

# Utilization of banana puree fermented by *Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* BB12 for the manufacture of synbiotic ice cream

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## Abstract

In this study, manufacturing synbiotic ice cream is containing fermented banana puree by *Lactobacillus acidophilus* LA5 and *Bifidobacterium* (Bif) *lactis* BB12 and probiotic ice cream containing fermented skim milk by the same bacteria under the same conditions. The Viability of probiotic starters, pH and sensorial properties were tested during the storage period, in addition to the overrun. Synbiotic ice cream showed high bacterial count for both species of probiotics, especially Ice.4 treatment (ice cream containing 10% fermented banana puree with same couple strains) which was 7.54 and 7.59 logarithmic (log) colony-forming unit (CFU)/ gram (g) for *Lactobacillus* (Lb). *Acidophilus* LA5 and Bif. *lactis* BB12, respectively, pH registered 5.7, sensorial attributes recorded 84% and overrun reached 78% compared to others trials that inoculated with 5% of the same puree or ice cream with fermented skim milk with (5,10) % or control treatment (without starters).

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## Introduction

Ice cream is a widely consumed product around the world. It is frozen foam of a mixture that includes water, air, non-fat solids, emulsifiers, fat, flavors, colorants and stabilizers (El-Samahy *et al.*, 2009). Probiotics defined as are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”, as the addition of probiotics to food increases the nutritional and functional value of products by enhancing the quantity and availability of nutrients and bioactive compounds arising from microbial metabolism, including organic acids, conjugated linoleic acid and exopolysaccharides. These microorganisms can be enhanced via a substrate that consumes it selectively can confer a health benefit called prebiotics (Villalva *et al.*, 2017). There are many probiotics strains belonging to *Lactobacillus*, *Bifidobacterium*, some of the *Bacillus*

species, the yeast *Saccharomyces cerevisiae*, and others that are known generally recognized as safe (GRAS) (Rasheed *et al.*, 2020). The strains belonging to the genus *Lactobacillus* and *Bifidobacterium* are among the most prominent species of probiotics added to ice cream and other dairy products. The combination of probiotics and prebiotics is called "synbiotics", it causes a synergistic effect that stimulates the growth of probiotics through the fermentation of prebiotics (Villalva *et al.*, 2017). The combination of probiotics and prebiotics improves the survival of the probiotics in the digestive tract as it enhances tolerate low pH, oxygen and temperature conditions (Al-Hassani and Mustafa, 2022).

Enriching the dairy products such as yogurt and milk with probiotics may achieve beneficial health effects, most notably reducing the level of cholesterol in the blood, contributing to the prevention of

colon cancer, relieving the pain of irritable bowel syndrome (IBS) and increasing antioxidants and inhibition compounds (Niamah *et al.*, 2017). Probiotics can also contribute to the prevention of ulcers, gastrointestinal infections, diabetes, heart disorders, and vitamin B production and stimulate the immune response (Mohammed *et al.*, 2021). Fermented foods with probiotics, such as yogurt, are also a suitable option for people who suffer from lactose intolerance, due to the ability of probiotics to produce high levels of the enzyme lactase, it also can modify the metabolic activities of colonic microbiota and thus alleviate lactose intolerance (Ibrahim *et al.*, 2021). When the arrival of probiotics to the digestive system of the host in sufficient quantities, they adhere to the special receptors on the surface of the intestinal epithelial cells, which prevents the adhesion of harmful microorganisms, which leads to excretion it outside the body. Thus, strengthening the beneficial bacteria that are mainly present in the gastrointestinal tract, and enhancing the body's immunity to confront pathological infections (Jasim and Taha, 2017). Recently, the enrichment of certain bacterial population in the digestive system with probiotics, prebiotics, and symbiotics has become alternatives to antibiotics (Aziz and AL-Hawezy, 2022).

The nutritional composition of banana puree presents a suitable substrate for the fermentation of probiotic products (Gallo *et al.*, 2021). Bananas are a common source of prebiotics that promote the growth of probiotics, as contain a sufficient amount of resistant starch, oligofructose, inulin, and others (Sidhu and Zafar, 2018). Therefore, the study aimed to produce a synbiotic ice cream that includes fermented banana puree via the probiotic *Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* BB12 and compare it with the probiotic ice cream includes the same probiotic species through different treatments with determining of the trail that ensures high viability of probiotics, appropriate pH, good overrun and better

sensory acceptance of the product to provide a desirable healthy product and at the same time achieve an adequate economic profit.

## Materials and Methods

Commercial lyophilized cultures of the probiotics *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB12 (supplied by Chr-Hansen) were activated with MRS broth medium three times and then activated with skim milk until the initial counts reached 11.45 and 11.42 log CFU/ml for *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB12, respectively.

Green bananas were purchased from a local market in Baghdad, Iraq, the puree was produced from the pulp of the fruit. Banana fruits were wiped with ethyl alcohol (70%), then they were peeled, and cut into small pieces (transverse slices), the slices were dipped in 0.5% (by mass per volume) citric acid solution for 10 min. then mixed using an electric mixer in order to obtain a homogeneous mixture, and then the puree was distributed in sterilized sealed glass cups (Alzobaay *et al.* 2013). Inoculation of banana puree by probiotic mixed cultures of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB12 (50:50) using 10 ml and incubation at temperature 37 °C for 48 hours until the initial counts reached 11.37 log CFU/g.

Ice cream mixture treatments were prepared by Mahmedand and Obeed's (2020) method. The ingredients used in making 1 kg of ice cream mixture included: 705 g full cream milk powder (NIDO/the French company Nestle), 130 g fresh cream (40% fat) (Dairy plant of College of the Agricultural Engineering Sciences/ University of Baghdad), 160 g table sugar (sucrose) was used as sweetener, and 5 g CMC (Carboxymethyl cellulose) stabilizer was used in the production of ice cream (10% fat). The ice cream mixtures were modified to include 5 and 10% fermented skim milk for the probiotic ice cream treatments (Ice.1 and Ice.2), respectively, as well as 5 and 10% fermented banana puree

for the synbiotic ice cream treatments (Ice.3 and Ice.4), respectively. Ice cream mixtures were incubated at 37 °C until the pH reached 5.90. Then cooled to 5°C, and left at 5°C overnight for aging. The mixtures were then frozen for 20 min in a CARPIGIANI freezer (Italy). The ice cream samples were packed into plastic containers (100 ml), at a temperature of 5 °C and stored at -18 °C until analysis (Al-Shawi and Ali, 2020).

Probiotic bacteria enumeration to ice cream treatments was done using tryptose proteose peptone yeast extract (TPPY) agar with added prussian blue which was prepared from the following ingredients: glucose (10 g), lactose (10 g), tryptone (7 g), peptone (7 g), yeast extract (2 g), tween 80 (1 ml), prussian blue (0.3 g), and 15 g of agar was added to it. All the ingredients were dissolved in a liter of distilled water. It was sterilized with an autoclave at 121 °C/15 minutes, cooled to 50 °C and then prussian blue was added. Ten grams of ice cream were diluted in 100 ml of sterilized peptone water solution. One ml of the diluted samples of ice cream treatments were poured on sterile TPPY-prussian blue agar. It was used for the differential enumeration of probiotic cultures. Incubation of plates was conducted under anaerobic conditions using a Gas-pack system at 37°C for 48-72 h. Results of enumeration were expressed as (log<sub>10</sub> CFU/g). Analysis of samples was conducted in duplicate. TPPY-prussian blue agar was proposed as a differential agar that allows *Lb. acidophilus* and *Bifidobacterium* to be enumerated on one medium. *Bifidobacterium* gave white colonies and *Lb. acidophilus* produced large pale blue colonies surrounded by a wide royal blue zone (Ashraf and Shah 2011; Teixeira, 2014).

pH values of ice cream treatments were measured by pH-meter (Hach Sension+ pH3) with a pH electrode Sension+ 5011T, firstly pH meter was calibrated using a buffer of pH 4 and pH 7, then 20 ml of the melted samples (20 ± 1.0°C) was putted in a

50 ml beaker, as pH values was determined by the glass electrode. Each measurement occurred in triplicate (Zagorska *et al.*, 2022).

The sensory assessments were conducted in the central laboratory of the Department of Food Science in the College of the Agricultural Engineering Sciences/ University of Baghdad. The panelists of nine raters were experts and well-known in this field. Panelists were provided with a glass of water and, instructed to rinse and swallow water between samples. They were given written instructions and asked to evaluate products for acceptability based on their taste, smell, color, texture and mouthfeel. Overall acceptability was determined by a special scale that we created according to the requirements of the experiment. Sensory evaluation was performed using a form modified (table 3) by Prashanth *et al.* (2018).

Overrun is the quantity of air absorbed by the ice cream mixture during manufacturing. High overrun values appear in the absorption of a high amount of air into the ice cream product, thus expanding the product (Asres *et al.*, 2022). The overrun values of ice cream treatments were determined in duplicate based on differences in weight (Wt.) after and before freezing as Elkot *et al.* (2022) stated by using the following equation:

$$\% \text{ Overrun} = (\text{Wt. of the mix} - \text{Wt. of the same vol. of ice cream}) / \text{Wt. of the same vol. of ice cream} \times 100\%$$

To detect the effect of different factors in study parameters, The Statistical Analysis System- SAS (2012) program was used (Cary, 2012). The least significantly difference (LSD) test was used to significant compare between means in this study

## Results and Discussion

### Probiotics count in ice cream

The results in Table 1 showed no statistically significant differences ( $P \leq 0.05$ )

in the viable counts of probiotic ice cream trials, which included two treatments for probiotic ice cream (Ice.1) and (Ice.2) with a concentration of 5 and 10%, respectively, and two treatments for synbiotic ice cream (Ice.3) and (Ice.4) at a concentration of 5 and 10%, respectively, during 120 days of storage.

Synbiotic ice cream treatments maintained higher viable probiotic counts than the probiotic ice cream treatments. Despite the gradual decrease in the viable counts in all the ice cream treatments, the decrease was less in the synbiotic ice cream treatments as compared to the probiotic ice cream treatments. The reason for this can be attributed to the fact that synbiotic ice cream treatments (Ice.3) and (Ice.4) contain 5 and 10% of fermented bananas, respectively, as banana puree is rich in prebiotics including, insoluble and soluble dietary fibers (DF), pectin, inulin and Fructo-oligosaccharides (FOS) (Kumar *et al.*, 2014) As these prebiotic compounds support the survival of probiotic cells. Al-Shawi and Ali, (2020) indicated the action of inulin as a protective mechanism for *Lb. acidophilus* cells during ice cream storage and FOS promotes the growth of *Lb. acidophilus*. Also, Prashanth *et al.* (2018) indicated that green bananas are rich in resistant starch, as resistant starch can act as a protective factor for probiotic cells during ice cream frozen storage.

The same applies to the viable counts of *Bifidobacterium lactis* BB12 bacteria, which were found to maintain their viability more in synbiotic ice cream treatments compared to probiotic ice cream treatments due to the effect of prebiotics sourced from fermented banana puree. The results of our study are consistent with Shori (2021), who mentioned that the addition of 2% inulin to ice cream containing 10% w/w fermented milk enhances the viability of *Bif. lactis*. It was observed that the sample (Ice.4) which included 10% fermented banana, was the best treatment for obtaining synbiotic ice cream with high viable probiotic counts, most likely due to the increase in the

proportion of fermented banana puree added to the ice cream compared to the sample (Ice. 3) which included 5% fermented banana. This means better support for the probiotic cells in the ice cream matrix due to the high proportion of probiotics. This assumption is supported by Krawęcka *et al.* (2021) who reported that adding bananas to ice cream helps maintain the viability of probiotics during freezing due to their polysaccharide content.

In most treatments, it was noticed that the counts of *Bif. lactis* BB12 were slightly lower than those of *Lb. acidophilus*, because *Bifidobacterium* spp. are strictly anaerobic cells, and ice cream is an environment that contains a rich concentration of oxygen, which makes the cells of these bacteria vulnerable to dissolved oxygen, which leads to the accumulation of toxic metabolites, including superoxide, hydroxyl radicals and hydrogen peroxide, hence causing cells death. *Bifidobacterium* spp is more susceptible to these toxic factors than *Lb. acidophilus* (Boza-Méndez *et al.*, 2012). However, it was observed that there was a gradual decrease in the count of viable probiotics with continued storage for 120 days because of the effect of freezing injury that leads to cells' death (Al-Shawi and Ali, 2020). Also, the effect of low temperatures and high concentrations of dissolved oxygen makes it difficult for probiotics to increase their count in ice cream during freezing storage (Boza-Méndez *et al.*, 2012).

Andriani and Wikandari (2022) support our finding as they stated the viability of lactic acid bacteria (LAB) can be maintained by adding skim milk to the product, for it can act as cryoprotectant (protective bacteria) during freezing. Our study showed that the counts of probiotics for both species were higher in the sample (Ice.2), which included 10% of the probiotic skim milk, compared to the sample (Ice.1), which included 5% of the probiotic skim milk. The increase in the proportion of the probiotic skim milk added to the ice cream mixture provides better protection for the probiotic

cells during the frozen storage of the product. Abdulrazzaq and Khalil (2022) mentioned that skim milk can act as a prebiotic source for probiotic bacteria. *Lb. acidophilus* is generally more tolerant to acidic conditions than *Bifidobacteria* whose growth retards at a pH less than 5.5. The tolerance of *Bifidobacteria* to acidic conditions varies according to the specific strain (Mohammadi *et al.*, 2011). It was found that *Lb. acidophilus* has a such high acid activity that it can tolerate higher acidic conditions than *Bif. lactis* (Zomorodi, 2019). Results agree with Nunes *et al.* (2018) who reported that *Lb. acidophilus* La-5 (ML) presented higher survival than *Bif. Bb-12* (MB) under these conditions.

The results showed there was a decrease in the counts of probiotics after frozen storage but still in a fairly high amount. It met the standard as a probiotic product according to Andriani and Wikandari, (2022) stating that the minimum amount of LAB contained is  $10^7$  CFU/g.

It is noted that the survival of probiotics during the storage period which lasted 120 days was without growth as viable cells in sufficient numbers with the possibility of cultivation when the appropriate conditions are available, as a result of the ability of

probiotic species to adapt in these conditions by regulating the osmotic stress in the presence of sugars like sucrose and lactose. In addition to the role of prebiotics in forming a matrix that protects probiotic cells, it reduces the damage caused by freezing (Tripathi and Giri, 2014).

Freezing causes a reduction in the cells' vital metabolic activities, and therefore cell growth does not occur under these conditions. Freezing leads to the death of some of the cells, but other numbers of cells resist freezing. The nature of damage to freezing depends on the conditions and velocity of the freezing process and the presence of protective materials from freezing such as prebiotics. The resistance of probiotics to the damage of freezing also varies between strains of probiotics. Also, one of the studies mentioned that the death rate of probiotics cells was greater during the freezing process than during storage. Probiotics show better survival in freezing conditions if they can dehydrate without breaking their cell membranes. Previous studies showed that the two strains of probiotics *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb-12 used in our study achieve high survival rates in ice cream (Mohammadi *et al.*, 2011)

**Table 1. Viable probiotics of ice cream treatments during the freezing storage period**

Days	Logarithm of Probiotics bacteria counts (CFU/g)									
	<i>Bifidobacterium lactis</i> BB12				LSD value	<i>Lactobacillus acidophilus</i> LA-5				LSD value
	Ice.1	Ice.2	Ice.3	Ice.4		Ice.1	Ice.2	Ice.3	Ice.4	
1	7.7	8.01	7.83	8.05	0.43 NS	7.82	8.06	7.88	8.07	0.48 NS
15	7.70	7.98	7.75	8.03	0.48 NS	7.79	8.04	7.85	8.02	0.41 NS
30	7.66	7.93	7.69	7.96	0.39 NS	7.70	7.95	7.81	7.98	0.37 NS
45	7.59	7.87	7.62	7.89	0.42 NS	7.65	7.91	7.71	7.94	0.38 NS
60	7.53	7.62	7.56	7.76	0.38 NS	7.49	7.80	7.59	7.78	0.38 NS
90	7.49	7.55	7.54	7.65	0.44 NS	7.44	7.61	7.53	7.72	0.41 NS
120	7.32	7.47	7.43	7.59	0.40 NS	7.41	7.43	7.46	7.54	0.39 NS

NS: Non-Significant

NS: Non-Significant statistical differences, Ice.1 and Ice.2 are probiotic treatments including probiotic skim milk in the ratio 5% and 10%, respectively. Ice.3 and Ice.4 are synbiotic treatments including banana pulp puree in the ratio 5% and 10%, respectively.

### pH values of ice cream

Results presented in Table 2 showed the difference in the pH values of the probiotic and synbiotic ice cream treatments on the first day, it is due to the addition of probiotic skim milk to the ice cream by 5% to treatment (Ice.1) and by 10% to treatment (Ice.2). The pH reached 5.36 in treatment (Ice.1) and decreased to 5.29 in treatment (Ice.2), and this finding is supported by Andriani and Wikandari (2022), which can be explained due to the accumulation of fermentation products, especially organic acids such as lactic and acetic acid.

The pH in (Ice.3) and (Ice.4) decreased to 5.89 and to 5.72 successively because of adding 5% and 10% of fermented banana puree to each treatment. This finding is supported by Prashanth *et al.* (2018). They emphasized pH increase as more green banana flour was added to the ice cream because it contains organic acids. A slight low decrease in the pH values was observed after 45 days of frozen storage in the probiotic and synbiotic ice cream treatments

that started at different pH values for each treatment and this could be attributed to the decrease in the viable counts of probiotics due to the freezing effect due to the cessation of metabolic activities.

The results of our study also agree with the study of Hasan *et al.* (2020) who indicated that replacing part of the skim milk with different types of dried fruit powder (including dried banana powder) resulted in a lower pH value for all treatments compared to the control group. Gheisari *et al.* (2016) found a gradual decrease in the pH of the probiotic ice cream containing *Lb. casei* during frozen storage for three months from 6.56 on the first day to 6.47 on the 90<sup>th</sup> day attributing the reason to the metabolic activity of the microflora by Embden- Meyerhof-Parnas pathway leading to the production of organic acids such as lactic and/or acetic acid. A decrease in pH in ice cream containing probiotics during storage, and the addition of dietary fiber and culture increased the acidity and decreased pH (Afzaal *et al.*, 2020).

**Table 2. pH values of ice cream treatments during storage**

Samples	pH values during storage (days)						
	1	15	30	45	60	90	120
Control Ice.	6.82	6.82	6.82	6.82	6.82	6.82	6.82
Ice.1	5.36	5.35	5.35	5.34	5.34	5.33	5.33
Ice.2	5.29	5.29	5.29	5.28	5.28	5.28	5.27
Ice.3	5.89	5.89	5.89	5.88	5.88	5.88	5.88
Ice.4	5.72	5.71	5.71	5.71	5.70	5.70	5.70
LSD value	1.04 *	1.18 *	1.06 *	1.14 *	1.22 *	1.22 *	1.26 *
* (P<0.05)							

\* Statistically significant differences (P<0.05), Ice.1 and Ice.2 are probiotic treatments including probiotic skim milk in the ratio 5% and 10%, respectively. Ice.3 and Ice.4 are synbiotic treatments including banana pulp puree in the ratio 5% and 10%, respectively.

### Sensory evaluation of ice cream

Table 3 showed statistically significant differences (p<0.05) in the results of sensory acceptance for all ice cream treatments during the freezing storage period for the first day, day 60 and day 120, respectively. Regarding the taste and smell of the synbiotic ice cream, it is noted that the highest sensory evaluation scores were in the trail (Ice.4), reaching 18 for both features from the first day to the end day of

frozen storage. It is higher compared with the trail (Ice.3). Hasan *et al.* (2020) indicated that replacing part of the skim milk with different types of dried fruit powder (including dried banana powder) scored high points for flavor, and the flavor score increased with the increase in the replacement rate due to the addition of more of fruit flavor.

Banana can improve the flavor of ice cream as containing inulin. Ozturkoglu-

Budak *et al.* (2019) stated that the addition of inulin in fermented milk supports an increase in the concentration of lactic and acetic acid from the probiotics *Lb. acidophilus* La-5 and *Bif. animalis* Bb-12 because inulin has a stimulating effect on the growth and viability of probiotics.

As for the probiotic ice cream, it is noted that the sensory evaluation scores were highest in the trail (Ice.2), reaching 18 from the first day to the end day during frozen storage. It is higher compared with the trail (Ice.1), and this difference is attributed to the high proportion of probiotic skim milk in the trail (Ice.2) which amounted to 10% compared to the trail (Ice.1) which included 5% of probiotic skim milk.

Probiotic and synbiotic ice cream trails showed an improved taste compared with

the control treatment for several reasons, most notably the presence of *Lb. acidophilus*, which gives an umami taste and contributes significantly to the acid taste due to lactic acid production (Cui *et al.*, 2019). Studies showed that the volatile compounds concentrations of acetaldehyde, acetone, and diacetyl were lower in the concurrent products supplemented with inulin, compared to the non-supplemented probiotic products (Ozturkoglu-Budak *et al.*, 2019).

Ice.4 sample registered high overall acceptance scores which included synbiotic banana puree 10% (fermented by *Bif. lactis* BB-12 and *Lactobacillus acidophilus*), especially with the stability of flavor criteria (taste and smell). This is due to the enhanced effect of the activity of probiotics and the presence of banana puree.

**Table 3. Sensory evaluation of ice cream treatments**

Properties (Every factor: 20 scores)	Treatments (After 1 day)					LSD value
	Control	Ice.1	Ice.2	Ice.3	Ice.4	
Taste	15	17	18	17	18	2.47 *
Smell	14	15	18	16	18	2.55 *
Color	18	18	18	17	18	1.52 NS
Texture	14	13	12	16	16	2.07 *
Mouthfeel	13	13	14	16	16	2.41 *
Total (100%)	74%	76%	80%	82%	86%	5.91 *
Treatments (After 60 days)						
Taste	15	16	18	16	18	2.08 *
Smell	13	15	18	16	18	2.17 *
Color	18	18	18	17	16	1.94 *
Texture	13	13	12	16	16	2.04 *
Mouthfeel	14	13	14	16	17	2.13 *
Total (100%)	73%	75%	80%	81%	85%	5.73 *
Treatments (After 120 days)						
Taste	15	16	18	16	18	2.19 *
Smell	14	15	17	16	18	2.33 *
Color	18	18	18	17	17	1.25 NS
Texture	12	12	13	15	15	2.09 *
Mouthfeel	13	13	13	16	16	2.41 *
Total (100%)	72%	74%	79%	80%	84%	5.69 *
Quality level based on scores						
1 to 4	5 to 8	9 to 12	13 to 16	17 to 20		
Awful	Bad	Regular	Good	Excellent		
* (P<0.05).						

\* Statistically significant differences (P<0.05), NS: Non-Significant statistical differences, Ice.1 and Ice.2 are probiotic treatments including probiotic skim milk in the ratio 5% and 10%, respectively. Ice.3 and Ice.4 are synbiotic treatments including banana pulp puree in the ratio 5% and 10%, respectively.

Ozturkoglu-Budak *et al.* (2019) indicated that synbiotic fermented milk products containing inulin with cultures of the probiotics *Lb. acidophilus* La-5 and *Bif. animalis* Bb-12 were preferred over probiotic products by the sensory panel in terms of appearance, body, and taste.

Regarding the color of the synbiotic ice cream, the highest scores were in the trail (Ice.4), reaching 18 on the first day, with a slight fluctuation until the last day during frozen storage, which is similar to the color in the trail (Ice.3), which amounted to 17 on the first day with a slight fluctuation until the last day during frozen storage. This difference is attributed to the high percentage of fermented banana puree in the trail (Ice.4), which amounted to 10% while the trail (Ice.3) included 5% of fermented banana puree.

As for the probiotic ice cream, the color scores for both treatments (Ice.2 and Ice.1) were close, reaching about 18 from the first day until the last day during frozen storage, because probiotic skim milk does not include special-colored compounds. The addition of 5% and 10% of fermented skim milk in (Ice.1) and (Ice.2) successively helped maintain the white color. The panellists did not notice any significant differences between the colors of the probiotic ice cream samples in the first day period and in the 120-day period. This is an expected result in these samples because they do not contain fruit pulp like those found in synbiotic ice cream containing fermented banana pulp. Significant differences are observed between all the samples under study on the 60th day, because of continuing freezing storage, which affects vitamin C and enzymes in the banana fruit. This is justified the color change in the ice cream containing the fermented banana puree, as vitamin C (ascorbic acid) is used to control enzymes in the frozen fruit. Although the banana is not heated before freezing, the oxidation of ascorbic acid (vitamin C) occurs at a rate slow during frozen storage. This leads to the

activation of the enzymes that cause the fruit to turn brown especially with a loss of vitamin C (Mieszczakowska-Fraç *et al.*, 2021). In treatment (Ice.4), the decrease in color values increased, which led to significant differences between all ice cream samples generally. Banana contains compounds that affect the color of ice cream, especially Violaxanthin and Cryptoxanthin, which are used as food colorants. They are also one of the fruits richest in trans- $\beta$ -carotene compounds that affect color properties (Sidhu and Zafar, 2018).

Regarding the texture trait of the synbiotic ice cream, the texture scores for both treatments (Ice.4 and Ice.3) were close, reaching about 16 since the first day. Then, they decreased slightly to 15/20 on the last day during the freezing storage, and thus it remained within the good range of the texture properties, despite the different added proportion of the fermented banana puree as in treatment (Ice.4), which amounted to 10% as compared to treatment (Ice.3) which included 5% of fermented banana puree. However, the panelists did not notice a significant difference between the two treatments regarding texture.

The results of our study agree with Hasan *et al.* (2020), who indicated that the use of ice cream samples containing 10% of apple powder gave the highest values for body and texture but decreased slightly with an increase in the percentage due to the roughness of texture and being granular. They also mentioned that adding prickly pear pulp by 5, 10 and 15% to ice cream resulted in an improvement in body and texture. In the same study, it was indicated that the use of bananas in ice cream causes a slight decrease in the texture properties of the product as a result of an increase in the total solids of the resulting ice cream, as the banana powder is characterized by its high content of carbohydrates. Yet. This differs from our study where fermented banana puree was used, not dried banana powder. The presence of FOS reduces the firmness

of the product as it contributes to better stability of the air bubbles embedded in the ice cream and increases the production of viscous gels, which leads to a better acceptance of the texture properties (Parussolo *et al.*, 2017).

As for the probiotic ice cream, the sensory evaluation scores for the texture trait were lower than those of the synbiotic ice cream. The texture scores for both treatments (Ice.2 and Ice.1) were close, reaching about 12-13 from the first day until the last day during the freezing storage period. Thus, they remained within the good range of the texture properties, because of using of 5% and 10% probiotic skim milk in treatment (Ice.1) and treatment (Ice.2) respectively which achieved an increase in the percentage of non-fat solids, in particular proteins, in the ice cream for both treatments. As milk proteins have a significant effect on the texture of ice cream by reducing the size of ice crystals and improving their stability when polysaccharides are present (El-Aziz *et al.*, 2015). The results of our study for treatment (Ice.2) agree Patel *et al.* (2006) who indicated that increasing the proportion of protein in the ice cream mixture leads to an improvement in the texture properties as a result of reducing the size of ice crystals. Our study showed that treatment (Ice.2) at the end of the freezing storage period improved texture properties as compared to the control.

Regarding the mouthfeel characteristic of synbiotic ice cream, the mouthfeel scores for both treatments (Ice.4 and Ice.3) were close, reaching about 16 from the first day to the last day during frozen storage. They remained within the good range of mouthfeel properties, despite the difference in the percentage of fermented banana puree added in the fourth treatment (Ice.4), which amounted to 10% compared to treatment (Ice.3) which included 5% of fermented banana puree. However, the panelists did not notice a significant difference between the two treatments regarding mouthfeel, and

both treatments were acceptable. The results of our study agree with Yanglar (2015) who found that adding 1% and 2% banana pulp flour to the ice cream mixture improved mouthfeel trait equally for both treatments.

The addition of fermented bananas to ice cream in treatments (Ice.3) and (Ice.3) improved the texture and the mouthfeel properties as compared to the control sample because they have inulin, which gives a distinctive viscosity due to the high molecular weight of inulin. Inulin is also a high hygroscopic substance and thus causes water-binding. Therefore, it generates a gel-like network which, together with other ice cream ingredients, affects the rheological properties of the product (Wood, 2011). Inulin is characterized by poor solubility depending on the length of its chain, and therefore, when it comes into contact with water or milk, it forms microcrystals, generating a creamy gel material, and result in a smooth, fat-like sensation in the mouth (Villalva *et al.*, 2017). Inulin also increases the syneresis of probiotics towards the production of more volatile fatty acids, which in turn improves sensory properties (Kamel *et al.*, 2021). The results of our study agree with Villalva *et al.* (2017) stated that the addition of the probiotic *Bifidobacterium lactis* Bb-12 with inulin in peach ice cream at a concentration of 10% did not result in negative effects on the sensory properties of the product.

The sensory evaluation scores of the mouthfeel sensation of the probiotic ice cream were lower than those of the synbiotic ice cream, the mouthfeel values for both treatments (Ice.2 and Ice.1) were close, averaging around 13-14 from the first day until the end of the frozen storage period. and thus remained within the good range of mouthfeel properties because of the use of 5% and 10% probiotic skim milk in treatment (Ice.1) and treatment (Ice.2) respectively increasing non-fat solids, in particular proteins, in the ice cream.

Al-Sahlany and Al-musafer (2020) indicated that a 10% replacement of wheat

flour with banana peel flour improved the color and most rheological properties of wheat flour samples after the replacement process. Also, the viable cells of

*Saccharomyces cerevisiae* were increased. Syed *et al.* (2018) indicated that an increase in milk proteins leads to an enhancement in the viscosity of ice cream due to increased water binding and the formation of smaller ice crystals. So the product has a better mouthfeel, creaminess, smoothness and the milkiness or opacity of the product is improved. Thus, the synbiotic ice cream scored mouthfeel points better than the probiotic ice cream. The results agree with Vacondio *et al.* (2013) who reported that adding 5% and 10% yacon extract to ice cream formulations gave sensory acceptance level of 82.82% and 82.71%, respectively.

#### **Overrun values of ice cream**

Overrun values in table 4 showed the control ice cream increased up to 71%, a good value, as it is within the appropriate range for the overrun in milk ice cream which ranges between 50 to 85%. The value indicates the correct homogenization of the mixture, which facilitated mixing and the incorporation of air with the mixture, especially with milk proteins thus generated suitable foam (Villalva *et al.*, 2017).

The overrun values of probiotics ice cream treatments were 73% and 77% for treatment (Ice.1) and treatment (Ice.2), respectively. Because the increase in total solids including protein and carbohydrates increase the ice cream overrun (Asres *et al.*, 2022). The overrun values of synbiotic ice cream treatments were 75% and 78% for treatment (Ice.3) and treatment (Ice.4), respectively. As the use of fermented banana puree by 5% for treatment (Ice.3) and 10% for treatment (Ice.4) means an increase in the percentage of total solids that are different from the nature of the known solids for ice cream. The reason may be due to the fact that bananas contain oligofructose and inulin which increase the incorporation

of air into the ice cream product. The ability of insulin is greater than oligofructose in this regard, which affects the ice cream overrun (Wood, 2011).

The addition of inulin to the ice cream mixture causes a large overrun in the product, reaching 81%, i.e. it's within the standard in the Argentine legislation, according to which the combined air should not surpass 120% and the ideal overrun value is from 70 to 100% (Villalva *et al.*, 2017). At this point, after achieving a good percentage of overrun, a conviction was generated that the proportion of banana puree added to ice cream should not exceed 10% in order to avoid an increase in the viscosity of the mixture, to achieve a significant impact on the whipping rate (El-Samahy *et al.*, 2009). Also, studies have also indicated that high levels of overrun negatively affect the survival of probiotics in ice cream, especially when probiotics are added to the mixture immediately before freezing with constant stirring (Boza *et al.*, 2012).

An increase in the viscosity of the ice cream mixture leads to an increase in the overrun, depending on the nature and percentage of the additive to the mixture. However, high viscosity may reduce the overrun of the product. Peasura *et al.* (2020) stated that adding pumpkin by 25% gave the overrun 73%, while it reached 84% in the control sample. Elkot *et al.* (2022) reported that the amount of black rice powder (BRP) used in the manufacture of synbiotic ice cream had a significant effect on the overrun and concluded that increasing the percentage of (BRP) led to increasing the overrun to 41.80%. EL-Sayed *et al.* (2014) reported that the using of prebiotic Fructooligosaccharides at a rate of 2% with different strains of probiotics in the manufacture of synbiotic ice cream gave an overrun range between 63.46 to 66.56%. Mykhalevych *et al.* (2022) stated that the addition of 1% oat beta-glucan to the ice cream mixture led to an increase the overrun to 72.3%. It seems that the fermentation

process has led to a change in the nature of the banana puree, which supports the achievement of the high overrun as shown in Table 4 as a result of reducing the viscosity to a degree. Srisuvor *et al.* (2013) stated that prebiotics with the

exopolysaccharides (EPS) produced by probiotic bacteria cause the casein particles to be covered and the casein aggregates are partially sterically stabilized, which leads to a reduction in the effective volume fraction and reducing viscosity.

**Table 4. Overrun values of ice cream treatments**

Treatments	Overrun%
Control (only ice cream)	71.2±0.50
Ice.1 (Probiotic ice cream) (5%)	73.3±0.45
Ice.2 (Probiotic ice cream) (10%)	77.1±0.27
Ice.3 (Synbiotic ice cream) (5%)	75.3±0.38
Ice.4 (Synbiotic ice cream) (10%)	78.2±0.82

Ice.1 and Ice.2 are probiotic treatments including probiotic skim milk in the ratio 5% and 10%, respectively. Ice.3 and Ice.4 are synbiotic treatments including banana pulp puree in the ratio 5% and 10%, respectively

## Conclusion

Possibility of using banana puree fermented by *Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* BB12 in improving some properties of synbiotic ice cream, included increasing viability of probiotics, acceptable pH, improving the sensory properties and additional to high overrun. Banana puree leads to better protection of probiotic cells during frozen storage compared to the case of free probiotic cells. Incorporation of probiotics with banana puree in ice cream is possible to obtain at least  $10^7$  viable cells/d.

## Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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