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RESEARCH ARTICLE

The Functional, Nutritional and Biological Properties of Ice Cream Produced Using Kefir Culture Inoculation

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ABSTRACT

In this study, changes in the physicochemical, microbiological and technological values of ice cream produced from ice cream mixes ripened with kefir culture inoculation at three different rates (0.5%, 1% and 1.5%) were investigated. Adding kefir culture to the mix decreased its firmness, cohesiveness, and viscosity index values and increased its consistency value. Furthermore, the pH, aw, % dry matter, first drop, complete melting, volume increase and all TPA (texture profile analysis) values were lower in ice creams produced using these mixes in comparison to the control sample. Furthermore, these changes increased in parallel with the amount of culture added. The ice cream produced using mixes ripened with 1.5% kefir culture inoculation yielded the lowest pH (5.74), aw (0.710), % dry matter (11.35), first drop (15.25 min.), complete melting (71.28 min.) and overrun (43.67%) values, as well as the lowest hardness (1759.32 N), springiness (0.90), cohesiveness (0.23), gumminess (742.42 N), chewiness (374.73 N) and resilience (0.698) results.
Keywords: Ice Cream, Fermentation, Kefir, Starter Culture

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Kefir Kültürü İnokulasyonu ile Üretilen Dondurmanın Fonksiyonel, Besinsel ve Biyolojik Özellikleri

ÖZ

Bu araştırmada üç farklı oranda (% 0.5, % 1 ve % 1.5) kefir kültürü inokülasyonu ile olgunlaştırılan dondurma mikslerinden üretilen dondurmaların fizikokimyasal, mikrobiyolojik ve teknolojik değerlerinde meydana gelen değişimler incelenmiştir. Dondurma miksine kefir kültürü ilavesi miksin sertlik, yapışıklık ve viskozite indeksi değerlerinin azalmasına kıvam değerinin ise artmasına neden olmuştur. Ayrıca bu mikslerden üretilen dondurmalarda kontrol örneğine kıyasla pH, aw, % kuru madde, ilk damlama, tamamen erime, overrun ve tüm TPA(tekstür profil analizi) değerleri de azalış göstermiştir. Ek olarak tespit edilen bu değişimler, ilave edilen kültür miktarına paralel şekilde artış göstermiştir. Örnekler arasında en düşük pH (5.74), aw (0.710), % kuru madde (11.35), ilk damlama (15.25 dk.), tamamen erime (71.28 dk.), overrun (43.67 %) değerleri en düşük örnek % 1.5 kefir kültürü inokülasyonu ile olgunlaştırılan mikslerden üretilen dondurma örnekleri olmuştur. Benzer şekilde en düşük sertlik (1759.32 N), esneklik (0.90), yapışıklık (0.23), yapışkanlık (742.42 N), çiğnenebilirlik (374.73 N) ve dayanıklılık (0.698) değerleri de aynı örneklerde tespit edilmiştir.
Anahtar Kelimeler: Dondurma, Fermentasyon, Kefir, Starter Kültür

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INTRODUCTION

Ice cream, thanks to its unique flavor, cooling effect, and nutritional value, is a popular dairy product consumed by people from all age groups around the world (Göktaş et al., 2022). Ice cream mixes mainly consist of milk, emulsifiers, vegetable oils, stabilizers, flavorings, and fruits. Artificial sweeteners, food dyes, pulp, dietary fibers, and probiotic bacteria are also added in some mixes (Marshall et al., 2003). The increasing number of consumers demanding natural, nutritious, and functional foods has encouraged producers to develop new ice cream products (Cruz et al., 2009).

Fermented dairy products have high nutritional value; they constitute a significant portion of fermented products and are preferred worldwide for their traditional and/or universal characteristics (Petrova et al., 2021). Fermented dairy products are categorized by considering production methods, animal species, and microbiological, physicochemical, and/or production technologies. Examples of these products include cheeses, fermented beverages, yoghurt, kefir, buttermilk, sour cream, and acidophilus milk (Shiby and Mishra, 2013).

Ice cream can be modified into a fermented product by introducing lactic acid bacteria and yeasts. This process enhances and changes its sensory, microbiological, and textural characteristics and increases its functional and nutritional value (Akarca et al., 2024).

Kefir is a fermented milk product produced through fermentation by lactic acid bacteria and yeasts (Gao and Li, 2016). Today, kefir can be produced in two ways: using kefir grains or a starter culture combination. Its aromatic, microbiological, and chemical characteristics are formed through the interaction of various bacteria and yeasts present in kefir grains or the combination of cultures (Farnworth, 2008).

This study aims to investigate alterations in the physicochemical, microbiological, and technological characteristics of samples produced using ice cream mixes ripened with kefir culture inoculation at three different rates.

MATERIALS and METHODS

Materials

The raw cow's milk was procured from a local producer in the Afyonkarahisar province. Salep, cream, and granulated sugar (beet sugar) were obtained from a local market in the same province.

Cultures

The Vivo Activ LLC 07400 (Ukraine) kefir cultures containing *Lactococcus lactis subsp. diacetylactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Leuconostoc mesenteroides subsp. cremoris,* and *Streptococcus thermophilus bacteria* and *Debaromyces hansenii* and *Kluyveromyces marxianus subsp. Marxianus* yeasts were used in this study. The culture contains 10 log CFU/g of living microorganisms.

Ice Cream Production

The ice cream production method used in this study (see Fig. 1) was modified from the method described by Akarca et al. (2024). Following production of the ice cream mixes, the kefir culture mixture in three different ratios (0.5%, 1% and 1.5%) was added to the mix, which was then ripened. The mixes were then processed into ice cream at -5 °C using a freezing machine (CRM-GEL 25C, Italy). Then, 250 g from each mix was placed in sterile glass containers and hardened at -24 °C. The resulting samples were kept at -18°C until analysis was completed.

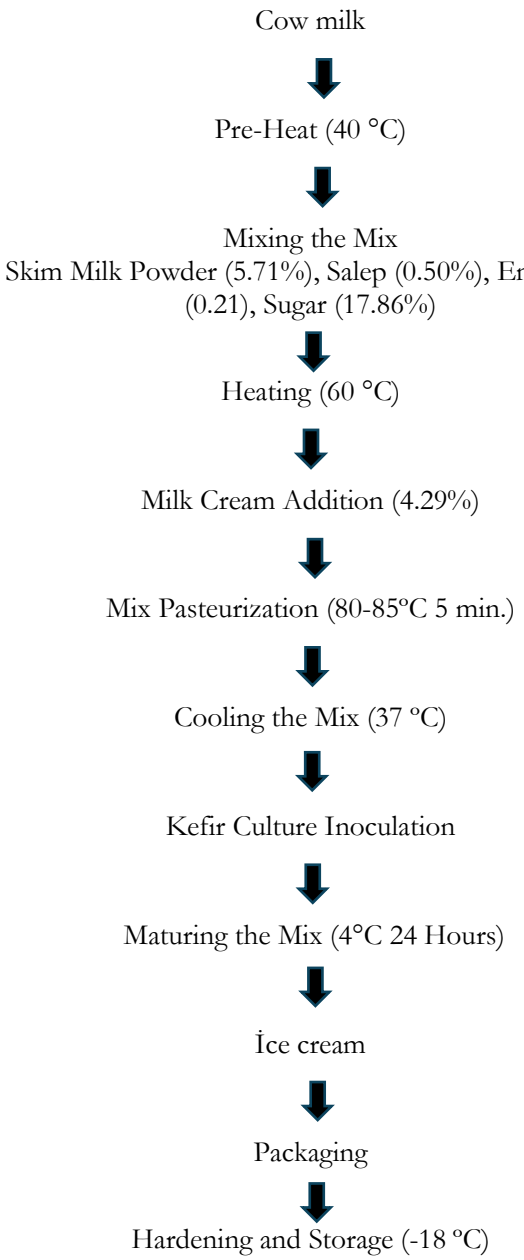

Figure 1: Ice Cream Production

Texture analysis of ice cream mix
Texture values (consistency, cohesiveness, viscosity index, and firmness) were measured utilizing a TA.XT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) with back extrusion rig hardware (Sert et al., 2017).

The first drip time
Ten grams of ice cream sample were put into a stainless-steel wire strainer over glass containers that had been weighed on a precision balance (Shimadzu AUX 220, Japan), and were left to melt at 20 °C. A stopwatch was then started, and the moment the ice cream began to melt and the first drops fell was recorded (Akarca et al., 2024).

Time to complete melting
Ice cream samples that had been hardened in a deep freezer (Uğur UDD560 BK, Turkey) at -18 °C were taken out and allowed to melt at 20 °C on a stainless-steel wire grid with 0.2 cm wires on a 500 ml glass beaker. The stopwatch was then started. The samples were then left to completely melt. The time (in minutes) taken for complete melting was recorded to determine the melting time (Güven and Karaca, 2002).

Overrun
The samples were first placed into a 500 ml tared glass measuring cylinder. The same samples were then placed into a 500 ml beaker and melted in a water bath. The resulting mixture was then transferred to a measuring cylinder of the same volume and weighed again (Jiménez-Florez et al., 1993).

pH value
The samples were first mixed with one-tenth of sterilized pure water, and then homogenized utilizing a homogenizer (Daihan Wisestir HS-30T, South Korea). The pH values were then measured using a pH meter (Hanna HI 2215 pH/ORP) (Akarca et al., 2024).

Water activity (aw)
These values were measure using a water activity analyzer (Novasina LabTouch-aw, Lachen, Switzerland) (AOAC, 2016a).

Dry Matter Analysis (%)
The % dry matter values of the ice cream samples were determined using an oven (Nüve, Turkey), according to the AOAC (2016b) method, and calculated using the following formula:
% dry matter = (m1-m2)/(m1-m0) x 100
m1: Sample container + weighed sample weight
m2: Weighed container + weighed sample weight after analysis
m0: Initially weighed sample weight

Color values (*L*, a*, b)**
The color values of the samples were measured by utilizing a colorimeter (Minolta Co., Osaka, Japan) by making use of the Hunter color measurement system (Akarca et al., 2024).

Texture analysis of ice cream
The samples were cut into 22±0.5 mm diameter and 20±0.5 mm length cylinders at -18 °C. TPA values were determined at room temperature using a texture analyzer (TA-XT2i; Stable Micro Systems, Surrey, UK) with a 30 kg load cell. Measurements were performed utilizing a spherical probe (1" Spherical Probe, Part Code: P/1S, batch no. 13155, Stable Micro Systems Ltd., Godalming, UK). The pre-test, test and post-test speeds were set at 1, 5 and 5 mm/s, respectively. The samples were compressed to 40 % of their original height, with an interval of 5 s between each compression. To determine the TPA profile of each sample, the measurements were performed three times and the values obtained were averaged (Isleroglu et al., 2015).

Lactic acid bacteria (LAB) count
De Man Rogosa Sharpe (MRS) agar (Merck, 110660, Germany) was used when counting lactic acid bacteria introduced to the mixes. Serial dilutions of samples were prepared before analysis by utilizing 0.1% buffered peptone water (Merck, 107228, Germany), and analyses were performed by following the spread plate method. The inoculated Petri dishes were incubated under anaerobic conditions in jars (Merck, 116387, Germany), supplemented with Anaerocult® A (Merck, 113829, Germany), at 37 °C for 72 hours in an oven (Shori et al., 2022).

Total number of yeasts
The number of yeasts added to the ice cream mixes was determined using potato dextrose agar (Merck, Germany). Prior to microbiological analysis, serial dilutions were prepared utilizing 0.1% buffered peptone water (Merck, 107228, Germany), and analyses were performed by following the smear plate method. The inoculated Petri dishes were incubated under aerobic conditions at 25 °C for 5-7 days in an oven (Campos and Cristianini, 2007).

Statistical Analyses
ANOVA and Duncan's test ($p<0.05$) were performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA) to determine the significance of differences in results obtained in this study. In addition, interactions between sample variation and results were determined by correlation and variation analysis.

RESULTS

The textural characteristics of the samples are presented in Table 1. The samples with the lowest and highest firmness and consistency values were found in the mixes fermented with kefir culture at 1.0 % (20.02

g and 294.43 g sec, respectively) and 1.5 % (14.69 g and 293.66 g sec, respectively).

Table 1. Textural Analysis Results of Ice Cream Mixes

Sample	Firmness (g)	Consitency (g sec)	Cohesiveness (g)	Index of Viscosity (g sec)
Control	15,03±0.42^{c}	283,28±1.80^{a}	-7,56±0.48^{a}	-2,65±0.16^{a}
0,5% C	17,18±0.68^{b}	276,99±2.30^{a}	-7,16±0.21^{a}	-8,21±0.85^{b}
1.0% C	20,02±0.21^{a}	294,43±12.28^{a}	-10,07±0.27^{b}	-12,81±0.56^{c}
1.5% C	14,69±0.47^{c}	293,66±2.12^{a}	-10,75±0.55^{b}	-14,38±1.93^{c}
P Value	0.001	0.130	0.0082	0.001
R	0.096	0.632	-0.885**	-0.961**

a - c (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant.

The cohesiveness and viscosity index values decreased in line with the kefir culture addition rate. The lowest values were found in the samples with 1.5% kefir culture, with sec values of -10.75 g and -14.38 g, respectively.

The physical analysis results are shown in Table 2. The lowest and highest first drip times were found in samples containing 1.5 % kefir culture (15.25 min.) and in the control sample (18.88 min.). Similarly, the lowest and highest complete melting times were found in the same samples (71.28 min. and 85.54 min.).

The sample with the lowest pH (5.74) was the one to which 1.5 % kefir culture was added, whereas the sample with the highest pH (6.46) was the control sample. The lowest and highest overrun values were found in the 1.5% kefir culture (43.67 %) and control (50.56%) samples.

Table 2. Physical Analysis Results of Ice Cream Samples

Sample	First Droplet (min.)	complete melting time (Min.)	Overrun
Control	18,88±0.37^{a}	85,54±1.24^{a}	50,56±2.30^{a}
0,5% C	17,97±0.67^{ab}	74,28±1.23^{b}	49,03±1.40^{ab}
1.0% C	16,88±0.45^{b}	73,62±0.28^{bc}	45,69±1.57^{ab}
1.5% C	15,25±0.35^{c}	71,28±0.87^{c}	43,67±2.19^{b}
P Value	0.006	<0.0001	0.069
R	-0.961**	-0.873**	0.244

a - c (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant,

The water activity and % dry matter values varied between 0.756 and 0.710, and between 32.22% and 31.35%, respectively, depending on the culture addition rate (p<0.05). The highest water activity and % dry matter values were found in the control sample, and the lowest ones were found in the sample to which 1.5% kefir culture was added (p<0.05).

Table 3. Physicochemical and Microbiological Analysis Results of Ice Cream Samples

Sample	pH	a_w	% Dry matter	LAB (log cfu/g)	yeast count (log cfu/g)
Control	6,46±0.03^{a}	0,756±0.01^{a}	32,22±0.06^{a}	1,87±0.11^{d}	2,27±0.05^{d}
0,5% C	6,19±0.02^{b}	0,735±0.01^{b}	32,05±0.18^{ab}	4,21±0.03^{c}	8,15±0.10^{c}
1.0% C	5,90±0.01^{c}	0,717±0.01^{c}	32,06±0.24^{ab}	5,29±0.01^{b}	8,44±0.01^{b}
1.5% C	5,74±0.02^{d}	0,710±0.01^{d}	31,35±0.46^{b}	5,80±0.01^{a}	8,61±0.01^{a}
P Value	<0.0001	<0.0001	0.109	<0.0001	<0.0001
r	-0.991**	-0.976**	-0.748*	0.951**	0.820*

a - c (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant,

The microorganisms in the culture mixture added to the ice cream mix metabolised the hexose sugars during fermentation, resulting in decreased aw and % dry matter values.

Adding kefir culture to ice cream samples increased the number of lactic acid bacteria and yeasts. This increase depended on the amount of culture added (Table 3; p<0.05). Before ripening, the mixture was cooled to 37 °C, inoculated with kefir culture, and then left to ripen at 4 °C for 24 hours. The number of microorganisms continued to increase until the temperature of the mixture dropped below the minimum limit for the growth of lactic acid bacteria and yeasts. However, some of these microorganisms were affected by the very low temperature and could not survive the ice cream production phase.

Adding kefir culture at different ratios increased the L* and b* values of the samples while decreasing the a* values (Fig. 2-4; p<0.05). The highest L* and b* values (89.14 and 5.93, respectively) were found in the sample to which 1.5% kefir culture was added, while the highest a* value (2.98) was found in the control.

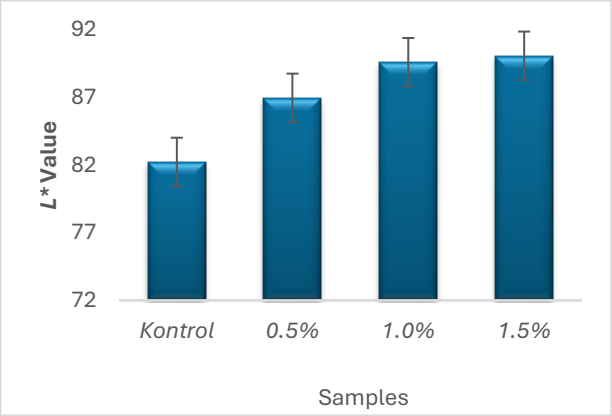

Figure 2. L* Values of Ice Cream Samples

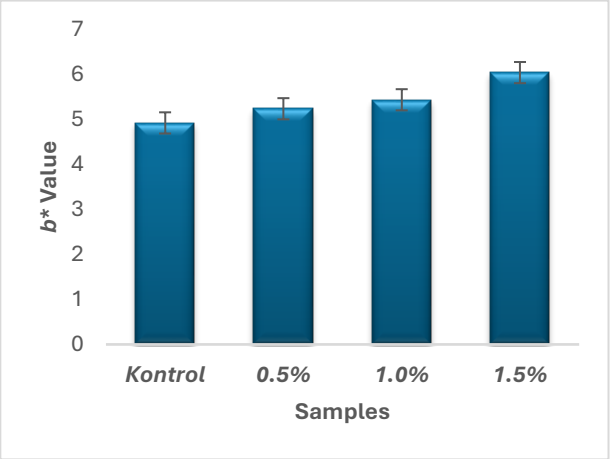

Figure 4. b* Values of Ice Cream Sample

The hardness, gumminess, chewiness and resilience interactions had a negative effect on sample diversity, while the adhesiveness interaction showed a positive correlation. Adding kefir culture decreased all TPA values (except springiness, p<0.05).

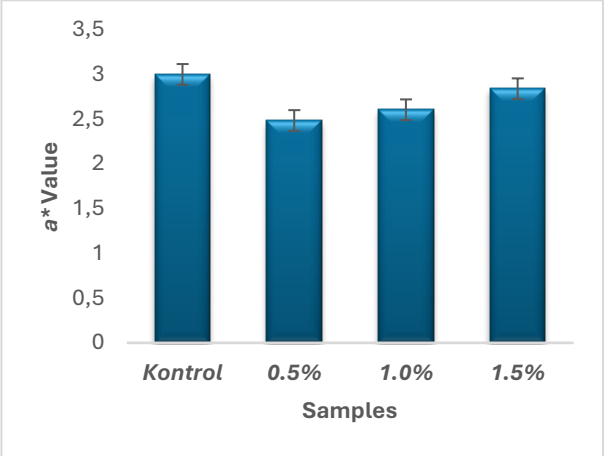

Figure 3. a* Values of Ice Cream Samples

This effect increased in line with the amount of culture added (see Table 4). The lowest values for chewiness, gumminess, adhesiveness, and hardness of the samples were determined to be 1759.32 N, -2.01 N, 742.42 N and 374.73 N, respectively, in samples to which 1.5 % kefir culture was added.

Table 4. Textural Analysis Results of Ice Cream Samples

Sample	Hardness (N)	Adhesiveness (g sec)	Springiness	Cohesiveness
Control	5386,35±10.15^{a}	-6,04±0.05^{c}	0,94±0.05^{a}	0,94±0.01^{a}
0,5% C	3175,51±330.82^{b}	-2,66±0.16^{b}	0,95±0.01^{a}	0,26±0.01^{b}
1.0% C	2459,03±30.70^{c}	-2,11±0.07^{a}	0,93±0.01^{a}	0,25±0.01^{bc}
1.5% C	1759,32±213.85^{d}	-2,01±0.01^{a}	0,90±0.01^{a}	0,23±0.01^{c}
P Value	<0.0001	<0.0001	0.519	<0.0001
r	-0.948**	0.853**	-0.535	-0.803*

a - d (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant,

Table 4. Textural Analysis Results of Ice Cream Samples (Continued).

Sample	Gumminess (N)	Chewiness (N)	Resilience
Control	815,27±11.10^{a}	736,04±10.90^{a}	0,959±0.02^{a}
0,5% C	785,95±6.52^{b}	626,54±8.76^{b}	0,871±0.02^{b}
1.0% C	760,45±8.93^{c}	472,66±10.26^{c}	0,741±0.01^{c}
1.5% C	742,42±6.07^{c}	374,73±9.03^{d}	0,698±0.01^{c}
P Value	0.004	<0.0001	<0.0001
r	-0.972**	-0.995**	-0.978**

a - d (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant,

DISCUSSION

The firmness and viscosity index values were found to be highly significant ($p<0.001$) in relation to sample variety. Additionally, the cohesiveness and viscosity index values exhibited a strong negative correlation with the sample variety interaction.

The addition of kefir culture to the mix during fermentation affected all the samples' viscosity values ($p<0.05$). Firmness and consistency values increased with the addition of kefir culture up to 1% addition level but decreased with the addition of more than 1% kefir culture ($p<0.05$). Akarca et al. (2024) stated that adding different lactic acid bacteria to ice cream mixes increases firmness and consistency values, similar to the results achieved in this study. Carbohydrates such as sucrose and lactose in the mix have a positive effect on its water-holding capacity. However, since the added lactic acid bacteria metabolized some of the carbohydrates in the mix, the water holding capacity decreased, as did the firmness and consistency values. In parallel with these results, Akarca et al. (2024) stated that lactic acid bacteria addition to mix samples decreased cohesiveness and viscosity index values.

Carbohydrates such as honey, sugar etc. have an increasing effect on stickiness and viscosity (Ozdemir et al., 2008). Yeast and lactic acid bacteria in the added kefir culture fermented the sugars in the mix and converted them into compounds such as organic acids with less density, causing a decrease in these two values of the mix. In parallel with the results achieved in this study, Alamprese et al. (2005) and Zhang et al. (2017) stated that *Lactobacillus plantarum* GG strains added to ice cream altered all textural properties of products. The complete melting time was strongly influenced by sample diversity ($p<0.0001$). Additionally, the interactions between the first drip time and complete melting time showed a very negative correlative effect on sample diversity. The first drip and complete melting times decreased with the addition of kefir culture ($p<0.05$), with the extent of the decrease depending on the amount of culture added. Consistent with these results, Akarca et al. (2024) found that adding lactic acid bacteria to mixes decreased the first drip and complete melting times. The melting rate is influenced by many factors, such as the mix composition, consistency coefficient, the amount of air included in the mix, and the structure of ice crystals, and the network of fat globules established during ice cream production.

Sugar and lactose addition increases the melting resistance due to their water-retention capacity and micro-viscosity-increasing properties (Bahramparvar and Mazaheri Tehrani, 2011; Muse and Hartel, 2004).

The addition of kefir culture containing yeast and lactic acid bacteria to the mix results in the metabolism of the carbohydrates in the mix. This increases the hydrolysis of milk proteins and the organic acid formation, such as lactic acid, decreasing the melting resistance.

Overrun values decreased in samples produced by adding kefir culture, with this decrease occurring in parallel with the amount of culture added ($P < 0.05$). Similar results were reported by Sarwar et al. (2021), Göktaş et al. (2022) and Akarca et al. (2024). The microorganisms (yeast and lactic acid bacteria) in the culture metabolized the sugars in the environment, yielding a decrease in viscosity and consequently in the overrun amounts.

The interactions between pH, a_w, lactic acid bacteria and yeast counts significantly affected sample diversity ($p<0.0001$). Similarly, the interactions between pH, a_w, and % dry matter had a negative effect on sample diversity, while the counts of lactic acid bacteria and yeast had a positive correlative effect (see Table 3). Adding kefir culture decreased the pH values, with a decrease in pH in parallel with the amount of culture added ($p<0.05$). The lower pH values in comparison to the control sample are due to the organic acids formed when the yeast and lactic acid bacteria in the added culture metabolize the hexose sugars in the mixture. Consistent with these findings, Zhang et al. (2014), Sarwar et al. (2021), and Göktaş et al. (2022) also reported that adding probiotic bacteria decreased the samples' pH values. Consistent with our research findings, Akarca et al. (2024) reported that the water activity (aw) values of ice cream samples decreased with the addition of lactic acid bacteria. Kılıç and Şevik (2021) reported L* values of 91.11, a* values of 1.39 and b* values of 8.07 for ice cream samples, which is consistent with the findings achieved in this study. The cohesiveness, resilience, chewiness, adhesiveness, and hardness had a significant effect on sample diversity ($p<0.0001$).

CONCLUSION

This study examined the alterations in the microbiological, physicochemical, and technological characteristics of ice creams produced using a mix ripened with kefir culture added at three different ratios (0.5%, 1% and 1.5%). Adding kefir culture to the mix decreased the firmness, cohesiveness, and viscosity index values, while increasing the consistency value. Additionally, the pH value, the time taken for the first drip, the time taken for complete melting, the overrun and all TPA values were lower in ice creams produced using these mixtures than in the control sample. Furthermore, these changes increased in parallel with the amount of culture added.

Ice cream is a popular dairy product consumed widely all over the world. It is also recognized as an important food thanks to its high nutritional value. The production of the mix after fermentation with kefir culture inoculation has also increased the functional and nutritional properties of the resulting products.

As well as being consumed as food, the fact that this valuable product is suitable for functional use reveals its potential for the natural treatment of intestinal and digestive diseases, especially food poisoning. Furthermore, consumer expectations were met by producing a functional and specialized food by enhancing the value of ice cream, which is already a valuable foodstuff.

Conflict of Interest: The authors have no conflicts of interest to report.

Authors' Contributions: MK contributed to the project idea, design and execution of the study, acquisition of data, analysed the data, drafted and wrote the manuscript, reviewed the manuscript critically.

Ethical Approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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