Chapter 4

Probiotic Butter

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Abstract

In recent years butter has seen a growth in its consumption because of the understanding of the physiological effects of dairy fat. The small-scale batch production of butter depends on the operator's hands-on skill and experience about cream aging, ending point of churning, and other parameters at each of the manufacturing stages. Large-scale processes are automated and allow better control to produce butter with constant characteristics. While the principles of butter making have not changed significantly over many decades, with greater understanding, control of key parameters during cream preparation and processing conditions have improved. In recent years there has been a lot of products from butter post-churning, mixing new ingredients like probiotics, flavors, and spices that will be beneficial in attracting and engaging consumers. Butter has shown to be a good matrix for adding probiotics and maintaining its viability throughout storage due to the fat protection effect. There are several ways of adding probiotics in butter, such as in microencapsulated form, together with traditional starters during cream fermentation or during the working step. Thus, this chapter is a practical guidance for probiotic butter manufacture.

Key words Milk fat, Butter, Probiotic, Functional food, Health

1 Introduction

The amount of fat in milk and the fatty acids content can be influenced by many parameters such as the diet of the cows, number of pregnancies, animal health, breed, and stage of lactation. The type of feed or pasture consumed by the animal has been used to modify the fatty acid profile of dairy fat to obtain beneficial nutrients as the increased polyunsaturated content. However, all changes in the percentage and types of fatty acids in milk can influence the processing and characteristics of manufactured products. The lipid fraction of milk corresponds to 4.2% of dairy solids and is composed of about 98% of triglycerides and other components in smaller amounts, such as phospholipids, sterols, lipopro-

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teins, and vitamins. It is important to remember that milk is a liquid emulsion of oil in water, which is very nutritious because it is a source of several essential compounds [1].

The butter production has grown worldwide [2] while the price of butter has doubled in the international market and its consumption grows by about 4% per year [3]. Around 40% of the consumers consulted on the research stated that they started to buy more butter because it is a healthier option [3]. This change in demand for butter is in function of changes in consumer perceptions of dairy fat. While in the past dairy fat has been associated with an increased risk of cardiovascular disease, there is growing evidence to suggest that regular consumption of dairy products with a regular fat content is not associated with an increased risk of these diseases [4]. In addition, some benefits of consuming dairy products with a regular fat content have been demonstrated in terms of the presence of bioactive nutrients and anti-inflammatory properties [5]. Butter is a complex of at least 400 types of fatty acids, the consumption of which, combined with a balanced diet and a healthy lifestyle, can protect against certain types of cancer, and reduce the risk of cardiovascular disease. Due to this new perspective on milk fat and its effect on human health, research in ruminant nutrition has generated results that demonstrate the production of bovine milk from tropical grasses can increase the level of beneficial fats. One of these fatty acids, known by the acronym CLA (conjugated linoleic acid), from its chemical name, has proven anticarcinogenic properties. Several works carried out by [3] have been demonstrating that cows fed with fresh elephant grass increased the CLA content in the milk, therefore, also in the butter.

Butter is one of the oldest dairy products that is still being made. Butter is obtained by concentrating milk fat in the form of cream, which is then churned until the oil-in-water emulsion is converted to water-in-oil emulsion. There are criteria for the composition of the product classified as butter. According to Codex Alimentarius [6] butter is a fatty product derived exclusively from milk that must contain at least 80% fat, a maximum of 16% moisture, and 2% defatted milk solids (proteins, lactose, and minerals). USDA [7] also defines this minimum fat content, in addition to other parameters, and indicates a three-level classification (AA, A, and B) based on the sensory attributes of the butter. In Brazil, there are also standards defined in the legislation to regulate the minimum fat content in butter, in addition to the maximum moisture content, sodium chloride and acidity, among other physical–chemical and microbiological parameters [8, 9].

Butter can be produced from sweet cream or fermented cream, with and without salt. It is used in several culinary preparations, such as an ingredient in the chocolate, confectionery, and bakery industries, in addition to the dairy industry. In cream fermentation, different probiotic or non-probiotic lactic ferments can be used.

The ferment can be defined as a microbial preparation containing a large number of cells of at least one microorganism that is added to a raw material (in this case, cream) to produce the fermented food and it can speed up and control the fermentation process [10].

The group of lactic acid bacteria (LAB) plays an important role in this process and has a long history of safe application and consumption in fermented foods and beverages. These bacteria generate rapid acidification of the raw material through the production of organic acids, mainly lactic acid, but also acetic acid, ethanol, aromatic compounds, bacteriocins, exopolysaccharides, and several important enzymes. With this, these bacteria increase shelf life, improve texture, and contribute to pleasant sensory characteristics in the final product [10]. Most of the lactic acid bacteria belonged to the genus *Lactobacillus*, which was recently reclassified [11]. The member species were distributed in 25 genera, including those that remained in the original genus *Lactobacillus.* Thus, species of probiotic lactic acid bacteria, or not, applied in butter studies may have been reclassified to other genera and have their gender designation changed [12]. Several LAB strains are recognized as probiotics. Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [13] indicating that viability is a necessary requirement to ensure the expected benefits. In turn, paraprobiotics, defined as non-viable cells, and postbiotics, which are substances generated by bacteria, respectively [14–16], have been studied and the health benefits are not all necessarily related only to viable cells. Thus, probiotics, but also paraprobiotics and postbiotics, can be present in butter, adding additional beneficial characteristics to the bioactivity of naturally present nutrients such as CLA, phospholipids, and vitamins. In this sense, this chapter's purpose is to describe the butter manufacturing and possible steps where probiotics can be added during the process.

2 Material and Methods

There are different butter varieties mainly involving the presence or absence of salt and primary fermentation of the cream. The type of ferment used in this fermentation will influence the flavor of the final butter (*see* **Note 1**). There are also different methods of making butter which include emulsification and the more well-known churning method. Figure 1 shows the process of making salted butter from cream using churning method. In addition, the main stages in the manufacture of butter will be presented so it can be verified in which stages the probiotics can be added.

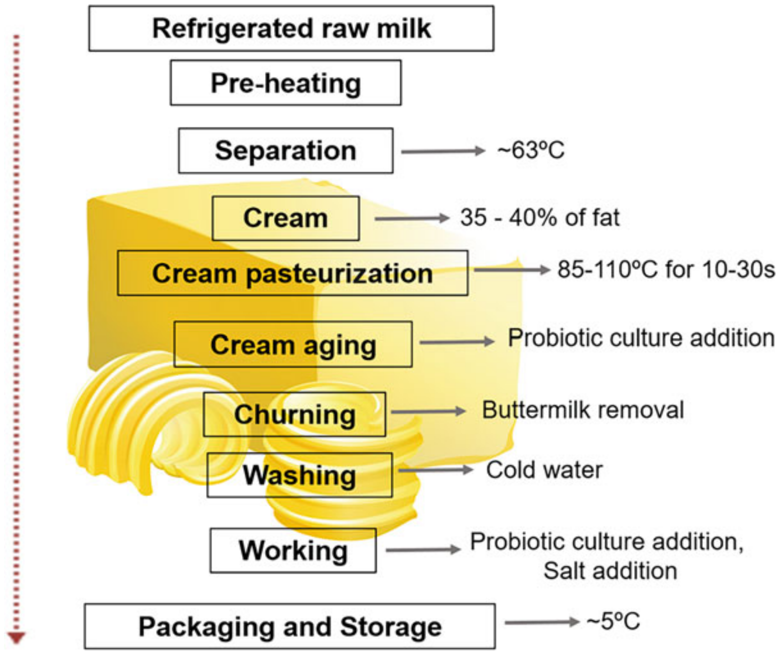

Fig. 1 Steps of the process of making salted butter

2.1 Cream Preparation

The cream can be extracted from milk kept at rest if it has not been treated in the double-piston homogenizer, as it separates naturally due to the fat particles being less dense and floating in the upper portion of the tank. It is more applied, however, the separation using centrifugation with the milk heated above 40 °C so that less damage occurs to the milk fat globule membrane (MFGM) since the fat will be in liquid form. An optimal temperature condition is 63 °C, for a cream with 35 to 40% fat or 45 to 48%, depending on the equipment and process [1]. There is a batch equipment called skimmer used to separate the cream from the milk and continuous separators for larger industrial volumes. After separating the cream, it undergoes thermal treatment in plate heat exchangers with time and temperature binomials between 85 and 110 °C for 10 to 30 seconds to eliminate pathogenic microorganisms and reduce the counts of deteriorating microorganisms (*see* Fig. 1). At this stage, vacuum can be applied to remove volatile compounds with unpleasant odors that can influence the sensory characteristics of the final product. After the thermal treatment, the cream is cooled to 4–5 °C so the maturation stage takes place during a minimum period of 4 hours or "overnight" when the milk fat crystallizes (*see* **Note 2**).

For the cream maturation, there are different combinations of time/temperature that influence the shape of the crystals that appear in the cream and it will affect characteristics as consistency and spreadability of the butter after churning. The greater efficiency of the different cream maturation time and temperature binomials are related to factors such as the season of the year and the levels of unsaturated fatty acids present [17, 18] (*see* **Note 3**). Slow and rapid cooling of the cream can result in rheological properties and crystals with similar polymorphic forms (α- and β'-), but with differences in their microstructure (*see* **Note 4**). Unlike butter produced from slowly cooled cream, rapid cooling results in butter with more uniformly sized crystals (*see* **Note 5**). When the amount of low melting fat is reduced (iodine number less than 35 I_2/100g) the butter will have a firm texture if the cream is cooled in stages. In these cases, the application of the "cool/heat/cool" procedure called "Alnarp method" can be beneficial for the butter final texture. Cooling to 8 °C after heat treatment, keeping at this temperature for 2 hours, heating to 20 °C, keeping for 2 to 3 hours at this temperature, and finally cooling to room temperature (*see* **Note 6**). Finally, the cream is cooled down to the churning temperature. There are several temperature sequences for chilling the cream before churning to separate the butter grains. For example, a combination is cool to 8 °C, raise to 18 °C and cool to 12 °C or cool to 8 °C, raise to 22 °C and cool again to 12 °C (*see* **Note** 7).

One of the ways of adding probiotics to butter is through their direct inoculation into the cream with or without a fermentation step (*see* **Note 8**). Several probiotic strains are available for use as DVS (Direct vat set) inoculums in butter production. The main cultures already used in probiotic butter and cell viability after storage are summarized in Table 1. The cell amount to be added into the butter varies between different strains to ensure the health benefits. The amounts of cells added by [19, 20], and others are adequate to keep cells active until the end of storage.

2.2 Batch Butter Making Process

1. The pre-treated cream is transferred to the mixer (*see* **Note 9**) where it is stirred at high speed to break the emulsion, and the grains of butter emerge as the drained buttermilk separates.
2. The temperature range for churning is 8 to 12 °C.
3. After the emulsion has broken, the buttermilk is drained from the tank and can be filtered to remove fat lumps.
4. Add fresh water to the churner and whisk to wash the grains and remove buttermilk residues until the water runs clear.
5. The working (mechanical treatment) of the butter takes place at low speed with the tank valve open to drain the buttermilk expelled from it. When the amount of water is sufficiently low, the valve is closed and, if necessary, salt is added.
6. As the working time is longer in the batch process compared to the continuous one, the salting process can take place by dry, wet, or brine salting (*see* **Note 10**).
7. When the butter appears to be dry, the working is stopped and the moisture content is measured and adjusted, continuing the mechanical work until all the free water has been absorbed.
8. Then, the butter is packaged.

Table 1
Information about probiotic strains added to butter formulations

Probiotic strain	Inoculation dose (log CFU/g)	Inoculation method	Inoculation temperature (°C)	Storage (days)	Probiotic viability after storage (CFU/g)	Reference
Lactobacillus acidophilus La-14	10.63	The microorganisms were encapsulated using the technique of extrusion and ionic gelation. The encapsulating matrix used was low viscosity sodium alginate at a concentration of 10 g/L, dripped onto the covering material, 0.1 Mol/L calcium chloride dihydrate. The capsules had an average diameter of 1.8 mm. The culture was lyophilized.	10.0	60	8.90	[19]
Bifidobacterium animalis subsp. *lactis* ATCC 27536 *Lactobacillus acidophilus* ATCC 4356	8.98	The spray-dried formulation was prepared by a combination of probiotic microorganisms, in a conjugated whey protein hydrolysate (WPH10-maltodextrin) matrix (1:1). Pure freeze-dried probiotic cultures were obtained from ATCC. Fresh cultures were obtained after two transfers in de man, Rogosa, and Sharpe broth (MRS) supplemented with 0.05% (w/v) L-cysteine at 37 °C for 72 h, under anaerobic conditions. Propagation of the cultures was continued, and the cells were harvested in their late log phase by centrifugation at 7000 × g for 10 min at 4 °C. the cell pellets were washed twice in phosphate-buffered saline (PBS) and suspended to achieve cell suspensions of 10 log CFU/mL.	–	–	5.09–8.22	[20, 21]
Lacticaseibacillus casei *Lactiplantibacillus plantarum* subsp. *plantarum* *Lacticaseibacillus paracasei* subsp. *paracasei* Lyofast CPR1	8.2–8.7	The probiotic culture was pre-activated in pasteurized milk for 15 h at 35 °C, with an initial concentration of 0.5% (w/v) of milk. Then, the pre-activated inoculum, with a probiotic level of 8.2–8.7 log CFU/mL was used to inoculate the cream applied to produce probiotic butter samples.	19.0	1.42	>6.0	[22]

Lacticaseibacillus casei LAFTI L 26; CSL3	7.0	After obtaining butter, the formulations with probiotic bacteria were supplemented using 0.1% of each probiotic (7 log CFU/g of butter).	4.0	90	4.81–5.80	[23]
Lactobacillus acidophilus La-5	6.0–8.0	The culture was used for fermenting cream. It was added to the cream at fermentation temperature, which varied according to the characteristics of the seasons. On I group the fermentation took place at 30 °C and physical maturing at 7 °C. on II group the fermentation took place at 37 °C and physical maturing at 7 °C. on III group to simulate autumn-winter season cream fermentation took place after physical maturing, at 20 °C. to simulate spring-summer the cream fermentation took place before physical fermentation, at 20 °C. IV group introduced the fermenting cultures in butter kernel. The samples were kept at 9 °C for 3 days for the increase of acid-creating activity.	20.0–37.0	42	>6.0	[24]
Bifidobacterium bifidum ATCC 29521 *Lactobacillus acidophilus* ATCC 4356	6.60–6.66	The cream was pasteurized, cooled to 18–20 °C, and divided in three parts for each replicate. All batches were inoculated with direct vat set culture (freeze-dried) at a level of 15 g/500 L.	18.0–20.0	60	6.57–6.68	[25]
Bifidobacterium lactis B100.6	>9.0	The butters were prepared in seven repetitions by churning the pasteurized cream (30% (v/v) fat) with the probiotic bacteria. The prepared butters were stored at 6 °C for 4 weeks.	–	28	>7.4	[26]

2.3 Continuous Butter Making Process

Most continuous butter production processes are based on the "Fritz method," the German scientist who designed the first equipment for this purpose. The current equipment have some points of difference in relation to the originally built one, but follow its operating principles containing a churning section, separation section, and working sections containing a vacuum chamber (*see* **Note 11**). At the output of the equipment, the butter is packaged.

2.3.1 Churning

The heat-treated cream is transferred to the churning section via a pump. There are studies carried out on the production of butter with probiotics that involve the addition of microencapsulated probiotics to the butter in the churning stage (Table 1). In this step, the cream is churned in a horizontal cylinder with an adjustable speed rotary beater. The phase inversion occurs in a few seconds and the speed of the beater regulates the size of the grains formed, which influences the fat loss to the buttermilk (*see* **Note 12**). Churning temperature is particularly important as phase inversion will only take place if there is enough liquid fat (*see* **Note 13**). In general, the churning temperature is around 10 to 12 °C. The fat content in the buttermilk should not be higher than 0.3 to 0.5% if there is an optimized churning (*see* **Note 14**).

2.3.2 Buttermilk Remotion

After phase separation, the buttermilk and buttermilk mixture are carried from the churning section to the separation section. This section consists of a horizontal, slow-rotating sieve drum with adjustable speed where the butter grains are retained, while the buttermilk passes through a finely meshed wire screen (*see* **Note 15**).

2.3.3 Working

The butter grains, now gathered in larger lumps, are then transferred into the first working section in which a pair of parallel contra-rotating endless screw transports the butter forward and "squeeze" most of the remaining buttermilk out of the product (*see* **Note 16**). This step allows for the water content to be adjusted so that probiotic culture addition and/or salting can be carried out if required without exceeding the limit of 16 g/100 g water in the final product. If the cream used in butter manufacturing has not been inoculated with probiotics, they can also be added at this stage (Table 1). Some butter machines are equipped with a couple of working units consisting of perforated plates interspersed with mixing vanes where the intensive working of the butter is performed. Another approach is to install a medium-shear mixer after the first working section for the same purpose. From the first working section the butter is conveyed either directly or indirectly via a butter pump to the second working section, where the final working takes place (*see* **Note 17**). The working temperature must be kept low (14–16 °C), as this temperature determines the size and the composition of the continuous fat phase, and thereby the extent of the three-dimensional crystal network (*see* **Note 18**).

2.3.4 Salting

Salt can be added in the last part of the first working section by a dosage pump with adjustable capacity and mixed into the butter by the working units. Subsequent working of the butter is accomplished in a short time that is insufficient for dissolving large salt grains (*see* **Note 19**).

3 Notes

1. The buttermilk resulting from the production of butter made with fermented cream is acidic, which restricts the possibilities of application compared to non-fermented one.
2. The temperature of the cooling step can be adjusted to achieve those apropriated to the multiplication of the probiotic or non-lactic ferment that wants to add. Instead, the probiotic can be added as an ingredient (without multiplying in the cream or butter) in the kneading step.
3. Studies such as those by [27] found that butter produced with sweet cream (without fermenting) from milk from cows raised on pasture had a softer texture and different melting properties than that produced from milk cream from stabled cows fed with feed. Milk from cattle on pasture has advantages in terms of increasing the content of polyunsaturated fatty acids such as omega 3, vaccenic acid, and CLA while reducing the levels of omega 6 and palmitic acid [28]. During the summer when cows are on pasture, dairy fat is less saturated and softer than winter cream, which contains higher levels of saturated fatty acids, resulting in harder fat. In general, the rapid cooling of the cream leads to the formation of crystals with low levels of liquid-free fat, which makes the texture of the butter harder and with lower spreadability characteristics. This characteristic can be improved with the method of maturation of the cream where it has its temperature increased in the initial stage and then reduced in two distinct stages. This technique involves cooling the cream to 20 °C, after heat treatment, keeping it for a few hours at this temperature, cooling it to 16 °C, keeping it for 2 to 3 hours, and, finally, cooling it down to the churning temperature. This maturation method leads to the separation of triacylglycerols with a high melting point from those with a low melting point, forming crystals that have laminated structures with the first ones in the center and layers of the second ones that are formed with the decrease in temperature. Butter produced with cream cooled in stages has a higher liquid fat content than that produced with cream cooled quickly and therefore has a softer and more spreadable consistency.

4. There may be differences in the polymorphism of milk fat crystals as a function of the cream's cooling speed [29]. It is noteworthy that the polymorphism is the phenomenon in which milk fat (solid) crystallizes in more than one form, presenting three-dimensional structures of crystalline packaging, giving it a distinct property.
5. Rapid cooling of the cream does not cause changes in the rheological profile and microstructure of the butter. However, slow cooling of aged cream will result in butter with a firm and brittleness texture, due to the formation of a denser crystal network. Butters produced from unripened creams are mainly formed by α- and β'- crystals and by few crystals in the β- form. Maturation results in a transition of crystals from the α- to β'-form and to the β- form which is formed by the recrystallization of fat. This alteration in the structure of the cream facilitates the next stage of obtaining butter, which consists of churning the cream [30]. The chemical composition and the mechanical treatment employed in obtaining butter can also affect the fat crystal network. Fat crystals are present both in the continuous fat phase and within milk fat globules [29].
6. This method favors the formation of crystals with a laminated structure, increases the liquid fat content, and reduces the firmness of the butter compared to the "heat–cool–cool" method. If the objective is to churn sweet cream, a frequently used cooling procedure is to start by chilling at 8 °C for at least 2 hours, regardless of the dairy fat composition. The cream is then heated to the temperature indicated by the composition of the fat considering the iodine index, and the higher this index, the lower the intermediate heating temperature in the cream maturation process.
7. These temperature variations are made to achieve adequate crystallization of the milk fat and to facilitate and/or optimize the separation of the butter grains. Different temperature combinations can be suitable for creams produced in summer or winter.
8. The addition of probiotics has also been widely discussed in terms of food safety since some strains can be used as protective cultures (bioprotection). This characteristic is associated with some species of *Lactobacilli*, *Streptococci*, *Enterococci*, *Lactococci*, and *Bifidobacteria* having a long history of safe use, proven antimicrobial properties, ability to naturally dominate the microflora, and occupy the ecological niche during the storage of products [31].
9. The process and equipment used to manufacture butter can be dependent on many factors, including production volume. For small-scale operations and artisanal production, the butter maker or churn is better adaptable and requires less investment.

In general, 100 L of raw milk yields 4 to 5 kg of butter. There are butter churns of different sizes ranging from those for around 5 kg of cream at 40% fat to those for processing several hundred kilos of cream. The typical mixer consists of a horizontal stainless-steel barrel that rotates around a shaft or a cylindrical tank with a rotating rod or paddle inside.

10. In wet salting, salt is moistened with water before being added to butter as a dough. This method leads to the rapid solubilization of salt in butter. Salting in brine requires the amount of water in the butter to be low. In this stage of working, it is common to use vacuum (20 kPa). After adjusting the moisture content, working continues, but with less vacuum intensity, as excess can lead to the migration of liquid fat and the presence of free oil droplets in the butter.
11. The capacities of these continuous equipment range from 500 to 15,000 kg/h.
12. Both very high and very low speeds will increase fat loss. The general rule is that the lowest speeds should be used and will result in grains with a diameter of 2 to 4 mm. The optimum churning speed depends on the fat content and temperature of the cream. Lower speeds are used for higher fat content and higher temperature.
13. If the liquid fat content is very low, high rotational speeds are needed to increase the temperature of the cream until there is enough liquid fat. However, raising the temperature by mechanical agitation consumes a lot of energy. However, if the churning temperature is too high, there is a loss of fat in the buttermilk and an increase in butter moisture.
14. If the sweet buttermilk (from unfermented cream butter) is to be reused in other dairy products such as cheese or powdered milk, the fat present in it will also be reused.
15. It is especially important to keep the temperature of the butter low through the entire process, and an efficient way to do that is to cool the butter grains in the separation section before they gather into larger lumps. This cooling can be achieved by spraying the butter grains either with chilly water or even better with recirculated cooled buttermilk, which will not cause dilution. Another way of controlling the temperature of the butter grains is circulating chilled water in the jacket of the separating section, but this is not as efficient as spraying the butter grains.
16. Different parameters influence the water content such as low-fat content of the cream, increased churning temperature, inadequate churning speed, size of butter grains, and inadequate working temperature. It should be noted that the interaction between these parameters is very strong, so careful adjustment of their effect must be done.

17. It is important that the working intensity is high enough to ensure a homogeneous texture in the butter. An intensity that is too low will result in loose or free moisture in the product, and an intensity that is too high will result in a greasy and sticky consistency.
18. The working temperature can be controlled by circulating chilled water in the jacket of the two working sections.
19. Undissolved grains will attract moisture during storage, which will result in free water droplets in the butter and reduced keeping quality. It is therefore necessary to use very fine-grained salt (average particle size around 15 mm), which could be added as a suspension (e.g., 100 g salt in 100 g water). It is critical that the salt is not contaminated, especially with copper and iron, as this will reduce dramatically the oxidative stability of the butter.

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