

Technical Note

Development and Characterization of a Functional Ice Cream from Sheep Milk Enriched with Microparticulated Whey Proteins, Inulin, Omega-3 Fatty Acids, and *Bifidobacterium* BB-12[®]

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Abstract: The aim of this work was develop a technological process for the manufacturing of an ice cream from sheep milk, enriched with both functional ingredients and probiotic bacteria. The studied process involved the use of an enriched milk (EM) obtained by mixing predetermined amounts of sheep skimmed milk concentrated by ultrafiltration (retentate), cream from sheep's milk and whey, microparticulated whey proteins (MWP), obtained by ultrafiltration of sweet sheep whey as a source of whey proteins, marine algal oil from *Schizochytrium* spp. as a source of the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), inulin as a prebiotic fiber, and locust bean gum as a stabilizer. The resulting EM was inoculated with starter and aroma cultures together with the probiotic culture of *Bifidobacterium animalis* subsp. *lactis* (BB-12[®]) in order to obtain a fermented functional product (FFP) with a physico-chemical composition similar to that of EM. FFP was the main ingredient (~80%, *w/w*) in the ice cream mixture. Two sucrose-alternative sweeteners (trehalose and erythritol), together with dextrose, were subsequently added to obtain the final ice cream formulation. The resulting ice cream met three nutritional claims: "Source of protein", "Source of fiber" and "High in omega-3 fatty acids" listed in Regulations (EC) No 1924/2006 and (EU) No 116/2010. Furthermore, the ice cream satisfied the requirement of "probiotic food" according to the Italian Ministry of Health's guidelines for probiotics. The nutritional characteristics of the ice cream, including the concentration of the probiotic culture, remained stable up to 120 days of storage at -20 ± 2 °C.

Keywords: functional foods; frozen dairy desserts; ovine; microparticulated whey proteins; probiotic; inulin; *Bifidobacterium*; ultrafiltration; fermentation



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1. Introduction

Frozen dairy desserts such as ice cream and frozen yogurt are very popular foods eaten all over the world [1]. Typically, frozen dairy desserts available on the market are characterized by high relative fat and sugar contents; thus, they are perceived as unhealthy foods because of their elevated caloric content [2]. However, growing consumer awareness of the role of nutrition in promoting health and wellbeing has led to an increasing interest in the development of innovative and functional frozen dairy desserts such as ice creams [3,4].

The term functional food refers to industrially processed or natural foods that when regularly consumed as part of a balanced diet can positively affect health and wellbeing and/or reduce the risk of disease for the consumer, beyond basic nutritional effects [5]. Ice cream is a suitable carrier for nutraceutical components both because it is a product consumed by all age groups and also because frozen storage can preserve its nutritional characteristics for a long time [4]. Ice cream can be functionalized by using different health-promoting ingredients such as probiotics, prebiotics, antioxidants, bioactive peptides,

low-calorie and low-glycemic-index sweeteners, whey and its products, and bioactive lipids (e.g., polyunsaturated fatty acids of the omega-3 series, PUFAs ω -3) [3,4]. In addition, the formulation of some functional ice creams may also be based on a product from microbial fermentation such as yogurt or other fermented milks [6–8]. In this case, the health benefits associated with the fermented product can derive either directly from the viable microorganisms (probiotic effect) or indirectly from the bioactive compounds synthesized by the lactic acid bacteria (LAB) during fermentation (biogenic effect) [9]. Typically, in the frozen yogurts available on the market, the amount of yogurt or fermented milk in the mixture is low (~20%, *w/w*) [1]. As a result, the nutritious characteristics deriving from the fermented product, which are also related to the availability of viable bacterial cells, undergo significant dilution.

Although the majority of ice cream produced in the world is made from cow's milk, the use of a different raw material, such as sheep's milk, can be an interesting alternative from a nutritional point of view. Indeed, compared to other ruminants' milk, sheep's milk shows interesting nutritional peculiarities. In particular, sheep's milk exhibits a favourable fatty acid profile, with a higher conjugated linoleic acid (CLA) content and a low omega-6/omega-3 PUFA ratio [10,11]. Sheep's milk shows a high content of proteins, minerals, and vitamins [12]. In addition, caseins (CN) and whey proteins (WP) from sheep's milk are a source of bioactive peptides with health-promoting properties [11]. Despite this, studies concerning the production of ice cream from sheep's milk are very scarce, and these deal mainly with the addition of probiotics, prebiotics, and WP concentrates [12–16]. Moreover, a few interesting studies look at the valorization of some by-products from the sheep's milk cheesemaking process, such as whey and second cheese whey (referred to as *scotta* in Italian) concentrated by ultrafiltration (UF) and used as a base for ice cream production, from the perspective of sustainability and reducing environmental impact [8,17]. Following this line of research, a possible alternative to whey valorization is its transformation into microparticulated whey proteins (MWP), which can be used as an ingredient in the production of ice cream [18,19]. MWP can improve the texture of low-fat products and are also an interesting ingredient from a nutritional point of view, due to their low caloric load and the high biological value derived from WP [20,21]. The literature regarding the use of MWP in the manufacturing of ice cream is rather scant, and no study deals with the use of a MWP self-produced from sheep whey; studies have focused only on commercial MWP from cow whey.

Based on these assumptions, the formulation of an ice cream obtained from a fermented dairy product, which is the main ingredient and whose nutritional and functional properties are transferred unchanged to the ice cream, could be of significant interest. Hence, the main objective of this work was to set up the technology to produce a functional and probiotic ice cream from sheep's milk. The nutritional properties of the obtained ice cream were derived directly from the functional product used as a "base", which resulted from the fermentation of a sheep's milk modified for the content of natural macro-components (fat, CN and WP), and from the inclusion of three ingredients with a functional value, together with the addition of a probiotic culture (*Bifidobacterium animalis* subsp. *lactis*, BB-12[®]). One of the functional ingredients was MWP (a source of WP), obtained from the recovery of a by-product of sheep milk cheesemaking. The other two functional ingredients were of non-dairy origin: a marine algal oil rich in the PUFAs ω -3 eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and inulin, a prebiotic fiber. In addition, trehalose and erythritol, two sucrose-alternative sweeteners of recent use in ice cream manufacturing which are interesting for their technological and nutritional properties, were used in the preparation of the ice cream mixture. The physico-chemical, nutritional, microbiological, and rheological characteristics of the sheep milk ice cream were evaluated during the frozen storage.

2. Materials and Methods

2.1. Manufacturing Procedure of Ice Cream

2.1.1. Preparation of the Fermented Functional Product (FFP)

The starting sheep's milk was modified based on a predetermined physico-chemical and nutritional composition (Table 1). The aim was to obtain an enriched milk (EM) that satisfied three nutritional claims listed in Regulation (EC) No 1924/2006 and in the subsequent amendment, Regulation (EU) No 116/2010:

- “Source of fiber”, if the product contains at least 3 g of fiber per 100 g or at least 1.5 g of fiber per 100 kcal [22];
- “High in protein”, if at least 20% of the energy value of the food is provided by protein [22];
- “High in omega-3 fatty acids”, if the product contains at least 0.6 g alpha-linolenic acid per 100 g and per 100 kcal, or at least 80 mg of the sum of EPA and DHA per 100 g and per 100 kcal [23].

Table 1. Target composition of the enriched sheep's milk (EM).

Parameters	Enriched Milk	Nutritional Claims ¹
Fat (%)	10.0	
Omega-3 (EPA ² + DHA ³) (%)	0.2	“High in omega-3 fatty acids”
Omega-3 (EPA ² + DHA ³) (g 100 kcal ⁻¹)	0.1	
Protein (%)	7.0	“High in protein”
Casein (%)	3.5	
Whey protein (%)	3.5	
Fat/Protein	1.4	
Inulin (%)	4.0	“Source of fiber”
Locust bean gum (%)	0.2	
Energy value (kcal 100 g ⁻¹)	140	
Calories provided by fat (%)	64	
Calories provided by protein (%)	20	“High in protein”

¹ Regulations EC No 1924/2006 and (EU) No 116/2010; ² EPA: eicosapentaenoic acid; ³ DHA: docosahexaenoic acid.

The composition of EM was designed so that the ice cream derived from it would retain two of the three starting claims. In fact, the reduction in nutrient level and the increase in caloric intake, both derived from the addition of the sugars in the ice cream, would not have changed the nutritional indications “Source of fiber” and “High in omega-3 fatty acids”. On the other hand, with regard to the nutritional claim about the protein content, since the indication “High in protein” could not be satisfied due to the caloric intake derived from the added sugars, the aim was to achieve the claim “Source of protein”. This nutritional indication is met if at least 12% of the energy value of the food is provided by protein [22].

EM was prepared by mixing predetermined amounts of six ingredients shown in Table 2. Three of them were derived from sheep's milk: cream from milk and whey, skimmed milk retentate (SMR), and microparticulated whey proteins (MWP) from whey. The remaining three ingredients were of non-dairy origin: marine algal oil, inulin, and locust bean gum. Among these six components, three can be considered functional ingredients (MWP, marine algal oil, and inulin). MWP is an ingredient rich in WP derived from sheep sweet skim whey, whose production process is described in more detail below. Marine algal oil is an ingredient wealthy in ω -3 fatty acids (EPA and DHA) and is extracted from heterotrophic microalgae. An oil derived from the marine microalga *Schizochytrium* spp. (Life's Omega™ 45, DSM, Heerlen, The Netherlands) was used in this study. Inulin is a prebiotic fiber containing fructosyl residues linked together with β -2,1 bonds and ending with a glucose molecule. The number of fructose units determines the different degrees of polymerization (DP). An inulin with DP \geq 20 (Fibruline™ XL, Cosucra, Warcoing, Belgium) was used in this work. Locust bean gum (Bongiovanni S.r.l., Villanova Mondovì, Italy) was added as a thickening and stabilizing agent.

Table 2. Composition and relative incidence of the ingredients, both derived from sheep's milk and of non-dairy origin, used to prepare the enriched milk (EM).

Parameters	Sheep's Milk-Derived Ingredients			Non-Dairy Ingredients		
	Cream ¹	MWP ²	SMR ³	Algal Oil ⁴	Inulin ⁴	LBG ^{4,5}
Fat (%)	72.0	0.6	0.5	99.9	-	0.7
Omega-3 (EPA ⁶ + DHA ⁷) (%)	-	-	-	45.0	-	-
Protein (%)	1.0	7.5	9.2	-	-	3.3
Fat/Protein	72.0	0.1	0.01	-	-	-
Casein (%)	0.8	-	7.3	-	-	-
Whey protein (%)	0.2	7.5	1.8	-	-	-
Inulin (%)	-	-	-	-	95	-
Lactose (%)	2.1	3.6	4.0	-	-	-
Energy value (kcal 100 g ⁻¹)	660	50	57	900	181	219
Relative incidence in EM (%)	12.6	36.0	46.5	0.5	4.2	0.2

¹ Cream resulting from the skimming procedure of milk and sweet whey; ² MWP: microparticulated whey proteins; ³ SMR: skimmed milk retentate; ⁴ values declared by the manufacturer; ⁵ LBG: locust bean gum; ⁶ EPA: eicosapentaenoic acid; ⁷ DHA: docosahexaenoic acid.

The production process of EM followed the procedure reported in Figure 1. Ingredients derived from sheep's milk (cream, MWP and SMR) were obtained from the same batch of raw whole bulk milk (about 50 kg) from Sarda sheep (composition: fat 7.6%, protein 6.3%, lactose 4.2%), in the amount required to replicate the trial 3 times, under the same operating conditions and at close temporal distance from each other. The milk was collected from the "Bonassai" experimental farm of Agris Sardegna (Sassari, Italy). Part of the whole sheep milk (about 11 kg) was filtered, heated to 40 °C, and skimmed using a centrifugal separator (Sartore, Vigone, Italy). From the skimming procedure, skimmed milk (9.6 kg) destined for UF concentration (in the production of SMR), and cream (1.1 kg) were thus obtained. The skimmed milk was firstly thermized (68 °C × 15 s), then cooled to 25 °C and concentrated by UF, until the predetermined volumetric concentration factor (VCR = 1.5) was reached. UF was carried out using a Sartocor[®] Slice Crossflow Filtration System (Sartorius, Gottinga, Germany) equipped with 4 Hydrosart[®] stabilized cellulose-based membrane (Sartorius, Gottinga, Germany) with a cut-off of 30 kDa and a nominal area of 0.1 m² each. The cross-membrane pressure, process temperature, and permeation flow rate were 0.1 MPa, 25 °C and 23.3 L h⁻¹ m⁻², respectively. The resulting SMR (5.6 kg) and cream were then pasteurized (72 °C × 15 s), rapidly cooled, and stored at 4 °C until further use.

The rest of the whole sheep milk (about 39 kg) was subjected to the cheesemaking process in the experimental cheese plant of Agris Sardegna, to obtain the sweet whey (23.0 kg) necessary to produce MWP. The sweet whey obtained was filtered, heated to 40 °C, and submitted to the skimming procedure using a centrifugal separator (Sartore, Vigone, Italy). The resulting cream (0.5 kg) was pasteurized (72 °C × 15 s) and stored at 4 °C until further use, while the skim whey (22.0 kg) was firstly thermized (68 °C × 15 s) then cooled to 25 °C and concentrated by UF using the same UF system utilized to produce SMR until the predetermined volumetric concentration factor (VCR = 4.4) was reached. The cross-membrane pressure, process temperature and permeation flow rate were 0.1 MPa, 25 °C and 45 L h⁻¹ m⁻², respectively. The obtained whey retentate (4.6 kg) was heated (80 °C × 10 min) in order to precipitate the macro-aggregates of WP. The resulting precipitate was then subjected to vigorous stirring using a high-speed disperser (ULTRA-TURRAX[®] T25, IKA, Staufen, Germany) and subsequently microparticulated by using a high-pressure homogenizer (Panther NS3006L, GEA Niro Soavi, Parma, Italy) in order to reduce WP macro-aggregates down to the average size of 1–2 µm. The MWP (4.3 kg) thus produced was quickly cooled and stored at 4 °C until subsequent use.

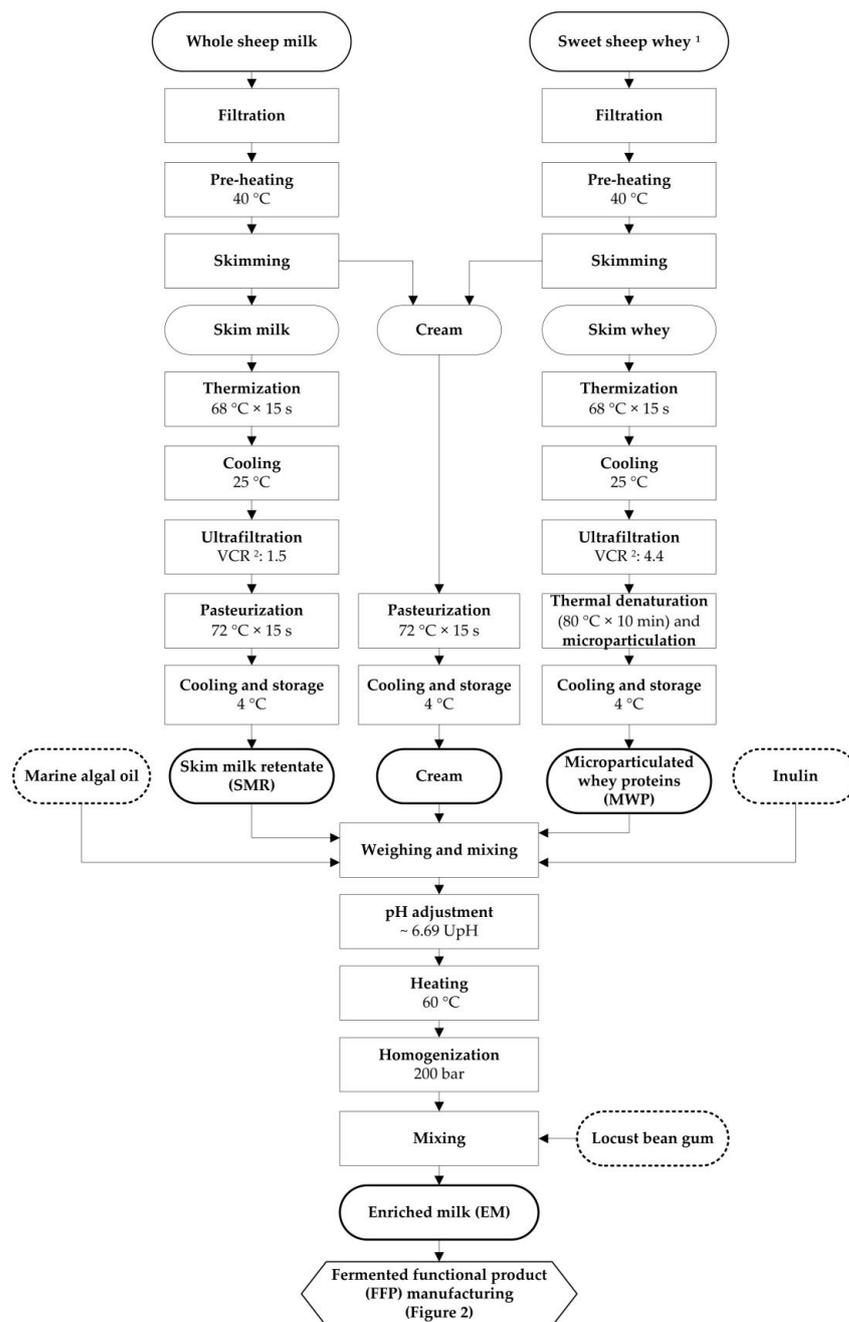


Figure 1. Flow chart of the enriched sheep milk (EM) preparation. ¹ Sweet sheep whey obtained from the cheesemaking process of whole sheep milk. ² VCR: volumetric concentration ratio.

To achieve the desired milk formulation of EM, the amounts of the single ingredients were determined by applying a mathematical system of equations, which was based on the pre-established composition of the final formulation (Table 1) as well as on the known composition of the ingredients themselves (Table 2). The relative incidence of each ingredient used in the preparation of EM is shown in Table 2. Cream (from milk and whey skimming process), MWP, SMR, marine algal oil, and inulin were thus weighed separately and mixed together. The resulting milk was standardized in terms of pH value (6.69 UpH), heated to a temperature of 60 °C, and homogenized at a pressure of 200 bar (Panther NS3006L, GEA Niro Soavi, Parma, Italy). At the end of the homogenization process, locust bean gum was added. The EM thus obtained was intended for the manufacturing process of the fermented functional product (FFP).

The production process of FFP is reported in Figure 2. EM was pasteurized at a temperature of $80\text{ }^{\circ}\text{C} \times 10\text{ min}$ and subsequently cooled down to incubation temperature ($44 \pm 1\text{ }^{\circ}\text{C}$). Hence, the following commercial freeze-dried cultures were added:

1. Thermophilic exopolysaccharide (ESP)-producing yogurt starter culture (YO-MIX™ T12, Danisco, Copenhagen, Denmark), including *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* at a concentration of $6\text{ log}_{10}\text{ CFU mL}^{-1}$;
2. Aroma culture (CHOOZIT™ MD 88, Danisco, Copenhagen, Denmark) consisting of a mesophilic heterofermentative species (*Lactococcus lactis* subsp. *lactis biovar diacetyllactis*) at a concentration of $5\text{ log}_{10}\text{ CFU mL}^{-1}$;
3. Probiotic culture of *Bifidobacterium animalis* subsp. *lactis* (BB-12®, Chr-Hansen, Hoersholm, Denmark) at a concentration of $7\text{ log}_{10}\text{ CFU mL}^{-1}$.

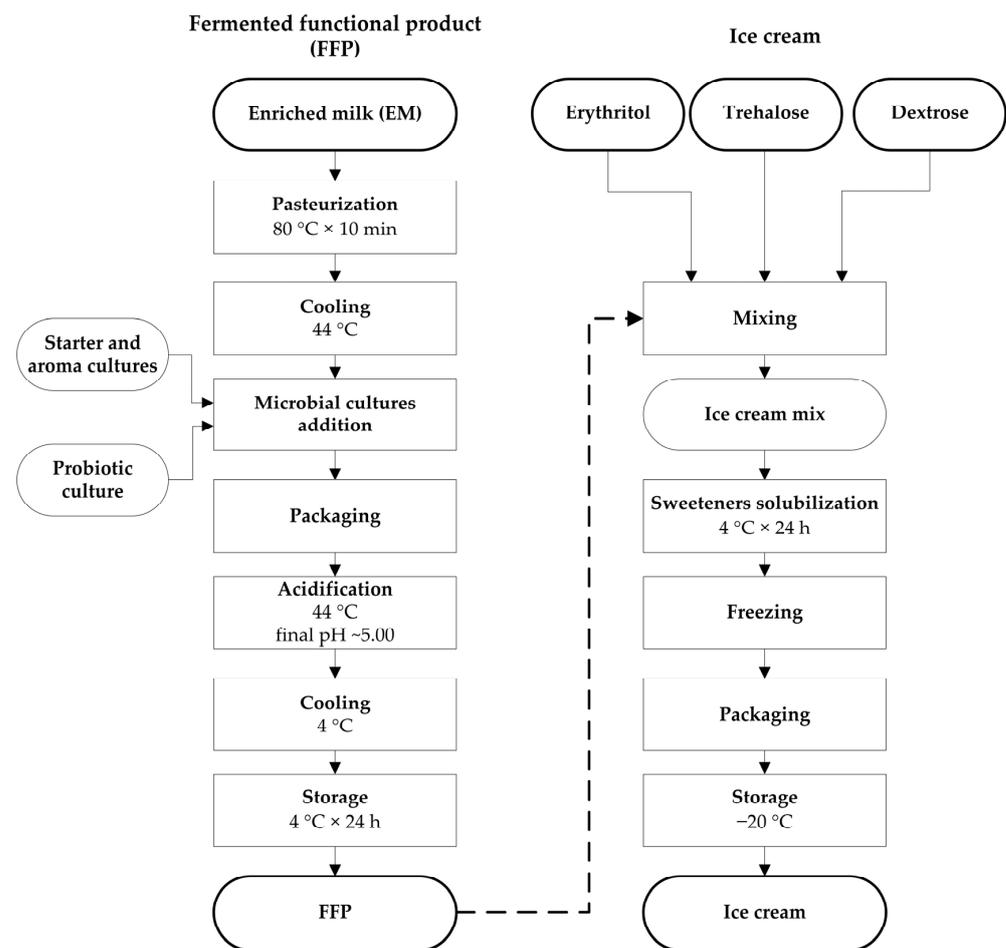


Figure 2. Flow chart of the manufacturing process of the fermented functional product (FFP) and ice cream.

The inoculated milk was packaged and incubated at $44 \pm 1\text{ }^{\circ}\text{C}$ for the time required for milk coagulation and for a final pH of 5.00 to be reached. The resulting FFP was rapidly cooled to $4 \pm 1\text{ }^{\circ}\text{C}$ and stored for 24 h at the same temperature.

2.1.2. Preparation of Ice Cream

The manufacturing process of ice cream is shown in Figure 2. At the end of the refrigeration storage period of FFP, the ice cream mixture (4 kg for each of the 3 trials) was prepared. Two sucrose-alternative sweeteners, trehalose (Hayashibara, Okayama, Japan) and erythritol (Giusto Faravelli S.p.A., Milan, Italy), were added to FFP together with dextrose monohydrate (Roquette, Lestrem, France). The technological and nutritional

properties of the selected sweeteners obtained from manufacturers or the literature are given in Table 3 [1]. The resulting ice cream mixture consisted of 80% (*w/w*) FFP (3.2 kg), 10% (*w/w*) trehalose (0.4 kg), 5% (*w/w*) erythritol (0.2 kg), and 5% (*w/w*) dextrose (0.2 kg). In order to allow the complete solubilization of all the added sweeteners, the mixture was rested at 4 ± 1 °C for 24 h. After this time, the freezing phase was carried out by using a batch freezer (Movì 30 Smart, INNOVA, Cerete, Italy). The extent of freezing affected the final consistency of ice cream. This parameter was automatically monitored by the machine, which recorded the increase in motor effort due to the change in ice cream viscosity during processing. On a scale of 1 to 12, a freezing grade of 9 was chosen, according to the machine indications for this product category. At the end of the freezing stage, the ice cream was completely extruded from the machine and packed in 200 mL (for physico-chemical and microbiological analysis) and 500 mL (for rheological analysis) plastic containers, and stored at -20 ± 2 °C for 120 days. The ice cream was sampled after 1, 30, 60, 90 and 120 days to monitor physico-chemical and microbiological features.

Table 3. Nutritional and technological properties of lactose, inulin (sugars provided by the fermented functional product, FFP), and the three sweeteners (dextrose, trehalose, and erythritol) used in ice cream manufacturing (values obtained from the manufacturers or the literature [1]).

Parameters	Lactose	Inulin	Dextrose	Trehalose	Erythritol
Energy value (kcal 100 g ⁻¹)	4	1.9	4	4	0.2
Glycemic index (GI) ¹	45	1	100	70	1
Sweetening power (POD) ²	16	0	70	42	70
Antifreeze power (PAC) ³	100	25	180	100	280

¹ The glycemic index (GI) is a number between 0 and 100 that represents how quickly blood sugar rises following the intake of 50 g of simple carbohydrates. Glucose is taken as the reference food with a value of 100; ² POD (sweetening power) is a parameter which indicates the relative sweetness of a sugar, compared to sucrose, whose POD value is 100; ³ PAC (antifreeze power) is a parameter which indicates the ability of a sugar to lower the freezing point of water, compared to sucrose, whose PAC value is 100.

2.2. Physico-Chemical Analysis

2.2.1. Compositional Characterization and pH Measurement

Samples of whole milk, skimmed milk, SMR, cream (after 1:10 dilution with skimmed milk, whose composition was used as a blank), MWP, and EM (before locust bean gum addition) were analyzed for fat, protein, casein, whey protein and lactose content using a Milkoscan FT+ (Foss, Hillerød, Denmark), and for pH (pH-meter Crison Basic 20+, Crison Instruments S.A., Alella, Spain).

Samples of FFP and ice cream were analyzed for pH (pH-meter Crison Basic+), dry matter (DM) [24], fat [25], total nitrogen [26], casein nitrogen [27], and non-protein nitrogen [28], while lactose content was determined by applying a gas chromatographic method, as described by Idda et al. [29], using a gas chromatograph (Varian 3600; Varian, Harbor City, CA, USA) equipped with a flame ionization detector and a split-splitless injector.

2.2.2. Omega-3 Fatty Acids Content and Peroxide Value (PV) Determination

Fat was extracted from ice cream samples according to the ISO-IDF procedure [30], using n-pentane and diethyl ether, after the addition of an ethanol and ammonium hydroxide solution. The n-pentane/diethyl ether layer containing the lipids was evaporated at 30 °C with a rotary evaporator to remove the solvent. The extracted fat was then used to determine fatty acid composition and PV.

In order to determine the change in ω -3 (EPA and DHA) content during storage, 50 mg of ice cream fat was trans-methylated through methanolic KOH, according to the ISO-IDF reference procedure [31]. The resulting fatty acid methyl esters (FAMES) were then analyzed using a gas chromatograph (8890 GC System, Agilent Technologies, Palo Alto, CA, USA), according to the procedure described by Lai et al. [32].

To evaluate the oxidative stability of ice cream fat during storage, PV was determined spectrophotometrically ($\lambda = 500$) using a UV-visible spectrophotometer (Cary 1E, Varian,

Harbor City, CA, USA), according to the ISO 3976:2006-IDF 74:2006 method [33]. PV was expressed as $\text{mEqO}_2 \text{ kg}^{-1}$ of fat.

2.2.3. Extrusion Temperature and Overrun

Upon extrusion from the batch freezer, a thermocouple (XS TEMP 70 Pt100 Professional, Vetrotecnica S.r.l., Padova, Italy) was used to measure the temperature inside the ice cream.

Overrun is a parameter that measures the increase in volume due to the incorporation of air into the ice cream mixture at the end of the freezing process. The method reported by Pires et al. [8] was followed and overrun was calculated based on the weight of the ice cream mix and that of the ice cream with the same volume, using the following equation:

$$\text{Overrun [\%]} = \frac{\text{weight of ice cream mix} - \text{weight of ice cream}}{\text{weight of ice cream}} \times 100 \quad (1)$$

2.2.4. Energy Density and Glycemic Load (GL)

The energy density of EM, FFP, and ice cream was determined indirectly, taking into account the content of macronutrients (fat, protein, and carbohydrates) in 100 g of product and the calories provided by each of them (fat: 9 kcal g^{-1} ; protein: 4 kcal g^{-1} ; lactose: 4 kcal g^{-1} ; inulin: 1.9 kcal g^{-1} ; dextrose: 4 kcal g^{-1} ; trehalose: 4 kcal g^{-1} ; erythritol: 0.2 kcal g^{-1} ; locust bean gum: 2.2 kcal g^{-1}).

GL is a measure of how quickly a specific food raises blood sugar based on its carbohydrate content and glycemic index. The GL of EM, FFP, and ice cream was calculated by multiplying the carbohydrates (lactose, inulin, dextrose, and trehalose) and erythritol contents in 100 g of product by the relative glycemic index and then dividing by 100.

2.2.5. POD and PAC

The concept of POD (from the Italian expression “Potere Dolcificante”) or sweetening power refers to the ability of sugars to provide sweetness [34]. The notion of PAC (from the Italian expression “Potere Anti Congelante”) or antifreeze power deals with the ability of sugars to lower the freezing point of water, thus affecting the hardness of ice cream [35]. Sucrose is conventionally taken as a reference, with a POD and PAC value of 100. POD and PAC levels in the ice cream were calculated taking into account the content of inulin, lactose, trehalose, dextrose, and erythritol in the ice cream mixture and the POD and PAC values of each of these ingredients (Table 3) by applying the following equations:

$$\text{POD} = \frac{\text{sum of [amount (g } 100 \text{ g}^{-1}) \text{ of each ingredient} \times \text{POD of each ingredient]}}{100} \quad (2)$$

$$\text{PAC} = \frac{\text{sum of [amount (g } 100 \text{ g}^{-1}) \text{ of each ingredient} \times \text{PAC of each ingredient]}}{100} \quad (3)$$

2.2.6. Textural Analysis

Hardness was evaluated after production using ice cream samples stored in 500 mL plastic containers at $-16 \text{ }^\circ\text{C}$ for 24 h. The test was performed using a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 10 mm diameter cylindrical probe and a 50 kg load cell. The test parameters were as follows: penetration distance from sample surface: 20 mm; and pre-test, test, and post-test speed: 1 mm s^{-1} . Each ice cream sample was penetrated at three different points on the surface. The results were determined using Exponent software version 6,2,1,0 (Stable Micro Systems, Surrey, UK) and hardness corresponded to the maximum force (N) measured during probe penetration.

2.3. Microbiological Analysis

The viable cell counts of total LAB and probiotic bacterium *Bifidobacterium animalis* subsp. *lactis* (BB-12[®]) were determined in EM upon inoculation, FFP 24 h after production,

and ice cream after 1, 30, 60, 90, and 120 days of frozen storage (-20 ± 2 °C). Total LAB were enumerated on plates at 37 °C for 72 h in aerobiosis, by using Milk Plate Count Agar (MPCA, Biolife Italiana S.r.l., Milan, Italy). The *Bifidobacteria* count was carried out by plating on Bifidus Selective Medium (BSM) agar (Merck, Darmstadt, Germany) supplemented by a BSM selective supplement (Merck, Darmstadt, Germany) and incubated under anaerobic conditions for 48 h at 37 °C. The results were expressed as \log_{10} CFU g^{-1} .

2.4. Mass Balance Evaluation

The mass balance regarding the production of 100 kg of ice cream was determined by taking into account the composition of the starting ingredients and that of the ice cream produced.

2.5. Statistical Analysis

The data obtained were statistically analyzed using the statistical package Minitab 16 (Minitab Inc., State College, PA, USA). The general linear model (GLM) procedure and Tukey's test for multiple comparisons of means were used to evaluate the effect of storage time on physico-chemical and microbiological parameters ($p < 0.05$).

3. Results and Discussion

3.1. Physico-Chemical, Nutritional, and Technological Properties

Table 4 shows the physico-chemical and nutritional characteristics of EM, FFP, and ice cream. The composition of EM was close to the predetermined target formulation (Table 1). This result demonstrated the validity of the standardization method developed by successfully adjusting the content of components in EM according to precise nutritional claims as well as technological requirements, which are mainly related to the ice cream manufacturing process.

Table 4. Physico-chemical and nutritional parameters of the enriched sheep milk (EM), fermented functional product (FFP), and ice cream after 24 h from production (mean \pm SD).

Parameters	EM	FFP	Ice Cream	Nutritional Claims ¹	
				FFP	Ice Cream
pH (UpH)	6.6 \pm 0.1	4.9 \pm 0.1	4.9 \pm 0.1		
Dry matter (DM) (%)	25.7 \pm 0.3	25.85 \pm 0.02	38.3 \pm 0.1		
MSNF ² (%)	11.4 \pm 0.1	11.62 \pm 0.02	8.4 \pm 0.1		
Fat (%)	10.2 \pm 0.4	10.19 \pm 0.04	8.20 \pm 0.01		
Omega-3 (EPA ³ + DHA ⁴) (%)	0.27 \pm 0.01	0.270 \pm 0.001	0.216 \pm 0.003	"High in omega-3 fatty acids"	"High in omega-3 fatty acids"
Omega-3 (EPA ³ + DHA ⁴) (g 100 kcal ⁻¹)	0.192 \pm 0.001	0.200 \pm 0.001	0.127 \pm 0.001		
Protein (%)	6.9 \pm 0.1	7.1 \pm 0.2	5.5 \pm 0.1	"High in protein"	"Source of protein"
Casein (%)	3.5 \pm 0.1	3.6 \pm 0.1	2.8 \pm 0.1		
Whey protein (%)	3.4 \pm 0.1	3.5 \pm 0.1	2.7 \pm 0.1		
Lactose (%)	3.50 \pm 0.01	1.6 \pm 0.2	1.2 \pm 0.2		
Inulin (%)	4.00 \pm 0.01	4.01 \pm 0.04	3.21 \pm 0.03	"Source of fiber"	"Source of fiber"
Glycemic load ⁵	1.637 \pm 0.003	0.83 \pm 0.01	12.71 \pm 0.01		
Energy density ⁶ (kcal 100 g ⁻¹)	141 \pm 4	134.7 \pm 0.2	168.5 \pm 0.2		
Calories provided by fat (%)	65 \pm 1	68.1 \pm 0.2	43.8 \pm 0.1		
Calories provided by protein (%)	19.6 \pm 0.3	21.2 \pm 0.2	13.2 \pm 0.2	"High in protein"	"Source of protein"
Calories provided by carbohydrates (%)	15.3 \pm 0.4	10.7 \pm 0.1	43.0 \pm 0.1		

¹ Regulations EC No 1924/2006 and (EU) No 116/2010; ² MSNF: milk solids-not-fat; ³ EPA: eicosapentaenoic acid; ⁴ DHA: docosahexaenoic acid; ⁵ glycemic load is determined by multiplying the carbohydrates (sugars, lactose, inulin, and trehalose) and erythritol content in 100 g of product by the relative glycemic index and then dividing by 100. ⁶ Energy density is determined taking into account the calories provided by each nutrient (fat: 9 kcal g⁻¹; protein: 4 kcal g⁻¹; lactose: 4 kcal g⁻¹; inulin: 1.9 kcal g⁻¹; trehalose: 4 kcal g⁻¹; erythritol: 0.2 kcal g⁻¹; locust bean gum: 2.2 kcal g⁻¹).

The fat content of EM was standardized to the predetermined value (10%) mainly by adding cream. The fat provided by this ingredient accounted for approximately 90% of the total fat in EM. However, as a result of the addition of algal oil, it was possible to modify the fatty acid profile of the lipid fraction compared to that of the starting sheep's milk, with an

increase in the content of the ω -3 fatty acids EPA and DHA. These essential fatty acids play an important role in the proper development of the fetus brain and retina during pregnancy as well as in reducing the risk of hypertension, inflammation, cardiovascular disease, and cancer in humans [36,37]. The Western diet is characterized by an inadequate intake of the ω -3 fatty acids EPA and DHA, compared with levels recommended by international organizations dealing with health and nutrition [38]. Therefore, fortification of dairy products with ω -3-rich oils is one of the possible strategies that can be used to increase the daily intake of these essential fatty acids. Ice cream in particular is a suitable product for this type of fortification both because of its wide range of consumers and optimal storage conditions.

The protein content of EM was standardized to the predefined value of 7% mainly with the use of SMR and MWP, which provided, respectively, 60% and 38% of the total protein. These two ingredients also allowed the CN:WP ratio to be changed from the natural value of the starting sheep's milk (~3.8) to the target value close to 1. Through the addition of SMR, it was possible to standardize CN content, since this component supplied more than 95% of CN in the final formulation. On the other hand, MWP, which derived from sweet whey concentrated by UF, was the ingredient that provided the highest amount of WP (76%). Increasing WP content in the dairy products is of great interest from a nutritional point of view, since WP have a higher biological value than CN, as they are rich in essential branched-chain amino acids with high bioavailability [20]. WP are well documented as a source of biologically active peptides that can exhibit a positive impact on health through several physiological effects, be they antioxidant, anti-inflammatory, anticancer, antimicrobial, immunomodulatory, antidiabetic, or antihypertensive [39,40].

The inulin content in EM derived entirely from the Fibruline™ XL product. Inulin is a soluble fiber that is partially digestible; therefore, compared to simple carbohydrates such as lactose, it is characterized by a reduced caloric value (about 1.9 kcal g⁻¹). This feature, together with its ability to reduce the feeling of appetite and its excellent technological properties, makes inulin a suitable ingredient for the production of foods with reduced calories. Inulin also acts as a prebiotic, promoting the selective growth and viability of probiotic bacteria such as *Bifidobacterium* spp. and *Lactobacillus* spp. thanks to the synergistic effect between prebiotic and probiotic, which is defined as symbiotic [41].

As shown in Table 4, FFP retained the unchanged composition and nutritional properties of EM as a result of the applied technology. In fact, the production process of FFP (Figure 2) occurred through a fermentation process, which did not involve changes in the initial volume of EM in the packages as a consequence of the low evaporation and lack of whey separation. This enabled the total recovery in FFP of the nutritional elements contained in EM. After curd breaking, FFP presented a particularly smooth texture free of clots and with a very creamy consistency (Figure 3a). The latter feature was due to the chosen yogurt starter culture (YO-MIX™ T12) that was able to ensure not only a rapid acidification (about 4 h to reach pH~5 in FFP) but also a smooth and very high-quality texture as a result of the ESP production. The simultaneous presence in the product of the thickening agent (locust bean gum) and substances with a “fat-replacer” effect, such as inulin and MWP, probably also played a role.

Table 4 also shows the composition of the ice cream. As expected, the content of the nutritional elements (fat, ω -3, protein, lactose, and inulin) in ice cream underwent a reduction compared to FFP. This effect was due exclusively to the addition of the sweeteners during the preparation of the ice cream mix. As a result, the addition of the sweeteners, which amounted to 20% of the ice cream weight, led to a linear increase in DM content from the initial value of FFP (25.85%) to that of the ice cream (38.3%). The fat (8.2%) and DM values of ice cream (38.3%) were within the range of variability for optimal values (fat, 5–8%; DM, 36–40%) found in good-quality ice cream produced in the world [1]. Ice cream DM and particularly the part represented by milk solids-not-fat (MSNF) and fat are important in improving the body and texture of ice cream as they affect the creaminess and melting rate. Ice cream production technology places limit values in the content of MSNF, fat and

sugars, which must absolutely be considered in the preparation of a mixture of ingredients to be used in the manufacturing of a good-quality ice cream (MSNF: min. 6%, max. 11%; fat: min. 5%, max. 14%; sugars: min. 14%, max. 20%) [42]. For example, an excess of MSNF can lead to the defect of sandiness, while an overload of sugars results in over-sweetness and a too-soft texture as a consequence of lowering the freezing point [1]. As shown in Table 4, in the ice cream, the values of these three parameters met the given limits.



Figure 3. Functional product after fermentation (a) and ice cream during extrusion from the machine at the end of the freezing process (b).

The developed procedure made it possible to keep two of the nutritional claims of FFP unchanged in the ice cream; these were “High in omega-3 fatty acids” and “Source of fiber”. In fact, despite the diluting effect of the sweeteners added to FFP, the ω -3 fatty acids and inulin content of the ice cream maintained the minimum values required by Regulations (EC) No 1924/2006 and (EU) No 116/2010 [22,23]. However, ice cream did not meet the nutritional claim “High in protein”, because the percentage of calories provided by protein within the total calories of 100 g of ice cream was less than 20% (13.2%). In any case, the ice cream satisfied the nutritional indication “Source of protein” ($\geq 12\%$, percentage of calories in the food provided by protein). Furthermore, the quality of the protein fraction of the ice cream produced in this study was certainly higher than that of the starting sheep’s milk, due to the use of MWP. It should also be pointed out that the physico-chemical and nutritional parameters of the ice cream remained almost unchanged during the frozen storage up to 120 days (Table S1).

Table 4 also reports the total energy value of the ice cream. As expected, the addition of the sugars resulted in an increase in the energy density of the experimental ice cream, compared to that of FFP (168.5 kcal 100 g⁻¹ vs. 134.7 kcal 100 g⁻¹). However, the energy value of the ice cream we obtained was lower than that of similar types of ice cream on the market. In fact, in the latter, the total caloric intake frequently exceeds 200 kcal 100 g⁻¹ [43]. The reduction of the energy value in the studied ice cream was an indispensable condition since the caloric content of a food is one of the fundamental parameters needed to meet the requirements of nutritional claims [22,23]. This objective was achieved by partially replacing sugars with the alternative sweetener erythritol, a natural polyalcohol found in small concentrations in fruits, vegetables, mushrooms and fermented foods. This sweetener is characterized by a close to zero caloric content (0.2 kcal g⁻¹) and a glycemic index of 1, as reported in Table 3, and it has a higher digestive tolerance than other polyols [44]. This approach allowed us to reduce the energy value of the ice cream by about 20 kcal 100 g⁻¹ and consequently to keep the nutritional claims of FFP almost unchanged in the ice cream.

GL is also reported in Table 4. The addition of sugars (trehalose and dextrose) resulted in an expected increase in the GL value of the ice cream, which showed a 15-fold increase,

compared to that of FFP (12.7 vs. 0.83). GL is the parameter that indicates the impact of carbohydrates on blood sugar, as it is calculated by considering both the quality (glycemic index) and quantity of carbohydrates provided by the food. Foods are classified as low-GL (≤ 10), medium-GL (11–19), and high-GL (≥ 20). Based on this classification, the obtained ice cream (GL~13) could be considered a medium-GL food, making it different from those commonly found on the market, which are generally classified as high-GL [45]. This result was a consequence of the partial substitution of dextrose with erythritol, which led to a reduction in the ice cream's GL by about 39%. The use of the sugar trehalose, which is characterized by a lower glycemic index than that of dextrose (70 vs. 100, Table 3), also contributed to achieving this outcome.

Table 5 shows the content of the ω -3 fatty acids DHA and EPA, together with PV, determined for ice cream samples during frozen storage (-20 ± 2 °C) up to 120 days. Although ω -3 fatty acids are known to be highly susceptible to oxidation, there was no significant reduction in the content of these fatty acids, which remained unchanged compared to the ice cream at 24 h after production, under the storage conditions applied. Consistent with this finding, PV, an indicator of fat oxidation, also did not change significantly during storage. Similar PV values were observed by Gowda et al. [46] in ice cream fortified with ω -3 fatty acids of different vegetable origins. This result could be related not only to the storage conditions applied to the ice cream, but also to the antioxidant activity explicated by trehalose, erythritol, and WP, as reported in the literature [47–49].

Table 5. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content, expressed as % of total fatty acid methyl esters (FAMES), and peroxide value (PV), expressed as mEqO₂ kg⁻¹ of fat, in ice cream after different storage time at -20 ± 2 °C (mean \pm SD).

Parameters	Storage Time (Days)				
	1	30	60	90	120
EPA	1.309 \pm 0.004	1.3 \pm 0.1	1.29 \pm 0.01	1.29 \pm 0.01	1.27 \pm 0.02
DHA	1.46 \pm 0.01	1.5 \pm 0.1	1.44 \pm 0.03	1.44 \pm 0.01	1.43 \pm 0.02
PV	2.24 \pm 0.04	2.5 \pm 0.2	2.22 \pm 0.04	2.06 \pm 0.04	2.5 \pm 0.3

Table 6 reports the parameters measured at the end of the ice cream manufacturing process, along with two key indicators for the correct balancing of ice cream (POD and PAC). The temperature of the ice cream, when extruded from the batch freezer, was -10.0 °C. The mean overrun value was 18%. This parameter measures the ability of the ice cream mixture to incorporate and hold air during the freezing process. In fact, air influences the product's quality by affecting the physical and sensory properties of ice cream, since it makes the texture soft, creamy and pleasant, and gives the product greater resistance to temperature changes, while reducing the feeling of coldness upon consumption. Typically, manufacturers prefer higher overrun values, as ice cream is sold on a volume basis. In discontinuous artisanal-type batch freezers, such as the one used in this study, the incorporation of air into the ice cream occurs only as a result of the stirring to which the mixture is subjected inside the freezing cylinder. For this reason, in artisanal ice cream, the overrun values are not high, varying between 20% and 40% [1]. However, a contained overrun value is an index of product quality, which differentiates it from industrial ice cream (in which this parameter is raised to values above 100% of the weight of the ice cream mixture through the forced insufflation of air during the freezing phase).

Table 6. Extrusion temperature, overrun, hardness, sweetening power (POD), and antifreeze power (PAC) of ice cream after 24 h from production (mean \pm SD).

Parameters	
Extrusion temperature ¹ (°C)	-10.0 ± 0.1
Overrun ² (%)	18.0 ± 0.8
Hardness (N)	27.7 ± 3.2
Sweetening power (POD) ³	11.40 ± 0.03
Antifreeze power (PAC) ⁴	35.1 ± 0.2

¹ Internal temperature of the ice cream when extruded from the machine. ² Overrun: [(weight of ice cream mix—weight of the same volume of ice cream)/weight of the same volume of ice cream] \times 100. ³ Sweetening power (POD) is calculated by multiplying the content of inulin, lactose, trehalose, dextrose and erythritol present in 100 g of ice cream by their POD value and dividing by 100. ⁴ Antifreeze power (PAC) is calculated by multiplying the content of inulin, lactose, trehalose, dextrose and erythritol, present in 100 g of ice cream by their PAC value, divided by 100.

The obtained ice cream presented a hardness value of 27.7 N after 24 h from production (Table 6). Hardness represents the resistance of ice cream to deformation from an applied external force, and it is an important physical aspect of ice cream as it affects the scoopability and the sensory quality of the product. This parameter is influenced by several factors such as temperature, sugar content, total solids, type of stabilizer, overrun, ice crystal size, and the extent of fat destabilization [1]. Typically, higher overrun values, smaller ice crystals, and greater fat stability at the same serving temperature, are associated with a softer ice cream texture [50]. The formulation studied and the process conditions applied seemed to limit the hardness of the ice cream despite the rather low overrun values.

The POD (sweetening power) and PAC (antifreeze power) values of the ice cream produced are given in Table 6. The ice cream was characterized by POD and PAC values of 11.4 and 35.1, respectively. These parameters need to be evaluated in the preparation of the ice cream base mixture through the appropriate balance of the added ingredients and depend on the soluble elements contained in the ice cream, in particular the sweeteners lactose, and inulin. Regarding the POD value, we chose to keep the ice cream at a low level compared to the desired sweetness, equivalent to that of an ice cream with a concentration of 13–16% sucrose (POD~15) [1]. The reduction in the sweetening power of ice cream was achieved through the use of trehalose (POD = 42, Table 3) in place of sucrose, which is commonly used in ice cream production (POD = 100). On the other hand, the relative antifreeze power of the ice cream was raised due to the use of erythritol, which is characterized by a very high PAC value compared to that of the other sweeteners (PAC = 280, Table 3). The lowering of the freezing point of the ice cream mixture (higher PAC values) is not automatically considered a negative feature. In fact, as the PAC value rises, the amount of free water available in the mixture is lower, and therefore, there is a reduction in ice volume and average crystal size during the freezing stage. The features of the ice cream when extruded from the batch freezer seemed to confirm this thesis (Figure 3b).

3.2. Microbiological Properties

Figure 4 shows the results of the microbial counts of the total LAB and probiotic culture of *Bifidobacterium animalis* subsp. *lactis* (BB-12[®]) in EM, in FFP 24 h after production, and in the ice cream during frozen storage for up to 120 days.

The inoculation procedure in EM resulted in a concentration of $6.4 \log_{10}$ CFU g⁻¹ for the yogurt starter culture and $7.6 \log_{10}$ CFU g⁻¹ for the probiotic culture (Figure 4a), values close to those set in the experimental protocol. The conditions under which the fermentation process was carried out allowed for an increase of about $2 \log_{10}$ CFU g⁻¹ for the starter culture ($8.4 \log_{10}$ CFU g⁻¹) and about $0.5 \log_{10}$ CFU g⁻¹ for the microbial count of *B. animalis* subsp. *lactis* ($8.1 \log_{10}$ CFU g⁻¹) in FFP after 24 h from production (Figure 4b). The slow growth of *Bifidobacteria* was widely expected. These bacteria, in fact, require essential amino acids for their growth, which *Bifidobacteria* are scarcely able to obtain in milk because of their low proteolytic activity [51]. Therefore, they are unsuitable for use as

single starter cultures in fermented milks. Furthermore, *Bifidobacteria* have an optimum pH for growth between 6 and 7, and they are unable to grow below pH values of 5, thus resulting in low acid tolerance [51]. Hence, the rapid acidification carried out by the starter culture in FFP (about 4 h to reach pH 5) did not allow for a greater growth of the probiotic culture BB-12[®]. An increase of 0.5 log₁₀ CFU g⁻¹ is similar to that reported by Valdes-Varela et al. [52] for *B. animalis* subsp. *lactis*, which was grown in monoculture on a specific growth medium in the presence of different prebiotic substrates. In our case, *Bifidobacteria* growth occurred in milk and under sub-optimal conditions of rapid acidification due to the presence of the starter culture. Therefore, this rate of growth within an incubation time of about 4 h (average time of FFP acidification) could be considered a satisfactory result. The presence of the prebiotic fiber inulin and WP in high concentrations may have played a role in this outcome, as reported by other authors [53,54]. Concerning the microbial counts in the ice cream during frozen storage, the results given in Figure 4 show the total LAB kept in ice cream, a concentration not significantly different from that of FFP (8.4 log₁₀ CFU g⁻¹) (Figure 4b) after up to 90 days of storage at -20 °C. The concentration of total LAB in ice cream showed a significant reduction ($p < 0.05$) from the initial value of FFP after 120 days of storage (7.7 log₁₀ CFU g⁻¹). Regarding the probiotic culture BB-12[®], a decrease in viable cell count was observed from the initial value of 8.1 log₁₀ CFU g⁻¹ detected in FFP (Figure 4b) to the value of 7.6 log₁₀ CFU g⁻¹ ($p < 0.05$) found in the ice cream after 1 day of storage at -20 °C (Figure 4c). Subsequently, *Bifidobacteria* maintained a count just over 7.0 log₁₀ CFU g⁻¹ until 120 days of storage, with no significant decrease ($p > 0.05$). This trend in probiotic count was similar to that reported by other authors [55,56]. In fact, the greatest decrease in probiotic survival usually occurs in the first few hours after the freezing process as a result of thermal shock, mechanical damage caused by ice crystals to bacterial cells, and oxygen toxicity due to the incorporation of air into the ice cream mixture (particularly toward strictly anaerobic bacteria such as *Bifidobacteria*) [15]. Subsequently, the viability of probiotics is more stable during frozen storage. Our finding was similar to that reported by other authors for *B. animalis* subsp. *lactis*, which in goat milk-derived ice creams showed a limited loss of viability over a storage period similar to that of the present study [55,57,58]. This survival rate was also similar to that found by Pinto et al. [59] using encapsulated BB-12[®] in frozen yogurt. To the best of our knowledge, there is only one work that has investigated the survival of *B. animalis* subsp. *lactis* in sheep's milk ice cream. However, in this study, BB-12[®] survival was evaluated over a shorter storage period (21 days) [15]. The stability of the probiotic culture during the frozen storage, as shown in our study, may be related to several combined factors. One of these could be the low overrun values observed in the ice cream we obtained, as reported by other authors [55]. In fact, it is well known that oxygen exposure is detrimental to strictly anaerobic bacteria such as *Bifidobacteria* [60]. Another contributing factor to the probiotic culture survival could be the cryoprotective effect of trehalose, which is widely reported in the literature [61,62]. Furthermore, the presence of the prebiotic fiber inulin may also have played a role, as reported in other studies [63,64].

The probiotic culture used in the present work (*B. animalis* subsp. *lactis*, BB-12[®]) is the best known and most studied in the world (with more than 370 scientific publications and 180 clinical studies), and its positive effects on gastrointestinal health and immune function have been demonstrated. In addition, it promotes the development of a healthy intestinal microbiota, preventing intestinal disorders and reducing the side effects of antibiotic therapy [65]. Regarding the recommended minimum amount of probiotic cells necessary to exert positive effects on health, the literature refers to viable cell values of at least 6–7 log₁₀ CFU g⁻¹ in the final product [60,66]. Probiotic standards are not currently harmonized at the European level. In Italy, the Ministry of Health's "Guidelines on probiotics and prebiotics" indicate at least 9 log₁₀ of live cells per day to be the minimum amount sufficient to achieve a temporary colonization of the gut by a probiotic bacterium [67]. This amount of live cells is provided by a 100 g serving of a product with a viable probiotic count of 7 log₁₀ CFU g⁻¹. Products that meet this requirement can be labelled as "probiotic

foods”, with an indication of the amount of live cells which must be provided until the end of shelf-life (with an error of $\pm 0.5 \log_{10}$). The experimental ice cream fully satisfied this condition, as the concentration of *B. animalis* subsp. *lactis* maintained the level of about $7 \log_{10} \text{CFU g}^{-1}$ up to 120 days of storage.

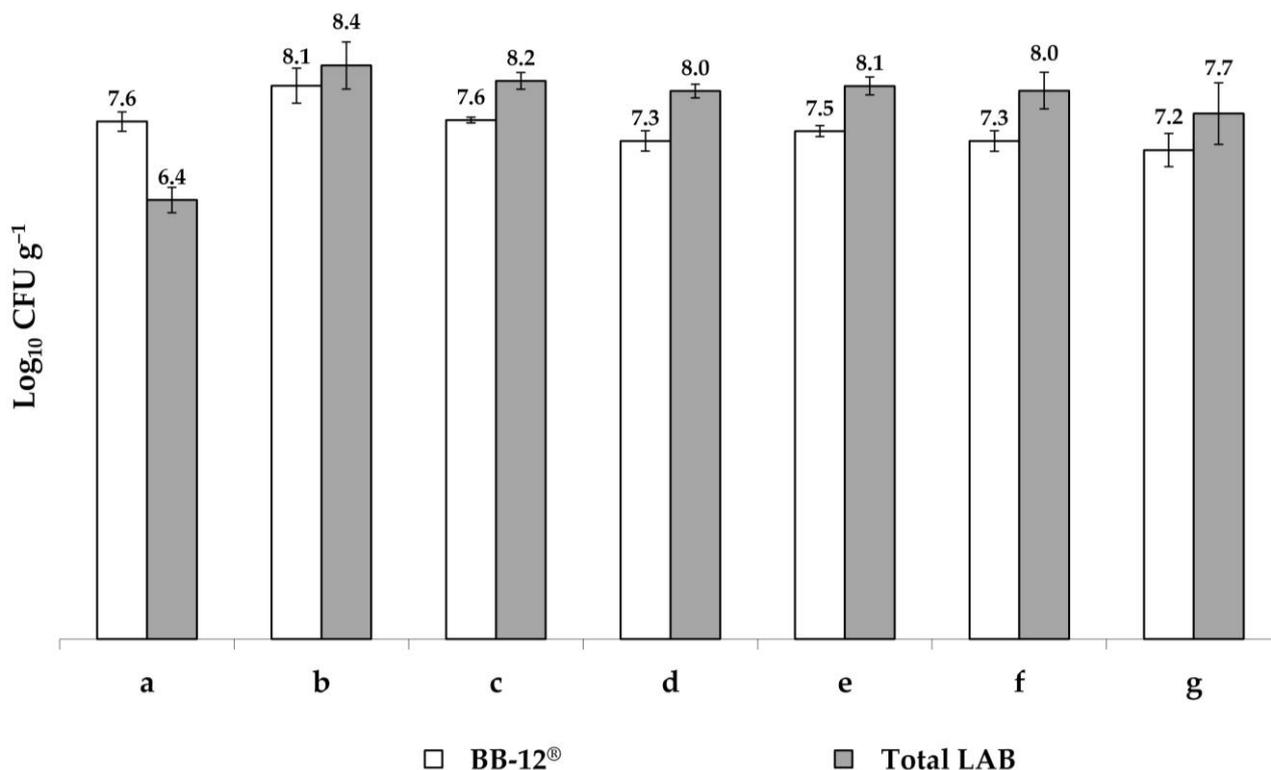


Figure 4. Viable counts ($\log_{10} \text{CFU g}^{-1}$) of *Bifidobacterium animalis* subsp. *lactis* (BB-12[®]) and total lactic acid bacteria (LAB) in inoculated enriched milk (EM) (a), fermented functional product (FFP) after 24 h from production (b), and sheep’s milk ice cream after 1 (c), 30 (d), 60 (e), 90 (f), and 120 (g) days of frozen storage ($-20 \pm 2 \text{ }^\circ\text{C}$). Error bars indicate standard deviations. Different letters within the histograms indicate significant differences between sampling points ($p < 0.05$).

3.3. Mass Balance Determination

Figure 5 shows the mass balance related to the production of 100 kg of ice cream, found by taking into account the composition of the ingredients given in Table 2 and the composition of ice cream reported in Table 4. Ingredients derived from sheep’s milk (SMR, MWP, and cream) and non-dairy ingredients (algal oil, inulin, locust bean gum, and sweeteners) are reported in the mass balance. The implemented ice cream production process was very efficient in recovering the natural components of milk (fat and protein). In fact, as reported in Figure 5, only a small amount of cream (about 0.1 kg) is surplus to the ice cream production needs, with respect to the use of about 70 kg of whole sheep milk and 144 kg of sweet sheep whey. In addition, the UF technology applied to this production process (Figure 1) allows the recovery in SMR and MWP of most of the proteins available in milk and whey, respectively. In fact, permeate is the only by-product of the UF process and consists mainly of water (about 94%) and part of the soluble elements contained in milk and whey (lactose, minerals, non-protein nitrogen, etc.). Since 30 kDa membranes were used in the present study, assuming an industrial application of the developed process, the use of UF membranes with a lower cut-off would allow for even more efficient protein recovery. Moreover, the whey resulting from cheesemaking is usually processed into whey cheeses in some countries, such as *Ricotta* in Italy, but a significant portion of this by-product is still used as animal feed or discarded as waste [17]. Therefore, the production of MWP

provides an alternative way of valorizing whey. MWP can also be used as an ingredient in the production of other foods in the dairy and non-dairy fields.

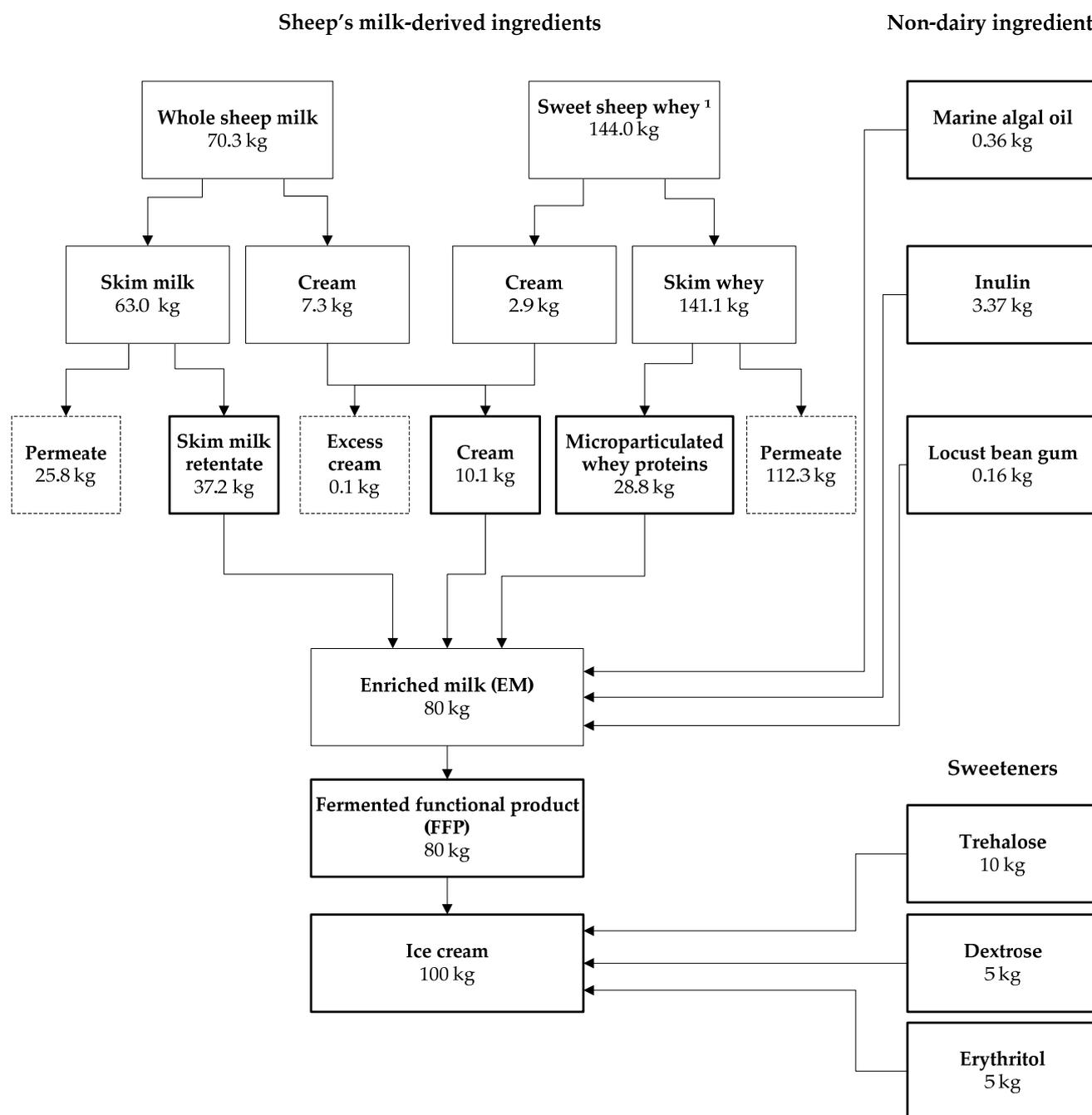


Figure 5. Mass balance for the production of 100 kg of ice cream. ¹ Amount of sweet sheep whey obtained from the cheesemaking process of about 250 kg of whole sheep milk.

4. Conclusions

In this study, we proposed a new protocol for manufacturing a functional and probiotic ice cream with specific nutritional characteristics consisting mainly of ingredients derived from sheep's milk. The obtained ice cream met some of the nutritional claims of the EU reference regulations along with the requirement of "probiotic food" according to the Italian Ministry of Health's guidelines on probiotics. These properties were retained during the frozen storage period of the ice cream. In addition, the process developed involved the concentration of milk and whey through the application of UF, a technique that allows for

the total recovery of the protein fraction available in these raw materials. In this way, it was also possible to valorize and recover a by-product of sheep's milk cheesemaking, that is, sweet whey in the form of MWP. Considering the outcomes of the present work, we clearly evidenced that an ice cream made from sheep's milk may be a suitable matrix for delivering health-promoting ingredients and probiotics. Further studies will be necessary to assess the adaptability of the studied process to an industrial scale.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/dairy5010011/s1>, Table S1: Physico-chemical and nutritional parameters of sheep's milk ice cream during frozen storage (-20 ± 2 °C) for up to 120 days (mean \pm SD).

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