compared to other dairy products. This was explained by the higher pH of ice cream compared to those of fermented milk products since the survival of probiotic bacteria in low pH environments is low (1).

Kefir, one of the richest products in terms of probiotic microorganisms, is a fermented dairy product in

which starter cultures or kefir grains consisting of ~83-90% lactic acid bacteria and ~10-17% yeast and acetic acid bacteria are used in the production (20, 39). These cultures usually include different strains of *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Acetobacter* genera and also lactose fermenting (*Kluyveromyces marxianus*) and non-fermenting yeasts (*Saccharomyces unisporus*, *Sacch. cerevisiae* and *Sacch. exiguus*) (15, 36). Since most of these microorganisms have probiotic properties, the evaluation of kefir in different ways has become an increasingly interesting issue.

The aim of this study is to enrich ice cream with probiotic bacteria and to obtain a functional product with increased nutritional value. For this purpose, kefir, was added to ice cream mix in different proportions and ice cream production was carried out. The study is considered to be important for obtaining an ice cream with probiotics that is not available in the market and has a higher nutritional value than both ice cream and kefir.

## Materials and Methods

**Kefir and ice cream preparation:** For the preparation of kefir, raw cow's milk (Ankara University Faculty of Agriculture Research and Application Farm, Ankara, Türkiye) was subjected to heat treatment at 90 °C/10 min and cooled to 25 °C. Immediately after the cooling process, kefir culture (CHOOZIT® Kefir DC, LYO 1000 L, Danisco, Germany) was inoculated (according to the ratio specified on the package, 5 g/1000 L) and the samples were incubated at 25 °C until pH 4.5-4.6 and kept at +4 °C for ~24 h until being used in ice cream mix preparation.

All ice cream mixes were formulated to contain 10% fat (derived from 65% fat cream; Ankara University Faculty of Agriculture Research and Application Farm, Ankara, Türkiye), 12% milk solids-not-fat (derived from cream and skim milk powder; Izi Sut A.S., Türkiye), 15% sucrose (derived from sugar obtained from local market) and 0.5% stabilizer-emulsifier mixture (Cremodan Sim Veg, Danisco, Germany). Mixing rates of kefir and ice cream mixes were shown in Table 1. In the preparation of the mixes, the amount of fat and milk solids-not-fat to be covered from kefir to be added to each sample was calculated separately, and these values were subtracted from the amount of the main substance desired to be in the final product, and the remaining amount was calculated from 2250 ml, 1500 ml and 750 ml mixes, respectively. The prepared mixes were heat treated at 80 °C for 20 min and homogenized with ultraturrax (DIAX 900, Heidolph, Schwabach, Germany) for 5 min. Subsequently, the mixes were promptly cooled to ~25 °C and then aged for approximately 19 h at +4°C. At this point, the aged mixes were inoculated with previously prepared kefir culture in

the amounts specified in Table 1 and the final mixture was re-homogenized with ultraturrax for 10 min. 3 L of ice cream mixes were frozen in a batch freezer (Triomaxx, Ada, Jiangmen, China) for 15 min and the samples were packaged and hardened at -25 °C for 20 h. All ice cream formulations were produced in duplicate.

**Table 1.** Mixing rates of kefir and ice cream mixes used in ice cream production.

Sample code	Kefir ratio (%)	Kefir used in mixture (ml)	Ice cream mix used in mixture (ml)
A (Control)	0	0	3000
B	25	750	2250
C	50	1500	1500
D	75	2250	750

### Determination of lactic acid, total solid, fat, total protein and ash contents:

Lactic acid contents of the ice cream samples were determined by using the titration method and the results were calculated as percent lactic acid (8). Fat contents were determined by Gerber method (6), total dry matter and ash contents were determined by gravimetric method according to AOAC (7) and Goff et al. (14), respectively. Kjeldahl method was used to determine the total protein content by multiplying the total nitrogen content by the factor of 6.38 (6).

### Rheological measurements:

The rheological properties of the samples were determined by Malvern Kinexus Pro-rheometer (Worcestershire, UK) with a cone and plate geometry (diameter: 40 mm, cone angle: 4°). The consistency index ( $K$ ) and flow behavior index ( $n$ ) of the samples were determined by dynamic rheometry at 2 mm gap, 0.1-300 s<sup>-1</sup> shear rate at 5 °C. The data obtained from the analysis were adjusted to the Herschel-Bulkley model based on the following equation:

$$\tau = \tau_0 + K \dot{\gamma}^n \quad (1)$$

where  $\tau$  is the shear stress (Pa),  $\tau_0$  is the yield stress (Pa),  $K$  is the consistency coefficient (Pa s<sup>n</sup>),  $\dot{\gamma}$  is the shear rate (s<sup>-1</sup>) and  $n$  is the flow behavior index.

All measurements were performed at least in duplicate.

**Overrun measurement:** Overrun was measured by comparing the weight of a certain volume of ice cream mix and the same volume of ice cream. Overrun results were calculated by using the weights recorded according to the equation below (14):

$$\text{Overrun (\%)} = \frac{(\text{Weight of mix} - \text{Weight of ice cream})}{(\text{Weight of ice cream})} \times 100 \quad (2)$$

**Melting characteristics:** The method specified by Mendez-Velasco et al. (22) was applied in order to determine the meltdown rates of ice cream. Samples were removed carefully from the containers and their weight were recorded. Ice creams were placed on a stainless steel wire with 2.5 mm<sup>2</sup> holes and a glass beaker placed underneath to collect the melted part. The analysis performed at room temperature (~22 °C) and the first dripping time of each sample was recorded in min. In addition, the weight of drained material through the wire was recorded every 20 min for 120 min, and the meltdown rates of ice cream were calculated according to the following formula:

$$\text{Meltdown rate (\%)} = (\text{Weight of drained material} / \text{Weight of ice cream}) \times 100 \quad (3).$$

In addition, the average melting rates were calculated considering the amounts of the dripped portion after a total of 120 min and expressed in g/min. Also the weight of the drained material of ice cream after 120 min was recorded and the percent mass retention was calculated by using the following equation (4):

$$\text{Mass retention (\%)} = 100 - \text{Drained material after 120 min (\%)} \quad (4).$$

**Hardness measurement:** The hardness values of the samples were determined by using a texture analyzer (TA.Xt Plus, Stabel Micro Systems®) equipped with a 5 mm diameter cylindrical stainless steel probe (Part Code: P/5, Stable Micro Systems®). The samples were kept at -15 °C for 24 h before analysis. Three measurements were recorded from three different containers for each sample and the average of these measurements was calculated. The parameters for analysis specified by Akalin et al. (4) were as follows: penetration distance = 15 mm, force = 5.0 g, probe speed during penetration = 3.3 mm s<sup>-1</sup>, probe speed pre- and post penetration = 3.0 mm s<sup>-1</sup>.

**Bacteriological analysis:** Ice cream samples (10 g) were diluted in 90 ml sterile Ringer solution (Merck, Darmstadt, Germany) and homogenized in a Stomacher (Bag Mixer 400 VW, Interscience, France) for 2 min. Subsequent serial dilutions were prepared in 9 ml sterile Ringer solution and poured onto plates of the various selective and differential agars in duplicate. M17 agar (Merck, Darmstadt, Germany) and MRS agar (de Man Ragoza Sharpe Agar, Merck, Darmstadt, Germany) were used for the enumeration of *Lactococcus* spp. and *Lactobacillus* spp. respectively. All plates were incubated at 37 °C for 24-48 h. The applied incubation conditions were aerobic and anaerobic for M17 agar and MRS agar respectively. Colonies were enumerated after the incubation and the results were expressed as log cfu/g.

**Sensory evaluation:** Approximately 25 g of ice cream were scooped into 50 ml plastic containers and kept at -15

°C for ~2 h before evaluation. For the sensory evaluation of ice cream samples, a scoring test with 7 experienced panelists from the academic staff of Ankara University Department of Dairy Technology were applied. The test form suggested by Meilgaard et al. (21) were modified and used for sensory analysis. Panelists were asked to evaluate the samples over 5 points in terms of appearance, texture and flavor characteristics. The panelists evaluated the four samples in the same session. Drinking water and unsalted crackers were provided to clean the mouth before and between the samples.

**Statistical analysis:** Total solid, fat, total protein, ash contents, rheological characteristics, overrun values, melting characteristics and hardness values were determined only on the 1st day of the storage. The other analysis were performed on the 1<sup>st</sup>, 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> days of the storage. All analysis were performed in duplicate for each parameter.

Analysis of variance (One-way ANOVA) method was used to evaluate the differences between kefir ratios (0, 25, 50, 75%) in terms of total solid, fat, total protein, ash, rheological characteristics, overrun, first dripping time, average melting rate, mass retention and hardness parameters. The Repeated Measures ANOVA method was used to determine whether the differences between the level means of the kefir ratios (0, 25, 50, 75%), time (40, 60, 80, 100, 120 min) factors and their interaction on the meltdown rates are statistically significant. The differences between the kefir ratios (0, 25, 50, 75%) and storage time (1, 30, 60, 90 days) and their interaction in terms of titratable acidity, bacterial counts and sensory characteristics were evaluated using the Factorial ANOVA method. Tukey multiple comparison test was used to separate means of data when significant differences (P<0.05) were observed. IBM SPSS Statistics 20 software was used for statistical analysis and the results were expressed as mean ± standart error.

## Results

**The chemical composition of ice cream samples:** The chemical composition of ice cream samples is given in Table 2 (P>0.05). The samples produced in this study were classified as fatty ice cream according to the Turkish Food Codex Communique on Ice Cream (35) since the total solid and fat contents of fatty ice cream should be at least 36% and 8% respectively.

**Rheological characteristics:** In Table 3, consistency index (*K*) and flow behavior index (*n*) values of the samples are given. All of the ice cream mixes were compatible with the "Herschel-Bulkley" behavioral model (correlation value - R<sup>2</sup> > 0.99) and no difference was found between the *K* and *n* values of the samples interpreted according to this model (P>0.05).

**Table 2.** The chemical composition of the samples (n=2).

	Samples*			
	A	B	C	D
Total solid (g/100 g)	36.87 ± 0.019	36.68 ± 0.019	36.61 ± 0.015	36.81 ± 0.027
Fat (g/100 g)	10.50 ± 0.000	10.25 ± 0.250	10.25 ± 0.250	10.25 ± 0.250
Total protein (g/100 g)	3.56 ± 0.035	3.56 ± 0.060	3.57 ± 0.055	3.54 ± 0.050
Ash (g/100 g)	0.87 ± 0.011	0.83 ± 0.015	0.85 ± 0.002	0.85 ± 0.009

\*A: 0% kefir - Control, B: 25% kefir, C: 50% kefir, D: 75% kefir.

**Table 3.** The consistency index (K), flow behavior index (n) and hardness values of the samples (n=2).

	Samples*			
	A	B	C	D
K (Pa.s)	0.23 ± 0.006	0.23 ± 0.002	0.23 ± 0.009	0.23 ± 0.003
n	0.72 ± 0.011	0.72 ± 0.024	0.72 ± 0.013	0.72 ± 0.008
Hardness (g)	15 539 ± 190	16 096 ± 315	15 628 ± 886	15 945 ± 390

\*A: 0% kefir - Control, B: 25% kefir, C: 50% kefir, D: 75% kefir.

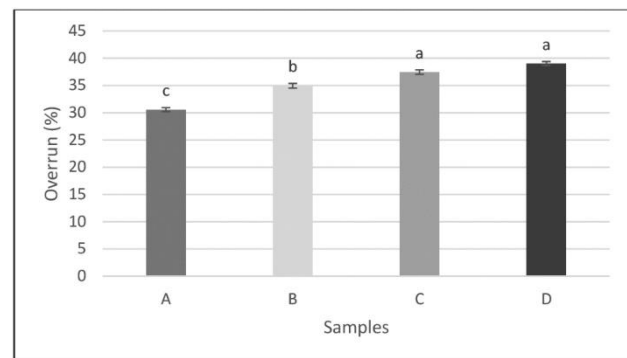
**Overrun:** As it is seen from Figure 1, the overrun values of the samples C and D with the highest kefir content were found to be the highest, while sample A without kefir was determined as the sample with the lowest overrun ( $P < 0.05$ ).

**Melting characteristics:** It was determined that there was no difference between the first dripping times, average melting rates, and remaining mass retentions at the end of 120 min ( $P > 0.05$ ) as it is seen from Table 4. Similarly, no difference was found between the meltdown rates of the samples at 40, 60, 80, 100 and 120 min ( $P > 0.05$ ) (Figure 2).

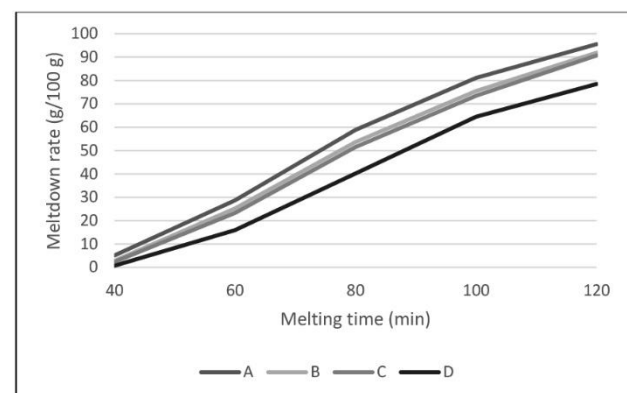
**Hardness:** Hardness values of the ice cream samples are given in Table 3. According to the results, kefir addition did not affect the hardness values of the samples ( $P < 0.05$ ).

**Lactic acid:** Use of kefir in ice cream mix affected the lactic acid content of the final product during 90-days of storage (Table 5). As the amount of kefir in mix increased, the lactic acid content of the product was also increased at all storage days.

**Bacterial counts:** As expected, *Lactococcus* spp. and *Lactobacillus* spp. counts increased for each day of storage ( $P < 0.05$ ) as the kefir amount increased (Table 5). In addition, *Lactococcus* spp. counts were higher compared to *Lactobacillus* spp. counts at all storage days. However, bacterial viability decreased for both probiotic bacteria during 90-days of storage ( $P < 0.05$ ), but all of the samples still maintained their probiotic properties since they contained at least  $10^7$  cfu/g (7 log cfu/g) of probiotic bacteria.

**Figure 1.** Overrun values of the samples.

A: 0% kefir - Control, B: 25% kefir, C: 50% kefir, D: 75% kefir. Values with the different letter are significantly different ( $P < 0.05$ ).

**Figure 2.** Meltdown rates of the samples at 40, 60, 80, 100 and 120 min.

A: 0% kefir - Control, B: 25% kefir, C: 50% kefir, D: 75% kefir.

**Table 4.** First dripping time, average melting rate and mass retention values of the samples (n=2).

	Samples*			
	A	B	C	D
First dripping time (min)	32.00 ± 2.000	33.00 ± 1.00	34.00 ± 1.000	40.00 ± 2.000
Average melting rate (g/min)	0.80 ± 0.030	0.79 ± 0.030	0.79 ± 0.025	0.66 ± 0.040
Mass retention (%)	4.48 ± 2.320	8.13 ± 3.230	9.32 ± 3.740	21.53 ± 6.050

\*A: 0% kefir - Control, B: 25% kefir, C: 50% kefir, D: 75% kefir.

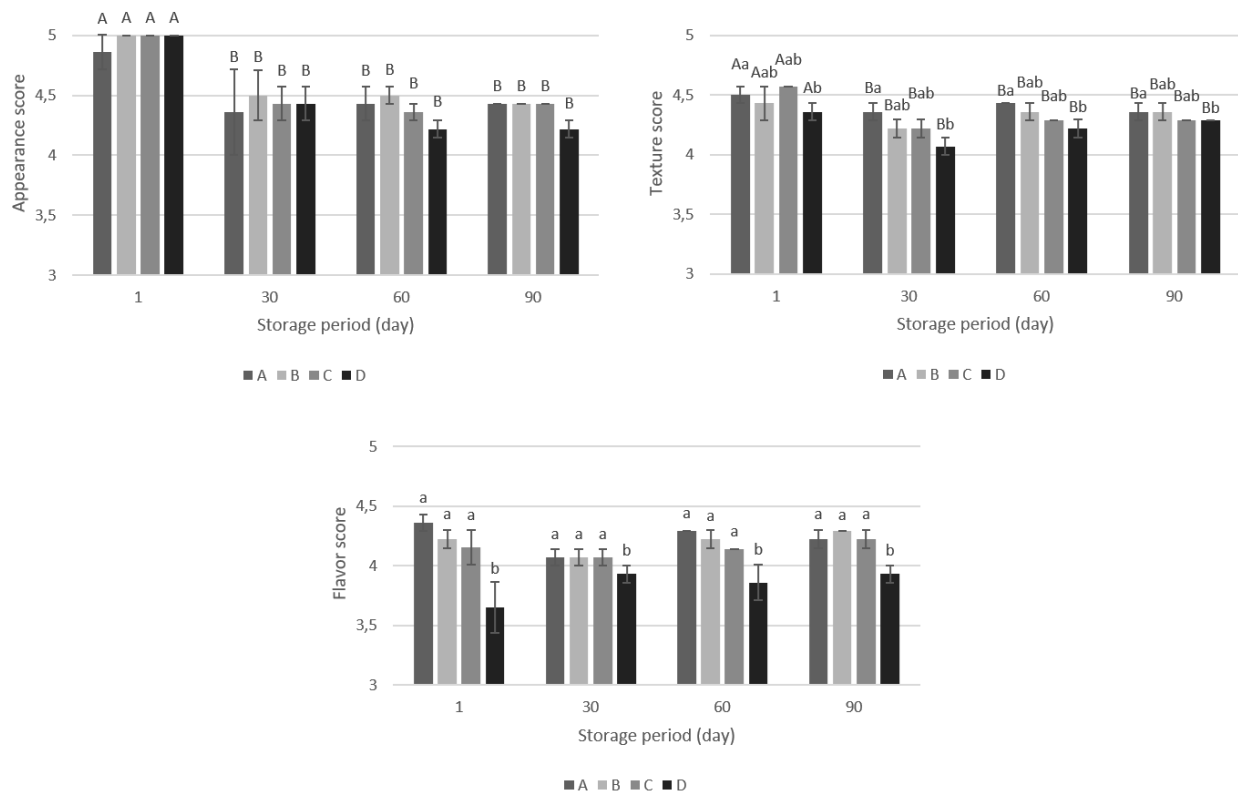
**Table 5.** Lactic acid content, *Lactococcus* spp. and *Lactobacillus* spp. counts of the samples during storage (n=2).

	Storage (Day)	Samples*			
		A	B	C	D
Lactic acid (g/100 g)	1	0.26 ± 0.005 <sup>d</sup>	0.41 ± 0.010 <sup>c</sup>	0.58 ± 0.010 <sup>b</sup>	0.69 ± 0.005 <sup>a</sup>
	30	0.25 ± 0.010 <sup>d</sup>	0.41 ± 0.005 <sup>c</sup>	0.58 ± 0.005 <sup>b</sup>	0.68 ± 0.010 <sup>a</sup>
	60	0.25 ± 0.000 <sup>d</sup>	0.41 ± 0.005 <sup>c</sup>	0.58 ± 0.015 <sup>b</sup>	0.69 ± 0.005 <sup>a</sup>
	90	0.25 ± 0.010 <sup>d</sup>	0.42 ± 0.005 <sup>c</sup>	0.58 ± 0.005 <sup>b</sup>	0.68 ± 0.005 <sup>a</sup>
<i>Lactococcus</i> spp. (log cfu/g)	1	ND <sup>A</sup>	8.27 ± 0.040 <sup>Ac</sup>	9.02 ± 0.07 <sup>Ab</sup>	9.34 ± 0.075 <sup>Aa</sup>
	30	ND <sup>A</sup>	8.16 ± 0.045 <sup>ABc</sup>	8.74 ± 0.060 <sup>Bb</sup>	9.15 ± 0.080 <sup>ABa</sup>
	60	ND <sup>A</sup>	8.00 ± 0.050 <sup>Bc</sup>	8.58 ± 0.110 <sup>BCb</sup>	8.92 ± 0.090 <sup>BCa</sup>
	90	ND <sup>A</sup>	7.92 ± 0.090 <sup>Bc</sup>	8.47 ± 0.0750 <sup>Cb</sup>	8.81 ± 0.055 <sup>Ca</sup>
<i>Lactobacillus</i> spp. (log cfu/g)	1	ND <sup>Ac</sup>	5.07 ± 0.055 <sup>Ab</sup>	5.79 ± 0.095 <sup>Aa</sup>	5.99 ± 0.085 <sup>Aa</sup>
	30	ND <sup>Ad</sup>	3.99 ± 0.065 <sup>Bc</sup>	4.67 ± 0.055 <sup>Bb</sup>	5.11 ± 0.050 <sup>Ba</sup>
	60	ND <sup>Ad</sup>	3.71 ± 0.080 <sup>Cc</sup>	4.27 ± 0.085 <sup>Cb</sup>	4.64 ± 0.075 <sup>Ca</sup>
	90	ND <sup>Ad</sup>	3.54 ± 0.060 <sup>Cc</sup>	4.06 ± 0.055 <sup>Cb</sup>	4.43 ± 0.045 <sup>Ca</sup>

\*A: 0% kefir - Control, B: 25% kefir, C: 50% kefir, D: 75% kefir.

Values with the different lower case letter within the same row and upper case letter within the same column are significantly different (P<0.05).

ND: Not detected.

**Figure 3.** Sensory evaluation results of the samples during storage (n=2).

A: 0% kefir - Control, B: 25% kefir, C: 50% kefir, D: 75% kefir.

Values with the different lower case letter within different samples in the same storage day and upper case letter within different storage days for the same sample are significantly different (P<0.05).



**Sensory evaluation:** Scoring test results performed by experienced panelists are given in Figure 3. The ratio of kefir did not affect the appearance of the samples ( $P>0.05$ ), however, the effect of storage was found to be significant in terms of the same property ( $P<0.05$ ). All of the samples had the highest scores on the 1st day of storage, and the scores did not statistically change after the 30th day until the end of storage. Unlike the appearance, both the ratio of kefir and the effect of storage duration were statistically significant on textural characteristic ( $P<0.05$ ). Sample D, which has the highest kefir ratio, had the lowest scores for texture property during the storage where samples B and C were not different from the control sample. All samples had the highest scores on the 1<sup>st</sup> day of storage, and the scores did not change on the 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> days. Considering the flavor characteristic, there was no difference between samples A, B and C ( $P>0.05$ ), however, sample D got the lowest scores for each day of storage ( $P<0.05$ ).

## Discussion and Conclusion

All of the ice cream samples were standardized in terms of total solid and fat contents. Therefore, there was no statistical difference between the chemical composition of ice cream samples ( $P>0.05$ ) (Table 2).

Similarly, there was no difference in the rheological properties of the samples examined. It is well known that the rheological properties of foods greatly affect the acceptability of the product by consumers (3). The rheological properties of ice cream are generally related to the dry matter content and the components with hydrocolloid properties such as stabilizers in the mix composition (14). Since the same type and amount of stabilizer was used in all ice cream samples in this study and no difference was found between the dry matter contents of the samples, it is an expected result that there was no difference in the rheological properties examined.

All of the ice cream mixes showed non-Newtonian flow characteristics since the  $n$  values were found to be below 1. Goff et al. (13) stated that the flow behavior index of a typical ice cream mix should be  $\sim 0.7$ . As it is seen from Table 3, the  $n$  values of the samples were determined close to this value as they should be.

The weight of ice cream per unit volume is one of the important physical properties that affect the quality of the product. The overrun of the product varies depending on the air given to the mix during the freezing process. It has been stated that the overrun of high-quality ice creams should be between 15% and 50% (33). According to this definition, the ice creams obtained in this study are of high quality (Figure 1). The main reason that the overrun values obtained in the study were not very high is that it is very difficult to exceed 35-40% overrun values of ice creams produced in batch type freezers (2).

The overrun value of ice cream can be affected by the state and denaturation level of the proteins in the product composition as well as the acidity and the freezing point of the product. Salem et al. (29) reported that the addition of different types of probiotics has an effect on the overrun of ice cream, and this is due to the change in the nature of the proteins and the freezing point of the product affected by the increase in acidity depending on the probiotic used. Therefore, in this study, it is thought that the destabilization of casein, which has an important role in the stabilization of air bubbles in the structure of ice cream, with the increase in acidity due to the addition of kefir, may have an effect on the overrun value of the product. It is probably due to fact that the emulsifying ability of casein increases with destabilization and therefore the overrun of ice cream increases due to the decrease of the interfacial tension (14).

The increase in overrun value with the increase in kefir ratio might also be related to the ability of microorganisms in kefir to produce Exopolysaccharides (EPS) during fermentation. It was stated that EPS can contribute to the formation of a matrix that can increase the amount of entrapped air and keep oxygen more efficiently. EPS are polysaccharides and contribute to keeping the air in the system of ice cream since they have the foam stabilizing ability (18).

Melting properties are one of the most important physical properties of ice cream, and the rapid melting of the product is undesirable particularly for consumers. Environmental conditions affect the melting properties of ice cream. When the ice cream is left to melt, the warm air in the environment penetrates into the ice cream and the ice crystals in the product begin to melt. The water occurred by the melting of ice crystals spreads into the unfrozen serum phase and as a result, the solution formed begins to flow from ice cream (24). In addition to environmental conditions, production conditions, type of milk, the composition of the mix, dry matter content, rheological properties, type and amount of stabilizer used are highly influential on the melting properties of ice cream (14, 19, 24, 30).

In this study, all production parameters including the composition of ice cream mixes and the rheological properties were the same. It is therefore no differences in melting behaviour of the ice cream samples were observed ( $P>0.05$ ) (Figure 2). In addition to this, it has become clear that the use of kefir in ice cream production did not affect the melting properties of the product. Agreeing with the result of previous studies (12, 30), the addition of probiotics did not affect the melting properties of ice cream. Additionally, all of the samples can be classified as good quality ice cream in terms of meltdown properties, since Arbuckle (5) stated that a good quality ice cream

should remain at room temperature for ~10-15 min without melting.

Hardness value refers to the strength required to create a certain deformation in ice cream (9) and it is a significant physical property for the acceptability of the product by consumers.

There was no difference in hardness values among the ice cream samples produced in this study ( $P < 0.05$ ) (Table 3). The hardness value of ice cream is closely related to the total dry matter content (14). Similar results were reported (19), indicating that there was no difference between the hardness values was due to the lack of difference between the soluble solid and fat in the product as in this study. Another reason could be speculated that the same stabilizer was used in the same amount in all of the samples yielded the similar results. As a matter of fact, it is known that the type and amount of hydrocolloid used in ice cream production affect the hardness of the product (32). Similarly, mix viscosity has a significant effect on the hardness value of ice cream as it is a measure of the viscosity of the unfrozen phase of the product (24). Therefore, the fact that there was no difference between the consistency index values obtained in this study was also effective on the hardness values.

The effect of the kefir ratio used on the acidity values during the storage period of ice creams was found to be significant ( $P < 0.05$ ). As in this study, ice cream mixes containing 12% non-fat dry matter are expected to have a titration acidity (lactic acid%) of ~0.2 (14) and sample A had approximately this value (Table 5). In addition, as the amount of kefir in ice cream increased, the acidity value of the product was found to be higher at all storage days (Table 5). This is related to the use of kefir, which consists various microorganisms that have a high ability to metabolize the lactose into lactic acid (37). There are other studies (26, 34) reporting that use of probiotic bacteria in ice cream increases the acidity of the product. The increased acidity of ice cream samples containing kefir can protect the product against spoilage microorganisms during storage, thus yielding longer shelf life of the product. Nevertheless, increased titratable acidity of ice cream, may adversely affect the sensorial acceptability of the product (18).

Normally the acidity of the fermented products is expected to increase during storage due to post-acidification (31). However, this study showed that the acidity values of the samples, even those containing kefir, did not change as the storage time progressed. This situation is likely to be caused by the slowing down of the metabolic activity of lactic acid bacteria present in the product with the storage of ice creams at very low temperatures ( $-25\text{ }^{\circ}\text{C}$ ). Farias et al. (11) and Turgut et al. (34) also reported that the lactic acid contents of probiotic ice creams did not change during the storage period.

*Lactococcus* spp. and *Lactobacillus* spp. counts were both increased as the amount of kefir in ice cream mix was increased. It was seen that *Lactococcus* spp. counts detected in the samples were higher in each day of storage compared to *Lactobacillus* spp. counts. This situation might be related to the culture used in kefir production, as well as due to the fact that *Lactobacillus* strains are more sensitive to low temperatures (19, 25). In addition, it is known that the growth of anaerobic *Lactobacillus* strains decreases with the increase of oxygen in the external medium (11). *Lactobacillus* spp. cannot synthesize ATP by respiration and their oxygen-scavenging systems are reduced or disappeared completely. As a result, oxygen is incompletely reduced to hydrogen peroxide and toxic oxygen metabolites ( $\text{O}_2^-$ ,  $\text{OH}^-$  and  $\text{H}_2\text{O}_2$ ) accumulate in the cell; and finally leads to cell death (10, 23). Therefore, it is thought that the amount of air given to the mix during freezing process and the fact that this air is kept in the product matrix during the storage period also negatively affect the growth of *Lactobacillus* spp.

Reduction in the both of bacterial strains counts during the storage period ( $P < 0.05$ ) is possible to be caused by the destruction of the microorganisms due to low temperature storage conditions. Osmotic pressure, which also changes with the decreasing temperature, causes dehydration in the bacterial cells, leading to cell damage (30). Ice crystals forming in ice cream could be another factor causing cell lysis by destroying the cell walls or membranes of microorganisms. The cells may be damaged as a result of mechanical stresses of ice crystals that may form inside the cell (23). Furthermore, toxic metabolites that may occur during storage may also cause cell lysis (31).

According to the International Dairy Federation, in order for food products to have probiotic properties, they must contain at least  $10^7$  cfu/g (7 log cfu/g) of probiotic bacteria during the storage period (16). The survival of probiotics in a food product varies depending on many factors such as acidity, the presence of other microorganisms in the medium, and bacterial metabolites; these bacteria can generally maintain their stability in ice during storage (26). As it is seen in Table 5, kefir containing samples (B, C and D) did not lose their probiotic properties during the storage. The number of probiotic bacteria were above the minimum required level at all times during the storage, although it tended to decrease towards the end of the storage. It was determined that only the control sample (A) did not contain *Lactococcus* spp. and *Lactobacillus* spp. Similarly, Ahmad et al. (2), Parussolo et al. (26) and Salem et al. (29) reported that although the ice cream samples containing various *Lactococcus* and *Lactobacillus* strains had a gradually decreasing bacterial count during storage, they still retained their probiotic properties.

In sensory evaluation, kefir addition did not affect the appearance property of the samples. However, the texture and the flavor characteristics were affected with the amount of kefir added. While there was no difference between samples A, B and C in terms of the mentioned properties, sample D with the highest kefir content had lower scores than the other samples. This was probably due to the fact that sample D had the highest acidity value. It was already mentioned that the increase in acidity in ice cream has a negative effect especially on the flavor properties since the product normally does not have a high acidity value (10). In addition, panelists detected fermented taste in kefir-containing samples, and this flavor was most intense in sample D. This was one of the reasons why sample D getting the lowest score. In similar studies (4, 19, 34), it was reported that the flavor scores of probiotic ice creams decreased with the increase in acidity. As it is seen from Figure 3, it has been determined that the storage time had no effect on the flavor scores of the samples ( $P>0.05$ ). As a matter of fact, no difference was observed in acidity values of the samples during storage.

All in all, all of the ice cream samples were found acceptable considering the chemical and physical properties. In addition, all of the samples maintained their probiotic properties during the 90-day storage period. However, due to the increase in acidity with the increase of kefir ratio, it was observed that sample D, which had the highest kefir ratio (75%), had lower scores from the panelists compared to the other samples in terms of texture and flavor properties. It was determined that samples B and C were not different from the control sample on each day of storage considering the same properties. Therefore, it is possible to produce probiotic ice cream with generally acceptable properties with the production method applied in samples B and C.

### Financial Support

This study has not been financially supported by any institution.

### Conflict of interest

The authors declared that there is no conflict of interest.

### Author Contributions

NK performed conceptualization, the production, all of the analysis, data interpretation and writing-editing the manuscript. BB contributed the microbiological analysis. RAD performed data analysis and interpretation. AG performed conceptualization and reviewing the manuscript.

### Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

### Ethical Statement

This study does not present any ethical concerns.

### References

1. **Acu M, Kinik O, Yerlikaya O** (2017): *Functional properties of probiotic ice cream produced from goat's milk*. Carpathian J Food Sci Technol, **9**, 86-100.
2. **Ahmad I, Khalique A, Junaid M, et al** (2020): *Effect of polyphenol from apple peel extract on the survival of probiotics in yoghurt ice cream*. Int J Food Sci, **55**, 2580-2588.
3. **Ak M** (1997): *Reoloji bilim dalı ve gıda endüstrisi*. Gıda ve Teknoloji, **2**, 36-46.
4. **Akalin AS, Kesenkas H, Dinkci N, et al** (2018): *Enrichment of probiotic ice cream with different dietary fibers: Structural characteristics and culture viability*. J Dairy Sci, **101**, 37-46.
5. **Arbuckle WS** (1986): *Ice cream*. Springer Science+Business Media, New York.
6. **Association of Official Analytical Chemists-AOAC** (1990): *Official methods of analysis of the association of analytical chemists*. AOAC, Washington.
7. **Association of Official Analytical Chemists-AOAC** (2013): *Official method 941.08 Total solids in ice cream and frozen desserts*. AOAC, Washington.
8. **Association of Official Analytical Chemists-AOAC** (2016): *Official methods of analysis of the association of analytical chemists*. AOAC, Rockville.
9. **Bahramparvar M, Tehrani MM, Razavi SMA** (2013): *Effects of a novel stabilizer blend and presence of  $\kappa$ -carrageenan on some properties of vanilla ice cream during storage*. Food Biosci, **3**, 10-18.
10. **Cruz AG, Antunes AEC, Sousa ALOP, et al** (2009): *Ice-cream as a probiotic food carrier*. Food Res Int, **42**, 1233-1239.
11. **Farias TGS, Ladislau HFL, Stamford TCM, et al** (2019): *Viabilities of Lactobacillus rhamnosus ATCC 290 and Lactobacillus casei ATCC 334 (in free form or encapsulated with calcium alginate-chitosan) in yellow mombin ice cream*. LWT-Food Sci Technol, **100**, 391-396.
12. **Favaro-Trindade CS, de Carvalho Balieiro JC, Félix Dias P, et al** (2007): *Effects of culture, ph and fat concentration on melting rate and sensory characteristics of probiotic fermented yellow mombin (Spondias mombin L) ice creams*. Food Sci Technol Int, **13**, 285-291.
13. **Goff HD, Davidson VJ, Cappi E** (1994): *Viscosity of ice cream mix at pasteurization temperatures*. J Dairy Sci, **77**, 2207-2213.
14. **Goff HD, Hartel RW** (2013): *Ice Cream*. Springer Science+Business Media, New York.
15. **Guzel-Seydim Z, Wyffels JT, Seydim AC, et al** (2005): *Turkish kefir and kefir grains: Microbial enumeration and electron microscopic observation*. Int J Dairy Technol, **58**, 25-29.
16. **Homayouni A, Alizadeh M, Alikhah H, et al** (2012): *Functional dairy probiotic food development: Trends, concepts, and products*. 197-212. In: EC Rigobelo (Ed), Probiotics. Intech Open Access Publishers, London.
17. **Inanc N, Sahin H, Cicek B** (2005): *The impact of probiotics and prebiotics on health*. Erciyes Med J, **27**, 122-127.



18. **Januario JGB, Oliveira AS, Dias SS, et al** (2018): *Kefir ice cream flavored with fruits and sweetened with honey: physical and chemical characteristics and acceptance*. *Int Food Res*, **25**, 179-187.
19. **Kesenkas H, Akbulut N, Yerlikaya O, et al** (2013): *A research on usage possibilities of soy milk for kefir ice-cream production*. *Journal of Agriculture Faculty of Ege University*, **50**, 1-12.
20. **Marquina D, Santos A, Corpas I, et al** (2002): *Dietary influence of kefir on microbial activities in the mouse bowel*. *Lett Appl Microbiol*, **35**, 136-140.
21. **Meilgaard M, Civille GV, Carr T** (1999): *Sensory Evaluation Techniques*. CRC Press, Boca Raton.
22. **Mendez-Velasco C, Goff HD** (2012): *Fat structures as affected by unsaturated or saturated monoglyceride and their effect on ice cream structure, texture and stability*. *Int Dairy J*, **24**, 33-39.
23. **Mohammadi R, Mortazavian AM, Khosrokhavar R, et al** (2011): *Probiotic ice cream: viability of probiotic bacteria and sensory properties*. *Ann Microbiol*, **61**, 411-424.
24. **Muse MR, Hartel RW** (2004): *Ice cream structural elements that affect melting rate and hardness*. *J Dairy Sci*, **87**, 1-10.
25. **Ozer B** (2006): *Yoğurt Bilimi ve Teknolojisi*. Sidas Medya Ltd, Izmir.
26. **Parussolo G, Busatto RT, Schmitt J, et al** (2017): *Synbiotic ice cream containing yacon flour and Lactobacillus acidophilus NCFM*. *LWT-Food Sci Technol*, **82**, 192-198.
27. **Roberfroid MB** (2000): *A European consensus of scientific concepts of functional foods*. *Nutrition*, **16**, 689-691.
28. **Sagdic O, Kucukoner E, Ozcelik S** (2004): *Functional characteristics of probiotics and prebiotics*. *Ataturk University Journal of Agricultural Faculty*, **35**, 221-228.
29. **Salem MME, Fathi FA, Awad RA** (2005): *Production of probiotic ice cream*. *Polish J Food Nutr Sci*, **14/55**, 267-271.
30. **Silva PDL, Bezerra MF, Santos KMO, et al** (2015): *Potentially probiotic ice cream from goat's milk: Characterization and cell viability during processing, storage and simulated gastrointestinal conditions*. *LWT-Food Sci Technol*, **62**, 452-457.
31. **Singh A, Bajwa U, Goraya RK** (2014): *Effect of storage period on the physicochemical, sensory and microbiological quality of bakery flavoured ice cream*. *Int J Eng Res Appl*, **4**, 80-90.
32. **Soukoulis C, Chandrinis I, Tzia C** (2008): *Study of the functionality of selected hydrocolloids and their blends with κ-carrageenan on storage quality of vanilla ice cream*. *LWT-Food Sci Technol*, **41**, 1816-1827.
33. **Tekinsen OC, Tekinsen KK** (2008): *Dondurma: Temel Bilgiler, Teknoloji, Kalite Kontrolü*. Selçuk Üniversitesi Basimevi, Konya.
34. **Turgut T, Cakmakci S** (2009): *Investigation of the possible use of probiotics in ice cream manufacture*. *Int J Dairy Technol*, **62**, 444-451.
35. **Turkish Food Codex-TFC** (2004): *Communique on ice cream (Number: 2004/45)*. Republic of Turkey Ministry of Agriculture and Forestry, Ankara.
36. **Turkish Food Codex-TFC** (2009): *Communique on fermented milk products (Number: 2009/25)*. Republic of Turkey Ministry of Agriculture and Forestry, Ankara.
37. **Turkmen N** (2017): *Kefir as a functional dairy product*. 373-381. In: RR Watson, RJ Collier, VR Preedy (Eds), *Dairy in Human Health and Disease Across the Lifespan*. Elsevier Inc, London.
38. **Turkmen N, Gursoy A** (2017): *Functional ice cream*. *Academic Food Journal*, **15**, 386-395.
39. **Wszolek M, Kupiec-Teahan B, Skov Guldager H, et al** (2006): *Production of kefir, koumiss and other related products*. 174-216. In: AY Tamime (Ed), *Fermented Milks*. Blackwell Science Ltd, Oxford.
40. **Yuennan P, Sajjaanantakul T, Goff HD** (2014): *Effect of okra cell wall and polysaccharide on physical properties and stability of ice cream*. *J Food Sci*, **79**, 1522-1527.

---

**Publisher's Note**

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

---