 doi:10.20944/preprints202102.0387.v1

Article

Probiotic sheep milk ice cream with inulin and apple fiber

Magdalena Kowalczyk*, Agata Znamirowska* and Magdalena Buniowska

Department of Dairy Technology, Institute of Food Technology and Nutrition, University of Rzeszow, Ćwiklińskiej 2D, 35601 Rzeszów, Poland; magdalenakowalczyk@poczta.fm (M.K.); aznam@univ.rzeszow.pl (A.Z.); mbuniowska@ur.edu.pl (M.B.)

* Correspondence: magdalenakowalczyk@poczta.fm (M.K.); Tel.: +48-17-785-4903; aznam@univ.rzeszow.pl (A.Z.); Tel.: +48-17-785-4946

Abstract: The aim of the study was to assess the effect of the addition of inulin and the replacement of part of inulin with apple fiber on the physicochemical and organoleptic properties. Moreover, the survival of *Bifidobacterium animalis* ssp. *Lactis* Bb-12 and *Lactobacillus rhamnosus* was studied in sheep milk ice cream. There was no effect of apple fiber and the type of bacteria on the number of bacteria of the probiotics after fermentation. As a result of freezing, mixture containing *Bifidobacterium animalis* ssp. *Lactis* Bb-12, there was a significant reduction in the bacterial from 0.39 log cfu g^{-1} to 0.46 log cfu g^{-1}. In all ice cream on the 21st day of storage, it exceeded 10 log cfu g^{-1}, which means that the ice cream retained the status of probiotic products. The *Lactobacillus rhamnosus* ice cream showed a lower yellow colour compared to the *Bifidobacterium* Bb-12 ice cream. The overrun of sheep's milk ice cream was within a range from 78.50% to 80.41%. The appearance of sheep's milk ice cream is influenced considerably by the addition of fiber and the type of bacteria and the interaction between the type of bacteria and the addition of fiber and storage time and fiber.

Keywords: ice cream, sheep's milk, probiotics, apple fiber, inulin, *Bifidobacterium, Lactobacillus*

1. Introduction

The growing awareness of consumers and their expectations regarding healthy and quality food contributed to increased demand for functional food production. Bioactive ingredients added to food positively affect the product's characteristics, quality, and human health. The trend of using sheep's milk for ice cream production has recently emerged. Compared to other mammals' milk, sheep's milk has a higher nutritional value, much more dry matter. Sheep's milk is a source of essential minerals and vitamins for the body [1].

The popularity of ice cream consumption and its availability contributed to developing functional ice cream recipes with increased nutritional value, enriched with probiotic bacteria and prebiotics [2]. According to FAO/ WHO, Probiotic bacteria are live microorganisms that provide health benefits to the host [3]. However, to maintain the probiotic effect, a minimum number of viable probiotic cells of 106 - 109 CFU is required [4].

Bifidobacterium animalis ssp. *Lactis* Bb-12 (*Bifidobacterium* Bb-12) and *Lactobacillus rhamnosus* (*Lb. rhamnosus*) are among the most commonly used probiotics from the group of lactic acid bacteria. Probiotics have antioxidant, anti-inflammatory, antibacterial, and antiviral effects [5]. They are used in disorders of the gastrointestinal tract [6], especially in irritable bowel syndrome [7], reduce bad cholesterol [8], and destroy cancer cells [9] and due to the production of β-D - galactosidase reduces the effects of lactose intolerance [10, 11]. However, dietary fiber intake is still low [12, 13].In such a

 doi:10.20944/preprints202102.0387.v1

case, synbiotic ice cream can be a proposition for supplementing fiber in the diet. Numerous studies show that prebiotics support beneficial health effects, including stimulating the absorption of minerals, especially iron and calcium [14], accelerating fats' metabolism, facilitating the treatment of obesity, and preventing constipation [15]. Clinical studies show that natural polysaccharides [16, 17, 18] affect mainly the growth and survival of bacteria *Lactobacillus* and *Bifidobacterium* among others: inulin, pectin, galactooligosaccharides (GOS), fructooligosaccharides (FOS) [19]. Inulin can be used in ice cream as a replacement for fat or sugar. Inulin plays a technological role by limiting ice crystals' growth during freezing and storage, changing the mixture's freezing point, and influencing ice cream's melting. Apple fiber [20], obtained from cleaning, micronization, and sterilization of dry apple pomace, also has a prebiotic potential. Apple fiber is a source of water-soluble pectin that is not digested by enzymes in the human digestive system [21]. After consumption, apple fiber reaches the small and large intestines relatively unchanged, nourishing the colonizing probiotics. Therefore, the study aimed to evaluate the effect of inulin addition and replacement of inulin with apple fiber on the physicochemical and organoleptic properties and the survival of *Bifidobacterium* Bb 12 and *Lb. rhamnosus* in sheep milk ice cream.

2. Materials and Methods

2.1. Materials

The material for the production of ice cream was raw sheep's milk (Farm "Owcza Zagroda," Wyżne, Poland), with the following chemical composition: protein: 5.34 ± 0.2%, fat: 6.20 ± 0.3%, lactose: 5, 01 ± 0.12% determined on a Bentley B-150 - milk and milk product analyser (Bentley, USA) and pH 6.8 ± 0.12 (FiveEasy pH-meter, Mettler Toledo, Greifensee, Switzerland).

For the production of ice cream, the following were used: white sugar (Polish sugar, Poland), mango-passion fruit flavor essence (Brown, Poland) with the following composition: natural and identical to raw mango and passion fruit flavors, citric acid E330 and mango juice, inulin (Orafti HP, Belgium), apple fiber (Aura Herbals Jarosław Paul, Poland) composed of 100% micronized apple fiber. To inoculated the sample, probiotic bacteria (Chr. Hansen, Denmark) were used: *Bifidobacterium* Bb-12 and *Lb. rhamnosus* (Pen, E/ N, Oxy).

2.2. Manufacture of ice cream mixtures

Sheep's milk (85%), sugar (11%), and flavor essence (0.1%) were mixed and divided into two batches. Inulin (4%) was added to the first batch and divided into a sample containing *Bifidobacterium* Bb-12 (Abb12) and *Lb. rhamnosus* (BLr). Inulin (2.5%) and apple fiber (15%) were added to the second batch, and they were also divided into two groups, AFbb12 and BFLr. The milk with additives was mixed and a homogenized using homogenizer (Nuoni GJJ-0.06/ 40, Zhejiang, China), with a pressure of 20 MPa at 60° C. After the milk was pasteurized at 85° C for 30 min and cooled to 37° C. The group Abb12 and AFbb12 was inoculated with a monoculture of *Bifidobacterium* Bb-12, while the group Blr and BFLr were inoculated with *Lb. rhamnosus*. Each of prepared milk samples was inoculated with a previously activated starter culture according to the method Mituniewicz-Małek et al. [22] with some modification (in the form of bulk activated at 40°C for 5 h, after 5 h inoculum consisted of log 9 cfu·g $^{-1}$ of bacteria, which was added to the milk in the amount of 5%. Prepared mixtures were fermented in an incubator (Cooled Incubator ILW 115, POL-EKO-Aparatura, Poland) at 37°C for 10 h, then cooled to 5°C and conditioned at this

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 February 2021 doi:10.20944/preprints202102.0387.v1

temperature for 12 h. Prepared ice cream mixes were frozen in a freezer (UNOLD AG, Germany) for 40 - 50 minutes. The produced ice cream was packed in 100 ml plastic containers and stored at -22°C for twenty-one days.

2.3. Physicochemical analysis

The chemical composition of ice cream and ice cream mixes were determined using a Bentley B-150 milk and dairy analyser (Bentley, USA). Measurement of the pH value of milk, ice cream mixes, and ice cream was performed with a FiveEasy pH meter (Mettler Toledo, Greifensee, Switzerland). Lactic acid content was determined by titration of samples with 0.1 NaOH using phenolphthalein as an indicator. The results are expressed in g L^{-1} [23]. Ice cream overrun was estimated as the air volume ratio in ice cream to the melted ice cream volume [24]. The melting and the first dropping time were assessed at an ambient temperature of 23°C by placing a defined ice cream sample on stainless steel grids.

2.4. Microbiological analysis

The number of probiotic bacteria *Bifidobacterium* Bb-12 and *Lb. rhamosus* were determined according to the method of Lima et al. [25]. The inoculation was done by the plate method using MRS agar (Biocorp, Poland) and then incubated anaerobically with GENbox anaer (Biomerieux, Poland) in a vacuum desiccator at 37°C for 72 hours [26]. The colonies were counted using a colony counter (TYPE J-3, Chemland, Poland). The number of viable bacterial cells was expressed as log cfu g^{-1}.

2.5. Colour of ice cream

The colour of the ice cream was determined by the instrumental method using a colorimeter (model NR 145, China) using the CIE LAB system [27]. The following values were analysed: L* as brightness, and as a* colour from red (+) to green (-), b* as the colours from yellow (+) to blue (-), C* as the purity and intensity of the colour, and h* as the shade of the colour.

2.6. Organoleptic analysis

The organoleptic evaluation was carried out by 20-person panel experts. The parameters were assessed on a 9-degree scale with structured and definitions.

2.7. Statistical analysis

The results from two independent studies were expressed as the mean and standard deviation in Statistica v. 13.1 (StatSoft, USA). One, two, and three-way ANOVA was performed, and the differences between the mean values were verified with the Turkey test, with $P < 0.05$.

3. Results

3.1. Physicochemical and organoleptic properties of ice creams mixtures and ice creams

The chemical composition of ice cream mixes based on sheep's milk before and after fermentation is presented in Table 1. There was no significant effect of fermentation and of apple fiber on the protein and fat concentration in the ice cream mixes. The protein content of all ice cream

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 February 2021 doi:10.20944/preprints202102.0387.v1

mixes was around 5%, while the fat content ranged from 5.9% to 6.1%. According to Fiol et al. [28], the standard parameters for milk-based ice cream are 64% water, 18% sugar, 10% non-fat milk dry matter, and 8% milkfat. Balthazar et al. [1] analysed ice cream made of sheep's milk with a higher fat content (10.03%) and lower protein (3.2 In the studies by Góral et al. [29], probiotic milk ice cream contained from 6.9% to 7.5% protein and from 5.1% to 5.6% fat.. Similar fat content (5.77% - 5.90%) in milk ice cream with strawberries and probiotic bacteria was shown by Vardar and Öksüz [30]. On the other hand, Akalin and Erişir [31] prepared probiotic milk ice cream with a fiber content of 4%, total solids 33%. Homayouni et al. [32] showed a higher content of dry matter and fat in synbiotic ice cream, accounting for 38.5% and 8.1%, respectively.

The carbohydrate content of ice cream blends before fermentation ranged from 19.45% in AFbb12 to 19.50% in Abb12. As expected, the carbohydrate content after fermentation decreased by 1.3% - 1.4%. There was no significant effect of apple fiber and the type of bacteria used for fermentation on the carbohydrate content in sheep's milk ice cream mixes. Also, in the study by Góral et al. [29], no significant effect of additives on the carbohydrate content (26.52% - 27.48%) in the ice cream was found. In sheep's milk ice cream in a study by Balthazar et al. [1], the carbohydrate content was determined in the range from 18.1 to 18.6%, i.e., similar to the results presented in Table 1. The effect of storage time, the addition of apple fiber, and the type of fermenting bacteria on the pH value and the concentration of lactic acid in sheep's milk ice cream are presented in Table 2.

Table 1. Chemical composition of ice creams mixtures samples.

Chemical composition	Storage time	Abb12	AFbb12	BLr	BFLr
Protein [%]	0	4.97^{Aa}±0.04	4.98^{Aa}±0.10	4.91^{Aa}±0.02	4.95^{Aa}5±0.04
	1	4.98^{Aa}±0.11	5.0^{Aa}±0.05	4.90^{Aa}±0.17	4.92^{Aa}±0.05
Fat [%]	0	6.08^{Aa}±0.23	6.00^{A}±0.04	6.07^{Aa}±0.02	6.04^{Aa}±0.03
	1	6.10^{Aa}±0.22	5.97^{Aa}±0.03	6.05^{Aa}±0.20	6.03^{Aa}±0.02
Carbohydrates [%]	0	19.50^{Ba}±0.14	19.45^{Ba}±0.02	19.49^{Ba}±0.04	19.46^{Ba}±0.02
	1	18.04^{Aa}±0.06	18.07^{Aa}±0.09	18.10^{Aa}±0.03	18.14^{Aa}±0.07

Mean±standard deviation.

n = 20;

$^{a, b}$ – Mean values denoted in rows by different letters differ statistically significantly at ($p \leq 0.05$);

$^{A, B}$ – Mean values in columns obtained for a given parameter denoted by different letters differ significantly ($p \leq 0.05$).

Abb12: sample with 4% of inulin and *Bifidobacterium* bb12, AFbb12: sample with 2,5% of inulin, 1,5 % apple fiber and *Bifidobacterium* bb12, BLr: sample with 4% of inulin and *Lb. rhamnosus*, BFLr: sample with 2,5% of inulin, 1,5 % of apple fiber and *Lb. rhamnosus*. Time: 0- before fermentation, 1- after fermentation.

Table 2. Lactic acid content and pH value of ice creams during storage

Properties	Storage time	Abb12	AFbb12	BLr	BFLr
pH	0	6.60^{Bb}±0.03	6.24^{Ba}±0.01	6.61^{Bb}±0.01	6.24^{Ba}±0.01
	1	5.19^{Ab}±0.08	4.93^{Aa}±0.03	5.97^{Ad}±0.04	5.75^{Ac}±0.03
	7	5.16^{Ab}±0.05	4.90^{Aa}±0.03	5.94^{Ad}±0.02	5.72^{Ac}±0.02

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 February 2021 doi:10.20944/preprints202102.0387.v1

	21	5.20^{Ab}±0.08	4.90^{Aa}±0.03	5.91^{Ad}±0.04	5.71^{Ac}±0.02
Lactic acid [g/l]	1	0.61^{Ac}±0.04	0.71^{Ad}±0.04	0.38^{Aa}±0.01	0.42^{Ab}±0.01
	7	0.62^{Ab}±0.08	0.74^{Ac}±0.06	0.39^{Aa}±0.05	0.41^{Aa}±0.02
	21	0.62^{Ab}±0.01	0.75^{Ac}±0.03	0.38^{Aa}±0.01	0.40^{Aa}±0.02

Mean±standard deviation.

n = 20;

$^{a, b, c, d}$ –Mean values denoted in rows by different letters differ statistically significantly at ($p \leq 0.05$);

$^{A, B}$ – Mean values in columns obtained for a given parameter denoted by different letters differ significantly ($p \leq 0.05$).

Abb12: sample with 4% of inulin and *Bifidobacterium* bb12, AFbb12: sample with 2,5% of inulin, 1,5 % apple fiber and *Bifidobacterium* bb12, BLr: sample with 4% of inulin and *Lb. rhamnosus*, BFLr: sample with 2,5% of inulin, 1,5 % of apple fiber and *Lb. rhamnosus*. Storage time: 0- before fermentation, 1- after fermentation, 7- after 7 days, 21- after 21 days.

Ice cream mixes with apple fiber (AFbb12 and BFLr) were characterized by a significantly lower pH value than the mixes made only with inulin ($p \leq 0.05$). As a result of the ten-hour fermentation, the pH value in all ice cream mixes significantly decreased from 0.49 in BFLr to 1.31 in AFbb12 ($p \leq 0.05$) compared to the pH before fermentation. In mixtures containing *Bifidobacterium* Bb-12, lower pH values were found than in the mixtures with *Lb. rhamnosus.* Ice-cream mixtures also showed lower lactic acid content of 0.23 g L^{-1} and 0.29 g L^{-1} than to Abb12 and AFbb12 blends. The conducted three-factor ANOVA (Table 3) shows that the pH value is significantly influenced by the three analysed research factors (a type of bacteria, storage time, apple fiber) and the interactions of these factors. The effect of the storage time on the pH value of ice cream mixes and ice cream is mainly due to the inclusion of the pH value before fermentation in this comparison. There was no significant effect of the storage time on the mixtures pH value after fermentation and ice cream after 7 and 21 days of storage. The addition of 1.5% apple fiber resulted in maintaining lower pH values in mixes and ice cream throughout the entire study period. In the study carried out by Akalin and Erisir [31], the pH value in the range of 5.35- to 5.45 was determined in probiotic ice cream with the addition of oligofructose and inulin. In milk ice cream containing *Lb. rhamnosus*, Pankiewicz et al. [33] determined the pH value from 5.73 to 5.83.

3.2 Microbiological analysis of ice creams mixtures and ice creams

The presented pH values of the product help to maintain the high survival rate of probiotic bacteria [34]. In addition, some studies confirmed a higher pH in fermented ice cream than in fermented milk or in fermented frozen desserts.

Mohammadi et al. [35]. Da Silva et al. [36] reported the pH value of 6.45 in unfermented ice cream with the addition of *Bifidobacterium* Bb-12. In addition, some studies have found a higher pH in fermented ice cream than in fermented milk or in fermented frozen desserts. Ozturk et al. [38] determined the pH value in fermented ice cream from 5.28 to 5.89, depending on the additives used. These low pH values determined by Dos Santos et al. [37] and Ozturk et al. [38] were associated with the addition of fruits, which lower the pH value.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 February 2021 doi:10.20944/preprints202102.0387.v1

The ice cream with the addition of AFbb12 and BFLr apple fiber also showed a higher content of lactic acid after 7 and 21 days than ice creams with inulin addition. The ANOVA analysis of variance indicates that the concentration of lactic acid was significantly influenced by the type of bacteria, the addition of fiber, and the interaction between the type of bacteria and fiber. Akalin et al. [39] reported that the presence of various dietary fibers influences the lactic acid content, especially in ice cream with orange, apple, and bamboo fiber. Those authors in probiotic ice cream with 2% apple fiber added as much as 3.65 g / 100 g of lactic acid.

As a result of the ten-hour fermentation of ice cream mixes from sheep's milk by *Lb. rhamnosus* and *Bifidobacterium* Bb-12, the number of bacterial cells exceeded 11 log cfu g $^{-1}$ (Table 4).

Tabela 4. Viable counts of probiotic bacteria in ice creams and ice creams mixture (log cfu g $^{-1}$).

Storage time	Abb12	AFbb12	BLr	BFLr
1	11.41^{Ba}±0.79	11.11^{Ba}±0.70	11.58^{Aa}±0.78	11.73^{Aa}±0.72
2	10.95^{ABa}±0.73	10.72^{ABa}±0.72	11.46^{Ab}±0.78	11.65^{Ab} ±0.80
7	10.77^{ABa} ±0.83	10.48^{ABa} ±0.74	11.34^{Ab} ±0.87	11.59^{Ab} ±0.79
21	10.68^{Aa} ±0.76	10.28^{Aa} ±0.73	11.22^{Ab} ±0.79	11.50^{Ab} ±0.77

Mean±standard deviation.
n = 20;
$^{a, b}$ – Mean values denoted in rows by different letters differ statistically significantly at ($p \leq 0.05$);
$^{A, B}$ – Mean values in columns obtained for a given parameter denoted by different letters differ significantly ($p \leq 0.05$).
Abb12: sample with 4% of inulin and *Bifidobacterium* bb12, AFbb12: sample with 2,5% of inulin, 1,5 % apple fiber and *Bifidobacterium* bb12, BLr: sample with 4% of inulin and *Lb. rhamnosus*, BFLr : sample with 2,5% of inulin, 1,5 % of apple fiber and *Lb. rhamnosus*. Storage time: 1- after fermentation, 2- directly after freezing, 7- after 7 days, 21- after 21 days.

In the process of ice cream manufacture, the ingredients used in the recipe may adversely affect the probiotic by changing the pH (e.g., pH 5.5 - 6.0 is optimal for the growth of *Lactobacillus acidophilus* and pH 6.0 - 7.0 is favorable for *Bifidobacterium*), titratable acidity or sugar content [35,40]. In this case, there was no effect of the addition of apple fiber and the type of bacteria on the number of viable cells after fermentation.

When the temperature was decreasing during the freezing of ice mixtures, changes in the osmotic pressure in the cells result in changes in the microorganisms, causing the loss of their metabolic properties. During the freezing process, the formed ice crystals can mechanically damage cell walls, and the condensation of harmful solutes or dehydration of cells additionally intensify the adverse changes [41, 42]. The adverse effect of oxygen due to the aeration process during freezing and high redox potential values on anaerobic bacteria, especially *Bifidobacterium* [35, 40], should also be mentioned. The survival rate of probiotic bacteria depends on the bacteria, production technology, temperature, storage time, and ice cream chemical composition. The results presented in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 February 2021 doi:10.20944/preprints202102.0387.v1

Table 4 indicate that as a result of freezing ice mixes fermented by *Bifidobacterium* Bb-12, there was a significant reduction in the bacterial number from 0.39 log cfu g $^{-1}$ to 0.46 log cfu g $^{-1}$ compared to the number of cells of these bacteria in the mixtures after fermentation ($p \leq 0.05$). The low pH of the mixtures and the high content of lactic acid contributed to reducing the *Bifidobacterium* Bb-12. The decrease in bacterial cell counts resulting from freezing is likely due to damage to the bacterial cell walls that led to the bacterial cells' death [43]. In the studies of Akalin and Erisir [31], during freezing of mixtures with *Lactobacillus acidophilus* and *Bifidobacterium* Bb-12, the number of bacterial cells decreased by 1.5- to 2 logarithmic units. Table 4 shows no significant effect of the freezing process on the number of *Lb. rhamnosus* cells in BLr and BFLr ice cream. The lack of this effect on the number of *Lb. rhamnosus* cells can be explained by significantly higher pH values and lower lactic acid content. According to Godward et al. [44] and Tamime et al. [45], probiotic bacteria's resistance to pH and acidity is bacteria-dependent. It was found that *Lactobacillus* has a broad cytoplasmic buffering capacity, resistance to pH (3.72-7.74), which enables its stability and resistance to changes cytoplasmic pH in an acidic environment.

Also, the 1.5% addition of apple fiber did not significantly affect the number of viable bacterial cells immediately after freezing (Table 4). Mohammadi et al. [35] obtained 8 log CFU in a milliliter of *Lactobacillus acidophilus* and 8 log CFU in a milliliter of *Bifidobacterium bifidum* immediately after freezing the ice cream. Akbari et al. [46] reported that after freezing, the viability of bacteria decreased by 0.28 (*Lactobacillus acidophilus*) and by 0.33 (*Lb. rhamnosus*) log units.

In these studies, ice cream storage at -22 ° C for 7 and 21 days resulted in not significant reduction in the bacterial of both types *Bifidobacterium* Bb-12 and *Lb. rhamnosus* (Table 4). However, after 7 and 21 days of storage, many bacterial cells were determined in ice cream with *Lb. rhamnosus* BLr and BFLr. The number of viable *Lb. rhamnosus* and *Bifidobacterium* Bb-12 cells in all ice cream on day 21 of storage exceeded 10 log cfu g $^{-1}$, which means that the ice cream maintained its probiotic status (Table 4). In the studies by Akalin and Erisir [31], a decrease in the bacterial number (from 0.3 to 0.9 log cfu g $^{-1}$) was found during freezing ice cream storage. The probiotic ice cream tested by Góral et al. [29] also showed a high number of bacterial cells in the range from 9 log CFU per milliliter to 11 log CFU per milliliter. According to the International Dairy Federation's recommendations, products defined as probiotic should contain at least 7 log cfu g $^{-1}$ *lactobacillus* or 6 log cfu g $^{-1}$ *Bifidobacterium* [47]. The studies of Balthazar [48] showed the number of *Lactobacillus acidophilus* cells exceeding 6 log cfu g $^{-1}$ in probiotic ice cream. Similarly, Akalin and Erisir [31] reported that probiotic cultures had an excellent ability to survive and maintain high cell counts in frozen foods.

The excellent survival rate of probiotic bacteria cells obtained in these studies ensures that the therapeutic level of synbiotic sheep's milk ice cream is maintained for at least 21 days. According to Jayamann and Adams [49], a bacterial level of 7 log cfu g $^{-1}$ is required to obtain a therapeutic (anti-diarrheal) effect.

The results of the colour of ice-cream mixes and ice cream during storage presents Table 5.

Table 5. Colour parameters of ice cream sample in ice cream mixture during storage.

Colour	Storage time	Abb12	AFbb12	BLr	BFLr

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 February 2021 doi:10.20944/preprints202102.0387.v1

L*	1	$70.58^{Ab}\pm0.35$	$61.52^{Aa}\pm0.49$	$71.60^{Ab}\pm0.32$	$61.89^{Aa}\pm0.55$
	7	$85.14^{Bb}\pm2.35$	$74.22^{Ba}\pm1.86$	$88.30^{Bb}\pm2.46$	$75.97^{Ba}\pm0.43$
	21	$86.78^{Bb}\pm1.28$	$74.93^{Ba}\pm2.86$	$88.70^{Bb}\pm0.94$	$77.29^{Ba}\pm1.05$
a*	1	$-0.04^{Bb}\pm0.09$	$4.29^{Ad}\pm0.15$	$-0.75^{Aa}\pm0.04$	$3.27^{Ac}\pm0.16$
	7	$-0.37^{Aa}\pm0.18$	$5.23^{Bb}\pm0.67$	$-0.26^{Ba}\pm0.15$	$5.65^{Bb}\pm0.11$
	21	$-0.47^{Aa}\pm0.28$	$4.81^{Bb}\pm0.48$	$-0.39^{Ba}\pm0.10$	$5.30^{Bb}\pm0.29$
b*	1	$17.43^{Ad}\pm0.31$	$15.79^{Ac}\pm0.55$	$9.49^{Aa}\pm0.07$	$10.15^{Ab}\pm0.12$
	7	$20.07^{Bc}\pm3.11$	$17.63^{Bc}\pm2.85$	$10.46^{Aa}\pm0.73$	$12.64^{Bb}\pm1.03$
	21	$19.13^{Bd}\pm1.80$	$14.62^{Ac}\pm0.55$	$12.11^{Ba}\pm0.45$	$12.91^{Bb}\pm0.23$
C*	1	$17.43^{Ad}\pm0.31$	$16.16^{Ac}\pm0.45$	$9.52^{Aa}\pm0.08$	$10.66^{Ab}\pm0.14$
	7	$20.07^{Bb}\pm3.11$	$18.21^{Bb}\pm3.04$	$10.46^{Aa}\pm0.73$	$16.28^{Cb}\pm0.29$
	21	$19.14^{Bc}\pm1.81$	$15.46^{Ab}\pm0.54$	$12.12^{Ba}\pm0.46$	$13.77^{Bab}\pm0.59$
h*	1	$90.14^{Ac}\pm0.28$	$74.63^{Bb}\pm0.22$	$94.49^{Bd}\pm0.26$	$71.9^{Ca}\pm0.83$
	7	$90.74^{Ac}\pm0.92$	$73.41^{ABb}\pm1.36$	$90.82^{Ac}\pm0.71$	$69.52^{Ba}\pm0.57$
	21	$91.34^{Ac}\pm0.76$	$70.55^{Ab}\pm1.96$	$91.83^{Ac}\pm0.42$	$67.39^{Aa}\pm0.75$

Mean±standard deviation.
n = 20;
$^{a, b, c, d}$ – Mean values denoted in rows by different letters differ statistically significantly at ($p \leq 0.05$);
$^{A, B, C}$ – Mean values in columns obtained for a given parameter denoted by different letters differ significantly ($p \leq 0.05$).
Abb12: sample with 4% of inulin and Bifidobacterium bb12, AFbb12: sample with 2,5% of inulin, 1,5 % apple fiber and *Bifidobacterium* bb12, BLr: sample with 4% of inulin and *Lb. rhamnosus*, BFLr : sample with 2,5% of inulin, 1,5 % of apple fiber and *Lb. rhamnosus*. Storage time: 1- after fermentation, 7- after 7 days, 21- after 21 days.

The L* parameter's highest values were recorded in the ice-cream mix with inulin and *Lb. rhamnosus*, and then in the mix with inulin and *Bifidobacterium* Bb-12. The lighter colour of Abb12 and BLr ice cream was maintained throughout the storage period. The 1.5% addition of apple fiber decreased the colour brightness by about 9 units in the AFbb12 and BFLr blends. After 7 and 21 days of storage, an increase in L* brightness was found in all ice cream sample. Extending the storage time from 7 to 21 days resulted in a further increase in L* brightness, but the differences were not significant. A significant effect of the storage time and addition of apple fiber and the interaction of these two factors on the brightness of the colour of ice cream was demonstrated (Table 3).

Ice cream mixes and ice cream with the addition of apple fiber (AFbb12 and BFLr) were characterized by a higher red colour (+ a*), which comes from phenolic compounds and pectin contained in the fiber [50, 51]. On the other hand, only ice cream with inulin addition had a higher green colour parametr (- a*). The ANOVA analysis of variance shows that a* parameter was influenced by the type of bacteria, storage time, the addition of apple fiber, and interactions between the storage time and the addition of apple fiber.

Analysed ice cream and ice cream mixture were characterized by a high value of yellow (+ b*) due to the mango-passion fruit essence used in their production. A significantly lower value of yellow colour was found in the ice cream fermented with *Lb. rhamnosus* throughout the storage

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 February 2021 doi:10.20944/preprints202102.0387.v1

period ($p \leq 0.05$). These results are confirmed by the analysis of variance, which showed that the type of bacteria, the addition of fiber, and the interaction of two factors significantly influenced the yellow colour intensity. The results for the intensity (C*) and hue (h*) of colour indicate that these colour coordinates are dependent on all factors tested (a type of bacteria, storage time, and fiber addition) and their interactions. The addition of apple fiber decreased the h* colour saturation parameter in AFbb12 and BFLr ice cream. The studies of Akalin et al. [39] also found that the addition of orange and apple fiber reduces the L* brightness of ice cream and intensifies the red and yellow colour. Also, in the studies of Crizel et al. [52], Dervisoglu and Yazici [53], ice cream with citrus fiber added lower L* values and higher a* and b * values, which indicates that the addition of fiber causes a reduction in brightness and gives a red and yellow. Ice cream made by Calligaris et al. [54] determined the following values of the colour parameters: L* 87.93, a* 2.41, and b* 6.91. In the studies carried out of Pankiewicz et al. [33], L* brightness in the range from 71.15 to 71.31 and the a* (0.15) and b* (13.29 - 13.64) colour coordinates were determined in milk ice cream fermented by *Lb. rhamnosus*. Table 6 shows the analysis of sheep's milk ice cream's physical properties for such features as overrun, first dropping time, and melting time. One of the most important parameters in assessing the quality of ice cream is the degree of air entrainment in the ice cream mixture, i.e., overrun. The ice cream mixture ingredients have the most significant impact on overrun, especially the content and protein proportion to fat [55].

Table 6. Overrun, first dropping time, and total melting rate in 7 and 21 days of storage.

Properties	Storage time	Abb12	AFbb12	BLr	BFLr
Overrun [%]	7	$79.15^{Aa} \pm 0.20$	$80.41^{Aa} \pm 0.22$	$80.50^{Aa} \pm 0.95$	$78.50^{Aa} \pm 0.12$
	21	$80.30^{Aa} \pm 0.47$	$80.41^{Aa} \pm 1.22$	$80.61^{Aa} \pm 0.40$	$79.1^{Aa} \pm 0.95$
First drop [s]	7	$972^{Ba} \pm 12.21$	$960^{Ba} \pm 10.26$	$991^{Bb} \pm 8.71$	$982^{Bb} \pm 10.12$
	21	$940^{Ab} \pm 14.40$	$906^{Aa} \pm 10.15$	$972^{Ac} \pm 7.91$	$911^{Aa} \pm 8.32$
Complete melting times [s]	7	$5469^{Bb} \pm 35.20$	$5230^{Ba} \pm 20.13$	$5913^{Bd} \pm 38.12$	$5692^{Bc} \pm 35.11$
	21	$4804^{Ac} \pm 33,85$	$4007^{Aa} \pm 25.42$	$5187^{Ad} \pm 40.00$	$4201^{Ab} \pm 15.32$

Mean±standard deviation.
n = 20;
$^{a, b, c, d}$ – Mean values denoted in rows by different letters differ statistically significantly at ($p \leq 0.05$);
$^{A, B}$ – Mean values in columns obtained for a given parameter denoted by different letters differ significantly ($p \leq 0.05$).
Abb12: sample with 4% of inulin and *Bifidobacterium* bb12, AFbb12: sample with 2,5% of inulin, 1,5 % apple fiber and *Bifidobacterium* bb12, BLr: sample with 4% of inulin and *Lb. rhamnosus*, BFLr : sample with 2,5% of inulin, 1,5 % of apple fiber and *Lb. rhamnosus*. Storage time: 1- after fermentation, 7- after 7 days, 21- after 21 days. Storage time: 7- after 7 days, 21- after 21 days.

The results in Table 6 shows that the ice cream's overrun was not affected by the storage time, the addition of apple fiber, and the type of bacteria used to ferment the mixture. Sheep's milk ice cream overrun was from 78.50% to 80.41% (Table 6). The studies of Akalin et al. [39] showed a lower overrun of ice cream from 25.55% to 30.60%, and the 2% addition of apple fiber increased the overrun of ice cream compared to ice cream without this addition. On the other hand, Akin et al. [42] indicate that ice cream's overrun depended on the content of sugar and inulin. Increasing the sugar content from 15% to 21% resulted in an increased overrun from 27.8% to 32.3%. However, Crizel et

 doi:10.20944/preprints202102.0387.v1

al. [52] prove that the addition of orange fiber as a fat replacement resulted in a significant reduction in the ice cream's overrun compared to the control sample, probably due to the lower fat content. In the conducted research, all ice cream groups contained about 6% fat; hence their overrun did not differ. Our study also proves that replacing inulin with apple fiber does not change the overrun of ice cream.

The melting rate of ice cream is influenced by many factors, including the total dry matter content, ice crystals, size, and the number of fat globules [29]. The first dropping time of ice cream drip time after 7 days of freezer storage differed significantly depending on the type of bacteria used to ferment the mixture. Ice creams containing *Lb. rhamnosus* (BLr and BFLr) had a longer time for the first dropping time after 7 days of storage than containing *Bifidobacterium* Bb-12 (Table 6). Extending the ice cream storage time to 21 days significantly reduced the first dropping time by 19 - 22 seconds in all ice cream groups.

A fast-melting product is undesirable, and a too slow melting rate can also be a disadvantage of ice cream [56]. The total melting time was shorter in the apple fiber ice cream (AFbb12 and BFLr) than their inulin only counterparts (Abb12 and BLr). Zhang et al. [56] found that pectin's addition reduced ice cream's melting rate and led to more excellent product stabilization. Pectin present in apple fiber may interact with other milk components to create a dense three-dimensional network structure and reduce the heat transfer rate [57]. Research by Akalin et al. [39] indicates that apple fiber is rich in pectin, known for its gelling properties that significantly increase ice cream mixes' viscosity. Soukoulis et al. [58] reported that apple fiber's addition increased the freezing temperature and led to a decrease in ice crystals and the percentage of frozen water. The analysis of variance carried out indicates that the total melting time was influenced by the interactions between the storage time and the addition of apple fiber and the interactions between the three examined factors (a type of bacteria, storage time, fiber). According to Criscio et al. [59] and El-Nagar et al. [60] samples containing 5% inulin had a significantly higher melting rate than to the controls and samples with 2.5% inulin. In the studies of Balthazar et al. [48], probiotic ice cream with 10% addition of inulin also had a longer melting time than probiotic ice cream without inulin. Akin et al. [42] in ice cream, depending on the content of sugar and inulin, noted that the values of the first dropping time and total melting time were within the ranges of 1780 seconds (15% sugar without inulin) - 2058 seconds (21% sugar with 2% inulin), respectively and 4806 seconds (21% sugar without inulin) - 5313 seconds (18% sugar with 2% inulin). In other yogurt ice cream studies, the addition of 5% inulin reduced the melting rate from 5% to 9% due to binding water, thus reducing the interaction of dry matter components with water [60]. On the other hand, Balthazar et al. [48] and Senanayake et al. [61] indicate that the amount of air introduced also determines the melting rate. Besides, the studies by Balthazar et al. [48]. The 10% addition of inulin did not significantly affect the aeration of probiotic ice cream made from sheep's milk compared to probiotic ice cream without inulin and [1] it was shown that fat destabilization influenced overrun. Muse and Hartel [62] indicated that the ice cream’s destabilized fat surrounds the air bubbles, stabilizing them and increasing the ice cream's aeration. According to our research (Table 1), all ice cream groups did not differ in fat content. Therefore, no differences in overrun were found. In this case, the addition of inulin had a more significant effect on extending the ice cream melting time. Analyses shows that ice cream with 2.5% inulin and 1.5% apple fiber (AFbb12, BFLr) is quickly melting than ice cream with

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 February 2021 doi:10.20944/preprints202102.0387.v1

4% inulin (Abb12, BLr). Also, in the studies of Akalin and Erisir [31], the addition of inulin increased the first dropping time and the total time of ice cream melting.

From a technological point of view, the use of fruit fiber in ice cream production causes significant changes in organoleptic characteristics, enhancing their taste and texture [63].

The addition of apple fiber significantly changed the appearance of sheep's milk ice cream. It increased sandiness and intensified the taste and smell of the expansion of mango-passion fruit essence, both on the 7th and 21st day of freezing storage (Table 7).

Table 7. Sensory characteristics of ice cream on 7 and 21 days of storage

Properties	Storage time	Abb12	AFbb12	BLr	BFLr
Appearance	7	8.07^{Ab}±1.33	5.71^{Aa}±1.50	7.43^{Ab}±1.51	5.57^{Aa}±1.76
	21	8.25^{Ab}±1.39	5.75^{Aa}±0.50	8.75^{Ab}±0.50	5.00^{Aa}±1.15
Hardness	7	6.00^{Aa}±1.35	6.71^{Aa}±1.36	6.14^{Aa}±1.04	6.71^{Aa}±1.89
	21	6.38^{Aa}±1.20	6.75^{Aa}±1.26	6.00^{Aa}±0.82	6.85^{Aa}±1.26
Smoothness	7	7.07^{Ab}±1.77	3.14^{Aa}±1.91	6.86^{Ab}±1.07	3.00^{Aa}±1.31
	21	6.38^{Ab}±1.06	3.75^{Aa}±1.26	6.50^{Ab}±1.29	3.50^{Aa}±1.29
Sweet taste	7	5.43^{Aa}±1.03	4.43^{Aa}±0.53	5.24^{Aa}±1.07	4.57^{Aa}±1.62
	21	5.25^{Aa} ±1.67	5.75^{Aa}±1.71	5.25^{Aa}±1.71	5.25^{Aa} ±1.50
Additives taste	7	5.29^{Aab}±1.77	6.71^{Ab}±1.60	4.00^{Aa}±1.21	6.57^{Ab}±1.72
	21	5.75^{Aab}±1.05	6.50^{Ab}±1.29	4.25^{Aa}±1.71	7.25^{Ab}±1.50
Off taste	7	1.00^{Aa}±0.00	1.00^{Aa}±0.00	1.00^{Aa}±0.00	1.00^{Aa}±0.00
	21	1.00^{Aa}±0.00	1.00^{Aa}±0.00	1.00^{Aa}±0.00	1.00^{Aa}±0.00
Odour additives	7	3.00^{Aa}±1.71	4.00^{Ab}±1.00	1.71^{Aa}±0.76	4.29^{Ab}±1.56
	21	3.88^{Aa}±1.30	4.50^{Ab}±1.52	2.00^{Aa}±0.82	4.75^{Ab}±1.06
Off odour	7	1.00^{Aa}±0.00	1.00^{Aa}±0.00	1.10^{Aa}±0.00	1.00^{Aa}±0.00
	21	1.00^{Aa}±0.00	1.00^{Aa}±0.00	1.00^{Aa}±0.00	1.00^{Aa}±0.00

Mean±standard deviation.

n = 20;

$^{a, b}$ – Mean values denoted in rows by different letters differ statistically significantly at ($p \leq 0.05$);

$^{A, B}$ – Mean values in columns obtained for a given parametr denoted by different letters differ significantly ($p \leq 0.05$).

Abb12: sample with 4% of inulin and *Bifidobacterium* bb12, AFbb12: sample with 2,5% of inulin, 1,5 % apple fiber and *Bifidobacterium* bb12, BLr: sample with 4% of inulin and *Lb. rhamnosus*, BFLr : sample with 2,5% of inulin, 1,5 % of apple fiber and *Lb. rhamnosus*. Storage time: 1- after fermentation, 7- after 7 days, 21- after 21 days. Storage time: 7- after 7 days, 21- after 21 days.

Akalin et al. [39], in the ice cream samples of prepared with apple and orange fiber, lower scores were given for flavor characteristics compared to the control ice cream. Crizel et al. [52] also showed lower taste scores for ice cream with 1.5% orange fiber than in the controls. The addition of microorganisms and inulin in the studies of Criscio et al. [59] did not significantly affect flavor intensity, texture, and smoothness. On the other hand, Akbari et al. [46] indicate that the introduction of inulin as a fat substitute made the evaluators prefer the taste of ice cream containing

 doi:10.20944/preprints202102.0387.v1

3% and 4% of inulin to taste ice cream without inulin. These authors state that the reason for the taste change in low-fat ice cream without inulin was most likely the crystal growth in the ice cream, which caused a change in taste and smell. Akin et al. [42] conducted studies on inulin and sugar influence on prebiotic ice cream's physical and sensory properties. They found that the addition of inulin does not affect the sensory properties of ice cream. However, Karaca et al. [64] found that reduced-fat, low-fat ice cream with carbohydrate-based fat replacers scored lower on flavor than the control. The analysis of variance performed shows that the storage time, type of bacteria, and interactions of all examined factors (storage time, kind of bacteria, fiber) do not affect the hardness, smoothness, sweet taste, taste, and smell of additives as well as foreign taste and smell. The research indicates that only apple fiber's addition contributed to a change in the smoothness additives, taste and smell.

 doi:10.20944/preprints202102.0387.v1

222 **Table 3**. Analysis of variance (ANOVA) P-values on the effects of storage time and type of bacteria and fibre on colour, pH, lacid acid, overrun, bacteria appearance, hardness,
223 smoothness, sweet taste, additives taste off taste odour additives, off odour of ice cream.

224

Properties	Type of bacteria	Storage time	Fiber	Type of bacteria * Storage time	Type of bacteria * Fiber	Storage time * Fiber	Type of bacteria * Storage time * fiber
L*	n.s. 0.1813	↑ 0.0007	↑ 0.0000	n.s. 0.4748	n.s. 0.9892	↑ 0.0012	n.s. 0.2122
a*	↑0.0037	↑ 0.0073	↑ 0.0000	n.s. 0.8549	n.s. 0.1225	↑ 0.0072	n.s. 0.6325
b*	↑0.0000	n.s. 0.2994	↑ 0.0496	↑ 0.0213	↑ 0.0004	n.s. 0.1295	n.s. 0.5593
C*	↑0.0000	↑ 0.0228	↑ 0.0128	↑ 0.0000	↑ 0.0000	↑ 0.0048	↑ 0.0145
h*	↑0.0000	↑ 0.0083	↑ 0.0000	↑ 0.0421	↑ 0.0000	↑ 0.0000	↑ 0.0279
pH	↑0.0000	↑ 0.0482	↑ 0.0000	↑ 0.0258	↑ 0.0350	↑ 0.0426	↑ 0.0498
Lacid acid [g L^{-1}]	↑0.0000	n.s. 0.3087	↑ 0.0000	↑ 0.0418	↑ 0.0323	n.s. 0.3110	n.s. 0.2388
Overrun [%]	n.s. 0.4132	n.s. 0.0786	n.s. 0.0786	n.s. 0.3532	n.s. 0.1096	n.s. 0.0701	n.s. 0.6300
First drop [s]	↑ 0.0012	↑ 0.0000	n.s. 0.0541	↑ 0.0001	n.s. 0.0531	n.s. 0.0620	n.s. 0.0714
Complete melting times [s]	↑ 0.0011	↑ 0.0000	↑ 0.0004	n.s. 0.0678	n.s. 0.0882	↑ 0.0000	↑ 0.0412
Bacteria	↑ 0.0096	↑ 0.0264	↑ 0.0390	↑ 0.0499	↑ 0. 0402	n.s. 0.2160	n.s. 0.1183
Appearance	↑0.0088	n.s. 0.3556	↑ 0.0158	n.s. 0.8324	↑ 0. 0426	↑ 0.01808	n.s. 0.2138
Hardness	n.s. 0.7617	n.s. 0.0870	n.s. 0.4301	n.s. 0.6735	n.s. 0.9116	n.s. 0.7844	n.s. 0.9940
Smoothness	n.s. 0.1067	n.s. 0.1559	↑ 0.0000	n.s. 0.1975	n.s. 0.1254	↑ 0.1860	n.s. 0.1103
Sweet taste	n.s. 0.4752	n.s. 0.3115	n.s. 0.5721	n.s. 0.8505	n.s 0.9699	n.s. 0.3590	n.s. 0. 5724
Additives taste	n.s. 0.5351	n.s 0.6157	↑ 0.0017	n.s. 0.7724	n.s 0.1524	n.s. 0.9151	n.s. 0.6372
Off taste	n.s. 0.1321	n.s. 0.9190	n.s. 0.9190	n.s. 0.9190	n.s. 0.9190	n.s. 0.1321	n.s. 0.1321
Odour additives	n.s. 0.2274	n.s. 0.3272	↑ 0.0022	n.s. 0.7721	n.s 0.0916	n.s. 0.9274	n.s. 0.7976
Off odour	n.s. 0.8243	n.s. 0.1248	n.s. 0.8243	n.s. 0.8243	n.s. 0.8243	n.s. 0.8243	n.s. 0.8243

*Storage time (days) = interaction ↑; Type of bacteria*Fiber = interaction ↑; Storage time * Fiber = interaction ↑; Type of bacteria * Storage time * fiber = interaction ↑; indicates significant effect P <0.05; n.s. no significant effect

3. Conclusion

Sheep's milk ice cream can be a good source of probiotic bacteria and dietary fiber. The addition of 1.5% apple fiber instead of inulin resulted in a change in ice cream's physicochemical and organoleptic characteristics. On an industrial scale, when using the addition of apple fiber for the production of probiotic ice cream, attention should be paid to selecting the strain and the survival of probiotic bacteria during the freezing of the mixture and storage of ice cream.

Author Contributions: Conceptualization, M.K. and A.Z.; methodology, M.K.; software, M.B.; validation, M.K., A.Z., M.B..; formal analysis, A.Z.; investigation, M.K.. and M.B.; resources, M.K. and A.Z.; data curation, M.K. and A.Z.; writing—original draft preparation, M.K. and A.Z.; writing—review and editing, M.K., A.Z. and M.B.; supervision, A.Z.; project administration, A.Z.; funding acquisition, M.K. A.Z. and M.B.; All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Balthazar, C.F.; Silva, H.L.A.; Vieira, A.H.; Neto, R.P.C.; Cappato, L.P.; Coimbra, P.T.; Raices, I.B.; Silva, R.S.L; Cruz, A.G. Assessing the effects of different prebiotic dietary oligosaccharides in sheep milk ice cream. *Food Res. Int.* 2017, 91, 38-46. https://doi.org/10.1016/j.foodres.2016.11.008
2. Ayar, A.; Siçramaz, H.; Öztürk, S.; Yilmaz, S.Ö. Probiotics properties of ice creams produced with dietary fibres from by-products of the industry. *Int. J. Dairy Technol.* 2017, 70, 1-9. https://doi.org/10.1111/1471-0307.12387
3. FAO/WHO Joint Expert Consultation Report: Evaluations of Health and Nutritional Properties of Probiotics in Food Including Powder Milk and Live Lactic Acid Bacteria, Cordoba, Argentina. 2001.
4. Gibson, G.R.; Hutkins, R.; Sanders, M.E.; Prescott, S.L.; Reimer, R.A.; Salminen, S.J.; Scott, K.; Stanton, C.; Swanson, K.S.; Cani, P.D. Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol*. 2017, 14, 491-502. https://doi.org/10.1038/nrgastro.2017.75
5. Lopez-Santamarina, A.; Lamas, A.; del Carmen Mondragón, A.; Cardelle-Cobas, A.; Regal, P.; Rodriguez-Avila, J.A.; Miranda, J.M.; Franco, C.M.; Cepeda, A. Probiotic effects against virus infections: New weapons for an old war. *Foods* **2021**, *10*, 130. https://doi.org/10.3390/foods10010130
6. Spence, C.; Youssef, J.; Navarra, J. Using ice-cream as an effective vehicle for energy/nutrient delivery in the elderly. *Int. J. Gastron. Food Sci.* 2019, 16, 100140. https://doi.org/10.1016/j.ijgfs.2019.100140
7. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Berni Canani, R.; Flint, H.J.; Salminen, S.; Calder, P.C.; Sanders, M.E. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Rev. Gastroenterol. Hepatol. 2014, 11, 506-514. https://doi.org/10.1038/nrgastro.2014.66
8. Rowland, I.; Gibson, G.; Heinken, A.; Scott, K.; Swann, J.; Thiele, I.; Tuohy, K. Gut microbiota functions: metabolism of nutrients and other food components. *Eur. J. Nutr.* 2018, 57, 1-24. https://doi.org/10.1007/s00394-017/1445-8

9. Legesse Bedada, T.; Feto, T.K.; Awoke, K.S.; Garedew, A.D.; Yifat, F.T.; Birri D.J. Probiotics for cancer alternative prevention and treatment. *Biomed Pharmacother*. 2020, 129, 110409. https://doi.org/10.1016/j.biopha.2020.110409
10. Sanders, M.E.; Merenstein, D.; Merrifield, C.A.; Hutkins, R. Probiotics for human use. Nutr Bull. 2018, 43, 212–225. https://doi.org/10.1111/nbu.12334
11. Kowalczyk M., Znamirowska A.. Próba zastosowania ß-D-Galaktozydazy do produkcji probiotycznych lodów mlecznych (In Polish). An attempt to use ß-d-galactosidase in the production of probiotic milk ice cream. *Żywn. Nauka Technol. Jakość*, 2020, 27, 2, 123, 50 -61. https://doi.org/10.15193/zntj/2020/123/334
12. U.S. Department of Health and Human Services and U.S. Department of Agriculture Dietary Guidelines for Americans 2015–2020. [(accessed on 4 August 2018)]; Available online: http://health.gov/dietaryguidelines/ 2015/guidelines/
13. Sah, B.N.P.T.; Vasiljevic, S.; McKechnie, O.N. Donkor,Physicochemical, textural and rheological properties of probiotic yoghurt fortified with fibre-rich pineapple peel powder during refrigerated storage. *LWT - Food Sci. Technol.* 2016, 65, 978–986. https://doi.org/10.1016/j.lwt.2015.09.027
14. Baye, K.; Guyot, J.P.; Mouquet-Rivier, C. The unresolved role of dietary fibers on mineral absorption. *Crit. Rev. Food Sci. Nutr*. 2017, 57, 949-957. https://doi.org/10.1080/10408398.2014.953030
15. Shah, B.R.; Li, B.; Al Sabbah, H.; Xu,W.; Mraz, J. Effects of prebiotic dietary fibers and probiotics on human health: With special focus on recent advancement in their encapsulated formulations. *Trends Food Sci. Technol*. 2020, 102, 178–192. https://doi.org/10.1016/j.tifs.2020.06.010
16. Tojo, R.; Suárez, A.; Clemente, M.G.; de los Reyes-Gavilán C.G.; Margolles A.; Gueimonde M.; Ruas-Madiedo P.. Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. World J. Gastroenterol. 2014, 20, 15163–15176. https://doi.org/10.3748/wjg.v20.i41.15163.
17. Salazar, N.; Dewulf, E.M.; Neyrinck, A.M.; Bindels, L.B.; Cani, P.D.; Mahillon, J. de Vos W.M.; Thissen J.P.; Gueimonde, M.; de Los Reyes-Gavilan, C.G.; Delzenne, N.M. Inulin-type fructans modulate intestinal bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. *Clin. Nutr.* 2015, 34, 501–507. https://doi.org/10.1016 / j.clnu.2014.06.001
18. Man, S.; Liu, T.; Yao, Y.; Lu, Y.; Ma, L.; Lu, F. Friend or foe? The roles of inulin-type fructans**.** *Carbohydr Polym*. 2021, 252, 117155. https://doi.org/10.1016/j.carbpol.2020.117155
19. Kumar, J.; Rani, K.; Datt, C. Molecular link between dietary fibre, gut microbiota and health. *Mol. Biol. Rep*. 2020, 47, 6229–6237. https://doi.org/10.1007/s11033-020-05611-3
20. Mateos-Aparicio I.; Armada, R.D.P.; Pérez-Cózar, M.L.; Rupérez P.; Redondo-Cuenca A.; Villanueva-Suárez M.J. Apple by-product dietary fibre exhibits potential prebiotic and hypolipidemic effects in high-fat fed Wistar rats. *Bioact. Carbohydr. Diet. Fibre* . 2020, pp 100219. https://doi.org/10.1016/j.bcdf.2020.100219
21. Rabetafika, H.N.; Bchir, B.; Blecker, C.; Richel, A. Fractionation of apple by-products as source of new ingredients: Current situation and perspectives. T*rends Food Sci. Technol*. 2014, 40, 1, 99-114. https://doi.org/10.1016/j.tifs.2014.08.004
22. Mituniewicz -Małek, A.; Ziarno, M.; Dmytrów, I.; Balejko, J. Short Communication: Effect of the Addition of Bifidobacterium Monocultures on the Physical, Chemical, and Sensory Characteristics of Fermented Goat Milk. *J. Dairy Sci*. 2017, 100, 6972–6979. https://doi.org/10.3168/jds.2017-12818.
23. Jemaa, M.B.; Falleh, H.; Neves, M.A.; Isoda, H.; Nakajima, M.; Ksouri, R.: Quality preservation of deliberately contaminated milk using thyme free and nanoemulsified essential oils. *Food Chem*. 2017, 217, 726-734. https://doi.org/10.1016/j.foodchem.2016.09.030

24. Rezaei, R.; Khomeiri, M.; Aalami, M.; Kashaninejad, M.: Effect of inulin on the physicochemical properties, flow behavior and probiotic survival of frozen yogurt. *J. Food Sci. Technol*. 2014, 51, 10, 2809–2814. https://doi.org/10.1007/s13197-012-0751-7
25. Lima, K.G.D.; Kruger, M.F.; Behrens, J.; Destro, M.T.; Landgraf, M.; Franco, B.D.G.M. Evaluation of culture media for enumeration of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium animalis* in the presence of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*. *LWT - Food Sci. Technol*. 2009, 42, 491–495.
26. Znamirowska, A.; Szajnar, K.; Pawlos, M. Probiotic Fermented Milk with Collagen. *Dairy* 2020, 1, 126-134. https://doi.org/10.3390/dairy1020008
27. Szajnar, K.; Znamirowska A.; Kuźniar P. Sensory and textural properties of fermented milk with viability of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* ssp. *lactis* Bb-12 and increased calcium concentration, International Journal of Food Properties, 2020, 23, 1, 582-598. https://doi.org/10.1080/10942912.2020.1748050
28. Fiol, C.; Prado, D.; Romero, C.; Laburu, N.; Mora, M.; Inaki Alavaa, J. Introduction of a new family of ice creams. *Int. J. Gastron. Food Sci*. 2017, 7, 5-10. https://doi.org/10.1016/j.ijgfs.2016.12.001
29. Góral, M.; Kozłowicz, K.; Pankiewicz, U.; Góral, D. Magnesium enriched lactic acid bacteria as a carrier for probiotic ice cream production. *Food Chem*. 2018, 239, 1151-1159. https://doi.org/10.1016/j.foodchem.2017.07.053
30. Vardar, N.B.; Öksüz O. Artisan strawberry ice cream made with supplementation of lactococci or Lactobacillus acidophilus. *Ital. J. Food Sci.* 2007, 19, 403-412.
31. Akalin, A.S.; Erisir D. Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream. *J. Food Sci*. 2008, 73, 4, 184-188. https://doi.org/10.1111/j.1750-3841.2008.00728.x
32. Homayouni, A.; Azizi, A.; Ehsani, M.R.; Yarmand, M.S.; Razavi, S.H. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chem*. 2008, 111, 50–55. https://doi.org/10.1016/j.foodchem.2008.03.036
33. Pankiewicz, U.; Góral, M.; Kozłowicz, K.; Góral, D. Application of pulsed electric field in production of ice cream enriched with probiotic bacteria (*L. rhamnosus* B 442) containing intracellular calcium ions. *J.Food Eng*. 2020, 275, 109876. https://doi.org/10.1016/j.jfoodeng.2019.109876
34. Cruz, A.G.; Antunes, A.E.; Sousa, A.L.O.; Faria, J.A.; Saad, S.M. Ice-cream as a probiotic food carrier. *Food Res. Int*. 2009, 42, 1233-1239. https://doi.org/10.1016/j.foodres.2009.03.020
35. Mohammadi, R.; Mortazavian, A.M.; Khosrokhavar, R.; da Cruz, A.G. Probiotic ice cream: viability of probiotic bacteria and sensory properties. *Ann Microbiol*. 2011, 61, 411-424. https://doi.org/10.1007/s13213-010-0188-z
36. Da Silva, P.D.L.; De Fátima Bezerra, M.; Dos Santos, K.M.O.; Correia, R.T.P. Potentially probiotic ice cream from goat's milk: characterization and cell viability during processing, storage and simulated gastrointestinal conditions. *LWT-Food Sci. Technol*. 2015, 62, 452-457. https://doi.org/10.1016/j.lwt.2014.02.055
37. Dos Santos Cruxen, C.E.; Hoffmann, J.F.; Zandoná, G.P.; Fiorentini, A.M.; Rombaldi, C.V.; Chaves, F.C. Probiotic butiá (Butia odorata) ice cream: development, characterization, stability of bioactive compounds, and viability of Bifidobacterium lactis during storage. *LWT-Food Sci. Technol*. 2017, 75, 379-385. https://doi.org/10.1016/j.lwt.2016.09.011

38. Ozturk, H.I.; Demirci, T.; Akın, N. Production of functional probiotic ice creams with white and dark blue fruits of Myrtus communis: the comparison of the prebiotic potentials on Lactobacillus casei 431 and functional characteristics. *LWT-Food Sci. Technol*. 2018, 90, 339-345. https://doi.org/10.1016/j.lwt.2017.12.049
39. Akalın, A.S.; Kesenkas, H.; Dinkci, N.; Unal, G.; Ozer, E.; Kınık, O. Enrichment of probiotic ice cream with different dietary fibers: structural characteristics and culture viability. *J. Dairy Sci*. 2018, 101, 37-46. https://doi.org/10.3168/jds.2017-13468
40. Śliwińska, A.; Lesiów, T. Lody jako żywność funkcjonalna - badania konsumenckie (In Polish). Ice cream as functional – consumer research. *Nauki Inż. Technol*. 2013, 1, 8, 65-76.
41. Tripathi, M. K.; Giri, S. K. Probiotic functional foods: Survival of probiotics during processing and storage. *J. Funct Foods* 2014, 9, 225-241. https://doi.org/10.1016/j.jff.2014.04.030
42. Akin, M.B.; Akin, M.S.; Kirmaci, Z. Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. *Food Chem.* 2007, 104, 93–99. http://dx.doi.org/10.1016/j.foodchem.2006.11.030
43. Al-Shawi, S.G.; Ali, H.I. Study the Possibility of Manufacturing Therapeutic Ice Cream by Adding Synbiotic and Study its Microbiological and Sensory Characteristics. *J. Pure Appl. Microbiol.* 2020, 14, 3, 2147-2156. https://doi.org/10.22207/JPAM.14.3.55
44. Godward, G.; Sultana, K.; Kailasapathy, K.; Peiris, P.; Arumugaswamy, R.; Reynolds, N. The importance of strain selection on the viability and survival of probiotic bacteria in dairy foods. Milchwissenschaft 2000, 55, 8, 441-445.
45. Tamime, A.Y.; Saarela, M.; Sondergaard, A.K.; Mistry, V.V.; Shah, N.P. Production and maintenance of viability of probiotic microorganisms in dairy products. Probiotic Dairy Products. 2005, 1, 39-63. https://doi.org/10.1002/9780470995785.ch3
46. Akbari, M.; Eskandari, M.H.; Niakosari, M.; Bedeltavana, M. The effect of inulin on the physicochemical properties and sensory attributes of low-fat ice cream. *Int. Dairy J.* 2016, 57, 52-55. https://dx.doi.org/10.1016/j.idairyj.2016.02.040
47. Champagne, C.P.; Gardner, N.J.; Roy, D. Challenges in the addition of probiotic cultures to foods. *Crit. Rev. Food Sci. Nutr*. 2005, 45, 61-84. https://doi.org/10.1080/10408690590900144
48. Balthazar, C.F.; Silva, H.L.; Esmerino, E.A.; Rocha, R.S.; Moraes, J.; Carmo, M.A.; Franco, R.M. The addition of inulin and Lactobacillus casei 01 in sheep milk ice cream. *Food Chem*. 2018, 246, 464–472. https://doi.org/10.1016/j.foodchem.2017.12.002
49. Jayamanne, V.S.; Adams, M.R. Determination of survival, identity, and stress resistance of probiotic bifidobacteria in bio-yoghurts. Lett. Appl. Microbiol. 2006, 42, 189-194. https://doi.org/10.1111/j.1472-765X.2006.01843.x
50. Mateos-Aparicio, I.; Matias, A. Food industry processing by-products in foods. In The Role of Alternative and Innovative Food Ingredients and Products in Consumer Wellness, 1st ed.; Galanakis, C., Ed.; Elsevier Inc.: London, UK, 2019, pp. 239–281.
51. Schieber, A.; Hilt, P.; Streker, P.; Endreß, H.U.; Rentschler, C.; Carle, R. A new process for the combined recovery of pectin and phenolic compounds from apple pomace. *Innov. Food Sci. Emerg. Technol*. **2003**, 4, 99–107. https://doi.org/10.1016/S1466-8564(02)00087-5
52. Crizel, T.d.M.; de Araujo, R.R; Rios, A.d.O.; Rech, R.; Flores, S.H. Orange fiber as a novel fat replacer in lemon ice cream. *Food Sci. Technol. (Campinas)* 2014, 34, 2, 332-340. https://doi.org/10.1590/fst.2014.0057
53. Dervisoglu, M.; Yazici F. Note. The effect of citrus fibre on the physical, chemical and sensory properties of ice cream. *Food Sci. Technol. Int*. 2006, 12, 2, 159-164. https://doi.org/10.1177/1082013206064005

54. Calligaris, S.; Marino, M.; Maifreni, M.; Innocente, N. Potential application of monoglyceride structured emulsions as delivery systems of probiotic bacteria in reduced saturated fat ice cream**.** *LWT-Food Sci. Technol. 2018,* 96, 329-334. https://doi.org/10.1016/j.lwt.2018.05.046
55. Florowska, A.; Wójcik, E.; Florowski, T.; Dłużewska, E. Wpływ dodatku preparatów błonnikowych na wybrane wyróżniki jakości lodów (In Polish). Infuence of the addition of fiber preparations on selected quality parameters of ice cream. *Zesz. Probl. Post. Nauk Roln.* 2013, 574, 11–18.
56. Zhang, H.; Chen, J.; Li, J.; Wei, C.; Ye, X.; Shi, J.; Chen, S. Pectin from citrus canning wastewater as potential fat replacer in ice cream. *Molecules.* 2018, 23, 4, 925. https://doi.org/10.3390/molecules23040925
57. Varela, P.; Pintor, A.; Fiszman, S. How hydrocolloids affect the temporal oral perception of ice cream. *Food Hydrocoll.* 2014, 36, 220–228. https://doi.org/10.1016/j.foodhyd.2013.10.005
58. Soukoulis, C.; Lebesi, D.; Tzia, C. Enrichment of ice cream with dietary fibre: Effects on rheological properties, ice crystallisation and glass transition phenomena. *Food Chem.* 2009, 115, 665-671. http://dx.doi.org/10.1016/j.foodchem.2008.12.070
59. Di Criscio, T.; Fratianni, A.; Mignogna, R.; Cinquanta, L.; Coppola, R.; Sorrentino, E.; Panfili, G. Production of functional probiotic, prebiotic, and synbiotic ice creams. *J. Dairy Sci*. 2010, 93, 4555-4564. https://doi.org/10.3168/jds.2010-335
60. El-Nagar, G.; Clowes, G.; Tudoricã, C.M.; Kuri, V.; Brennan, C.S. Rheological quality and stability of yog-ice cream with added inulin. *Int. J. Dairy Technol*. 2002, 55, 89-93. https://doi.org/10.1046/j.1471-0307.2002.00042.x
61. Senanayake, S.A.; Fernando, S.; Bamunuarachchi, A.; Arsekularatne, M. Application of *Lactobacillus acidophilus* (LA 5) strain in fruit-based ice cream. *Food Sci. Nutr.* 2013, 1, 428-431. https://doi.org/10.1002/fsn3.66
62. Muse, M.R.; Hartel, R.W. Ice cream structural elements that affect melting rate and hardness. *J. Dairy Sci. 2004, 87,* 1–10. http://dx.doi.org/10.3168/jds.S0022-0302(04)73135-5
63. Al-Sheraji, S.H.; Ismail, A.; Manap, M.Y.; Mustafa, S.; Yusof, R.M.; Hassan, F.A. Prebiotics as functional foods: A review. *J. Funct. Foods* 2013, 5, 1542–1553. https://doi.org/10.1016/j.jff.2013.08.009
64. Karaca, O.B.; Güven, M.; Yasar, K.; Kaya, S.; Kahayoglu, T. The functional, rheological and sensory characteristics of ice creams with various fat replacers. *Int. J. Dairy Technol.* 2009, 62, 93-99. https://doi.org/10.1111/j.1471-0307.2008.00456.x

 doi:10.20944/preprints202102.0387.v1