Chapter 3

Probiotic Ice Creams

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Abstract

The growing consumer demand for healthier and more functional foods has led to the introduction of new ingredients in ice cream formulations with nutritional and physiological properties, such as probiotics. Incorporating probiotic bacteria into an ice cream should not affect the quality of the product. Therefore, its quality parameters such as air incorporation, melting rate, and sensory characteristics must be the same or better when compared to conventional ice cream. This chapter is a practical guidance for probiotic ice cream manufacture, presenting the steps and amount of probiotic addition into ice cream production.

Key words Ice cream, Probiotic, Functional food, Health

1 Introduction

Dairy products such as ice cream and frozen desserts can serve as vehicles for delivering probiotics to humans. In addition, ice cream can be kept in storage for longer time than other dairy products [9]. In this context, consumers' perception of healthy and functional foods led to the introduction, in the manufacture of ice cream, of ingredients with nutritional and physiological properties, such as probiotics [2, 24], dietary fibers [6–8], and synbiotics [9]. Ice cream is a complex colloidal system consisting of air cells, ice crystals, and partially destabilized fat globules dispersed in a continuous aqueous phase within polysaccharides, lactose, sugars and mineral salts [15]. As well the high level of total solids in ice cream provides protection to probiotic bacteria [20].

Probiotic ice cream is an acidified dairy frozen dessert of partially frozen structures. Acidification of the ice cream mixture can be carried out through direct inoculation of probiotic cultures, for example, *Bifidobacterium* spp. and *Lactobacillus* spp., the mixture of acidified milk or probiotic yogurt mixed with ice cream [3]. The therapeutic value of probiotic bacteria usually depends on the

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viability of these bacteria. Therefore, the International Dairy Federation [21] suggested that a minimum of 7 $\log_{10}$ probiotic bacterial colony forming units should be viable at the time of consumption per gram of the product (CFU/g), which is in accordance with the latest International Scientific Association for Probiotics and Prebiotics consensus statement [19]. The viability of probiotic bacteria in frozen dairy desserts is limited due to intrinsic environmental parameters, such as high redox value, oxygen toxicity, rupture of bacterial cell membranes during the freezing process, and the vulnerability of bacteria to acidic conditions [10]. Therefore, the efficiency in adding the probiotic depends on the inoculated dose, temperature, type of dairy foods, and the presence of air, and its viability must be maintained throughout the shelf life and intestinal environment, in addition to resisting at gastric pH [9].

Akin et al. [2] investigated the effects of inulin on the viability of probiotic bacteria in ice cream and found that their survival was higher in samples with inulin, probably due to the effect of the prebiotic. *L. acidophilus* and *B. lactis* counts were less than 5 $\log_{10}$ CFU/g in the control samples, while in the inulin-supplemented samples they were 5 $\log_{10}$ CFU/g. These results suggested that the addition of inulin stimulated the growth and improved the viability of those probiotic bacteria. Indeed, similar results were also verified by Balthazar et al. [9] in fermented sheep milk ice cream, in which synbiotic ice cream presented 7.61 $\log_{10}$ CFU/g and 5.18 $\log_{10}$ CFU/g against 6.89 $\log_{10}$ CFU/g and 5.02 $\log_{10}$ CFU/g from probiotic version viability after 150 days of frozen storage and in vitro simulated digestion, respectively, explained by inulin protection during storage and in vitro simulated digestion.

Homayouni et al. [20] verified in ice cream with microencapsulated or non-microencapsulated probiotics that there was a loss of only 0.7 and 0.4 $\log_{10}$ CFU/g of *Lacticaseibacillus casei* and *B. lactis* in the free state, respectively, and 0.3 and 0.2 $\log_{10}$ CFU/-gin the encapsulated state during the first month of storage. In the following five months, probiotic counts remained with a loss of 2.7 and 2.5 $\log_{10}$ CFU/g for the free state and 1.1 and 0.5 $\log_{10}$ CFU/g for the encapsulated state, and after the sixth month, the final drop was 3 0.4 and 2.9 $\log_{10}$ CFU/g in the free state of *L. casei* and *B. lactis*, respectively, against 1.4 and 0.7 $\log_{10}$ CFU/g in the encapsulated state. The numbers of viable probiotic bacteria in all types of ice cream were between 8 and 9 $\log_{10}$ CFU/g after three months of storage, the normal shelf life of ice cream. Pandiyan et al. [22] noticed that the melting rate of probiotic and symbiotic ice cream is faster and this behavior was attributed to the technological characteristics of the product such as freezing point and viscosity. This chapter is a practical guidance for probiotic ice cream manufacture, presenting the steps and amount of probiotic addition into ice cream production.

2 Materials

2.1 Ice Cream Ingredients

Ice cream can be processed with a variety of ingredients, including:

- Milk.
- Yogurt (*see* **Note 1**).
- Fat (*see* **Note 2**).
- Protein (*see* **Note 3**).
- Milk solids-not-fat (*see* **Note 4**).
- Water.
- Sweeteners (*see* **Note 5**).
- Stabilizers (*see* **Note 6**).
- Emulsifiers (*see* **Note 7**).
- Flavoring (*see* **Note 8**).
- Coloring.
- Probiotics—examples Table 1.
- Fruits.
- Nuts.
- Bakery pieces.
- Candy pieces.

Table 1 shows a traditional ice cream formulation. This formulation can be adapted with higher or lower values than those shown in Table 1, in addition to the addition of others, for specific purposes.

2.2 Ice Cream Equipment

The main equipment needed to produce ice cream are described below, along with suggestions for equipment to be purchased.

- **Doser:** Ingredient Doser A3 (Tetra Pak).
- **Mixer:** High shear blender (Bredoo Likwifier); High shear mixer B200–300 A (Tetra Pak).
- **Pasteurizer:** Pasteurizer D (Tetra Pak).
- **Homogenizer:** Industrial five-piston homogenizer (Tetra Pak); Homogenizer 250 (Tetra Pak).
- **Maturator:** Incubator (cooled Incubator ILW 115, POL-EKO-APARATURA.
- **Freezer:** Tetra Hoyer Frigus-KF freezer (TetraPak), WCB Ice Cream freezer (WCB Ice Cream); Ice Cream Machine (Delonghi, Il gelato, ICK5000), Ice Cream Machine (−5 °C; L/30–3, SEVEL Cooling INC.); Continuous Freezer S300 M2 (Tetra Pak).

Table 1
Traditional ice cream composition

Composition (%)	Amount (%)
Milk solids-not-fat	9.0–11.0
Milk fat	10.0–16.0
Sucrose	10.0–16.0
Corn syrup solids	2.0–5.0
Stabilizer	0.15–0.35
Emulsifier	0.10–0.15
Total solids	36.0–42.0
Water	58.0–64.0

Source: Adapted from [7]

- **Filler:** Rotary-type filler for cups and round nested containers (Huhtamaki, Inc.); In-line filler for square-round packages (TD Sawvel Co., Inc.); Ice Cream Smart Filler A1 (Tetra Pak).
- **Wrapper:** Ice Cream Wrapper A2 (Tetra Pak).
- **Hardening:** Super Deep Chest Freezer LY450LD (−35 °C) (Snow-MY).

2.3 Probiotic Strains

Inoculation of probiotics can be performed by Direct Vat Set (DVS) or by Propagation. The DVS method promotes the use of standardized freeze-dried cultures, with low amounts sufficient for inoculation. The propagation method is carried out by cell cultivation in a specific medium, purification of the culture, and subsequent incorporation into the product. Table 2 presents the species of probiotics used in the preparation of ice cream and describes the inoculation method used.

3 Methods

The processing of ice cream is divided into two distinct stages, the production of the mixture and the freezing operations. To produce quality ice cream, the steps must be carried out in a controlled manner, adapting the desired final characteristics. The elaboration steps include mixing, heat treatment, homogenization, maturation, freezing, packaging, and hardening (*see* Fig. 1).

1. Mix the ingredients, as described in Table 1, in a tank with agitation and heating, heating them to 50 °C to facilitate solubilization (*see* **Note 9**) [12].
2. After the complete incorporation of the ingredients, pasteurize the mixture at 70–85 °C from 30 s to 30 min (*see* **Note 10**) [5].

Table 2
Information about probiotic strains added to ice cream formulations

Probiotic strain	Inoculation dose (CFU/mL)	Inoculation method	Inoculation temperature (°C)	Storage (days)	Probiotic viability after storage (CFU/mL)	Reference
Lactobacillus acidophilus La-5®	9.0	90% of the milk was transferred into two sterile jars, and the milk samples were inoculated with freeze-dried *L. acidophillus* La-5® and incubated at 37 °C/4 hours until the pH reached 4.7.	4.0	60	7.37	[4]
Bifidobacterium bifidum	7.56–7.60	Freeze-dried *Bifidobacteria* was prepared in 200 mL of reconstituted skimmed milk (10% w/w), and it was incubated at 37 °C/24 hours. The probiotic was reactivated into 300 mL of milk and incubated at 37 °C until the pH became 4.6.	4.0	60	6.20–6.28	[5]
Lactobacillus acidophilus La-5®	8.20	The *L. acidophilus* La-5 was inoculated in the ice cream mix and incubated at 37 °C until the pH reached 5.80.	37	60	7.25	[13]
Lactiplantibacillus plantarum subsp. *plantarum* ATCC 8014	6.0–7.0	About 1% of *L. plantarum* subsp. *plantarum* inoculum was cultured in sterile man, Rogosa, and sharp broth and incubated overnight at 37 °C. after that, the cells were centrifugated and washed twice with sterile peptone water. Approximately 1 mL of *L. plantarum* subsp. *plantarum* after 20 h of incubation was added to 150 mL of pasteurized full cream milk.	40	60	>7.46	[16]
Saccharomyces boulardii *Lacticaseibacillus rhamnosus* GG	7.34 10.11	The *S. boulardii* was incubated in YPD broth for 48 h, and the *L. rhamnosus* was incubated in MRS broth for 24 h at 37 °C. both strains were grown in 5 L volumes to obtain them at desired levels before the inoculation of the ice cream	37	28	6.2 9.2	[26]

(continued)

Table 2
(continued)

Probiotic strain	Inoculation dose (CFU/mL)	Inoculation method	Inoculation temperature (°C)	Storage (days)	Probiotic viability after storage (CFU/mL)	Reference
		mix. After the incubation, the cultures were centrifuged, and obtained pellets were washed twice with PBS and inoculated to the pasteurized ice cream mix to 37 °C before and after the aging steps.				
Lacticaseibacillus paracasei subsp. *Paracasei* L-26 *Lacticaseibacillus casei* 431 *Lactobacillus acidophilus* La-5®	9.0	The starter probiotic cultures were previously inoculated in milk at 40 °C for 5 hours. 5% of inoculum was added to the ice mixes.	37	21	10.18–10.17 9.88–9.85 8.83–9.68	[17]
Bifidobacterium lactis (Bl-04)	9.0	The culture was dissolved into UHT milk and activated to obtain 10^9 CFU/mL of bacteria cells in MRS broth.	2	120	7.16	[14]
Lacticaseibacillus paracasei subsp. *Paracasei* L-26 *Bifidobacterium longum* + *Bifidobacterium bifidum* B-94	8.0–9.0	The probiotic cultures were inoculated in the ice cream mix by the commercial company's recommendations. The inoculated mixtures were left to incubate at 37 °C, which was carried out until the pH values reached 4.8–4.9.	37	120	8.19 8.15	[1]
Lactiplantibacillus plantarum subsp. *plantarum* LP299v *Lacticaseibacillus casei* ATCC 393	8.50–9.0	An aliquot of each probiotic culture was individually transferred into MRS broth and placed in an incubator at 37 °C for 24 hours. After, the 10 mL culture was diluted to 100 mL and re-incubated at 37 °C/48 h. it was centrifuged to isolate the probiotics from the MRS.	37	60	>7.50	[25]

Lactobacillus acidophilus *Bifidobacterium lactis*	8.0	Freeze-dried cultures of probiotic strains were inoculated separately in glass tubes containing MRS broth and incubated at 37 °C/24 h under aerobic conditions. Then they were centrifuged, and washed twice with sterile saline. The resulting pellet was diluted in sterile saline.	37	180	5.95 >6.0	[18]
Lacticaseibacillus casei 01	6.0	100 mg (w/w) of freeze driedLactobacillus casei-01 in 1 L (v/v) of skimmed sheep milk (w/v) for 6-h incubation. Subsequently, fermented sheep milk was added by sheep milk fat and skimmed sheep milk to totalize 2 L of mix added with inulin, sugar, and stabilizer/emulsifier.	37.0	150	> 7.0	[9]

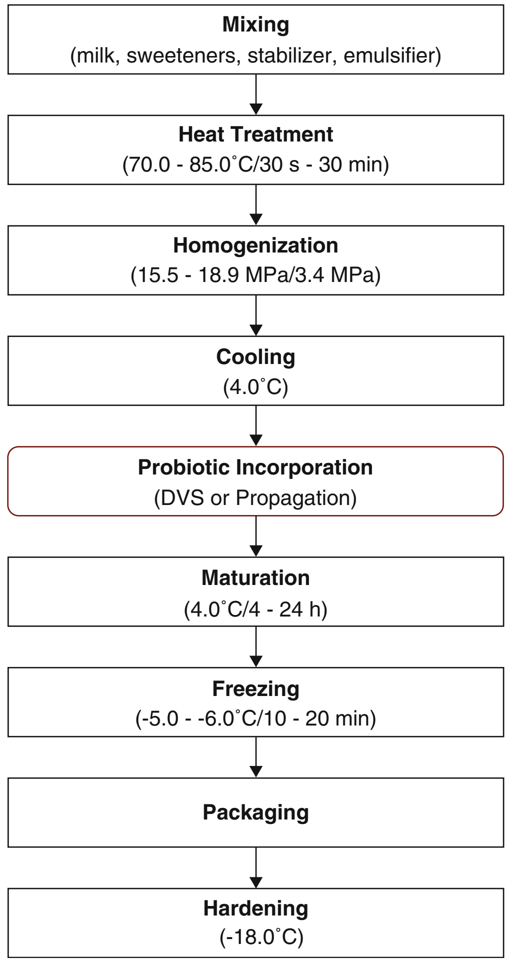

Fig. 1 Flowchart of ice cream making

3. Then homogenize the pasteurized mixture in two steps: at high pressure (15.5–18.9 MPa) and after low pressure (3.4 MPa) (*see* **Note 11**) [23].
4. After homogenization, incorporate probiotic microorganisms into the mixture (*see* **Note 12**) and cool until you reach 4 °C to start the maturation stage.
5. Perform maturation by stirring the mixture at 4 °C temperature for a time of 4–24 h (*see* **Note 13**). After complete maturation, the mixture proceeds to freeze.
6. Freeze the ice cream in equipment containing a rotary stirrer, for air incorporation, up to an overrun of 50%, at a temperature of −5 to −6 °C for 10–20 min (*see* **Notes 14, 15,** and **16**).
7. Then fill the ice cream and proceed to the final hardening at −30 °C or lower (*see* **Note 17**), with subsequent storage at −18 °C (*see* **Note 18**).

4 Notes

1. Yogurt is used in the preparation of frozen yogurt.
2. Fat sources can come from milk fat, such as cream milk, butter, and butter oil, fats, and oils from plants, such as corn, sunflower, canola, and peanut, and blends of oils [15].
3. Protein sources may or may not be milk. Whey proteins and caseins are used, in addition to soy proteins and nuts [15]. Proteins are also used to give ice structuring.
4. The milk solids-not-fat contribute to the flavor and texture. The industry usually uses concentrated milks, dried skim and whole milk, milk power blends, and whey products [15].
5. Sweetening sources may be corn sweeteners, maple sugar, honey, invert sugar, fructose, molasses, malt syrup, brown sugar, lactose, sugar alcohols, sorbitol, mannitol, xylitol, and other nonnutritive sweeteners such as saccharin, aspartame, and sucralose [15].
6. Examples of stabilizers used: carob gum, guar gum, xanthan gum, sodium carboxymethylcellulose, sodium alginate, microcrystalline cellulose, carrageenan, gelatin, and pectin [23].
7. The emulsifiers added in ice cream formulations are of two types: mono- and diglycerides and sorbitan esters, as polysorbate 80. Some factories also use eggs or egg yolk.
8. The most used flavors are chocolate, vanilla, and strawberry. But, neopolitan, lemon, nut, pear, rum and raisin, cookies and cream, and others can also be added.
9. Automatic dosing pumps or tanks in load cells can add liquid ingredients. Dry ingredients are added by pumping at high speed or with high shear mixers to prevent the formation of lumps. Dry ingredients should be incorporated into the mixture at a temperature below 30 °C [12, 15].
10. The four methods to pasteurize ice cream can be low-temperature long-time (LTLT—69 °C/30 min), high-temperature short-time (HTST—83 °C/15 s), higher-heat shorter-time (HHST—90 °C/1–3 s), or ultra-high temperature (UHT—138 °C/≥2 s) [11]. Batch pasteurization uses double-shirt tanks, in which the mixture is heated, with steam circulation or hot water inside, performing heat changes. Continuous pasteurization is performed in heat exchangers, and there may be a preheating of the mixture between 30 and 40 °C to mix the ingredients.
11. Homogenization is performed in two stages so that in the first stage fat globules tend to group and form agglutinated. In the second stage, the adsorption of proteins occurs on the fat surface, avoiding further regrouping, and making the emulsion more stable.

12. The cultures can be added to ice cream in several ways, of type DVS (direct vat set), for direct addition of the product in a pasteurized mixture, or in the use of milk as a substrate for fermentation [4].

    The freeze-dried probiotic culture can be added to the mixture before maturation and freezing, which presents advantages related to the easy insertion of the same in the mixture; however, as it is not in its active form, it may be that the probiotic remains inactive. In addition to freeze-dried culture, there is the possibility of incorporating probiotic biomass into the mixture before maturation [5]. The added biomass ferments the mixture, which then proceeds to maturation.

    When milk is used as substrate for probiotic incorporation, the step can be performed by means of a partial mixture of milk with probiotic, so that 10 to 30% of milk proceeds to a fermentation stage with probiotic culture, at 37 °C, for up to 12 hours in anaerobiosis, and then that fermented milk is incorporated into the rest of the mixture before freezing. This partial fermentation of milk promotes the activation of probiotic culture, besides not significantly altering the organoleptic characteristics of ice cream [4].

    Also, from the use of milk, it can be entirely fermented by probiotic culture and, after, the other ingredients are added. However, this type of process ends up resulting in more acidic sensory characteristics due to the high production of lactic acid [4].

13. In the maturation stage, the addition of flavorings and dyes that are sensitive to the heat of thermal processing is carried out aseptically. The additives added, both in the mixing stage and in maturation, cannot interfere with the action of the probiotic, nor cause any kind of damage. At this stage occurs the development of the sensory characteristics of flavor and aroma of the product.

14. The freezing stage is one of the main parts of the preparation of ice cream because there is the incorporation of air in the ice cream, also known as overrun, a step that gives the characteristic of the body and texture of the ice cream. The amount of overrun should be between 2.5 to 3 times the total solids of the ice cream. The presence of this stage in the preparation of ice cream causes risks to the survival of probiotics, due to oxygen toxicity, and the use of aerotolerant strains is necessary. In addition, the size of the particles should be monitored, and the ice crystals should have sizes of 30 to 50 um, air bubbles from 20 to 80 um, the agglomerated fat globules from 2 to 20 um, and the isolated fat globules of 0.1 to 15 um [23].

15. The freezing step takes place in two stages. The first step takes place by passing the mixture in a high-beat shaved surface heat exchanger to allow extensive nucleation of ice crystals and air incorporation. The second step is freezing the ice cream packed in reduced time to prevent the formation of large crystals [23].
16. The probiotic cultures are usually sensitive to freezing, so the incorporation of air and the reduction at shallow temperatures become a lethal medium for these bacteria. The use of cryoprotection, such as sugars, fats, or proteins, promotes the improvement of the resistance of these bacteria to the frozen environment, considering freezing time. The encapsulation of probiotics with these cryoprotectants, in addition to protecting against freezing, also has improved viability during passage in the gastrointestinal tract.
17. During storage, it is extremely important that there is no great variation in temperature, to the point of releasing water in the ice cream, because this failure promotes recrystallization, with the increase of ice crystals, leading to an unpleasant texture in the product. In ice cream, small and quite numerous crystals are sought, the opposite of this is considered a manufacturing defect [23].
18. The formation of ice crystals occurs in two stages. Nucleation is the step that occurs on the wall of the heat exchanger, with small and numerous ice crystals. The low temperature during hardening promotes the continuous growth of the formed crystals. When hardening is slow, the remaining water in the ice cream migrates to the crystals already formed, causing large crystals, which promote the disruption of the cell membrane of probiotics and lead to their inactivation [23].

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