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Innovative Dairy Products Development Using Probiotics: Challenges and Limitations

Esteban Boza-Méndez, Rebeca López-Calvo and Marianela Cortés-Muñoz

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1. Introduction

Probiotic foods are food products that contain a living probiotic ingredient in an adequate matrix and in sufficient concentration, so that after their ingestion, the postulated effect is obtained, and is beyond that of usual nutrient suppliers (Saxelin et al., 2003).

Probiotic delivery has been consistently associated with foods (especially dairy). However, nowadays there is an increasing trend toward using probiotics in different food systems despite its original sources and even as nutraceuticals, such as in capsules. According to Ranadheera et al. (2010) this changing trend in delivering probiotics may lead to a reduction in functional efficacy due to the exclusion of the potential synergistic effect of the food. Selection of the adequate food system to deliver probiotics is a vital factor that should be considered when developing functional products.

Foods are carriers for the delivery of probiotic microorganisms to the human body. The growth and survival of probiotics during gastric transit is affected by the characteristics of the food carriers, like chemical composition and redox potential. Same probiotic strains could vary in functional and technological properties in the presence of different food ingredients or in different food environments (Ranadheera et al., 2010). Thus, variation between different strains' behavior in different conditions would be expected.

Dairy products have been considered as a good carrier for probiotics since fermented foods and dairy products have particularly a positive image. A major advantage is that consumers are already familiar with them and many believe that dairy products are healthy, natural products. Table 1 shows some of the beneficial physiological properties that have been associated with milk components.

Others advantages of dairy products as vehicles for probiotics are that fermentation acts to retain and optimize microbial viability and productivity, while simultaneously preserving

the probiotic properties. Consumers are familiarized with the fact that a fermented dairy product contains living microorganisms, and they are also able to protect probiotics through the gastrointestinal transit. This protection comes as a result from the buffering capacity that increases survival chances. The refrigerated storage recommended for these products helps to stabilize probiotic bacteria (Ross et al., 2002; Stanton et al., 2003).

Ingredient	Source	Claim areas examples
Minerals	Calcium Casein peptides	Optimum body growth and development, dental health, osteoporosis
Fatty acids	Conjugated linoleic acid	Heart disease, cancer prevention, weight control
Prebiotics/carbohydrates	Galactooligosaccharides Lactulose Lactose	Digestion, pathogen prevention, gut flora balance, immunity, lactose intolerance
Probiotics	Lactic acid bacteria Bifidobacteria	Digestion, immunity, vitamin production, heart disease, antitumor activity, remission of inflammatory bowel disease, prevention of allergy, alleviation of diarrhea
Proteins/Peptides	Caseins, whey proteins, immunoglobulins, lactoferrin, glycoproteins, specific peptides	Immunomodulation, body growth, antibacterial activity, dental health, hypertension regulation (angiotensin inhibitors)

Table 1. Selection of ingredients and claims associated with functional dairy foods (adapted from Shortt et al., 2003).

Besides, according to Shortt et al. (2003) significant opportunities exist for dairy products whose functionalities have widespread appeal. This means that a product encapsulating the needs of every member of a family is extremely likely to be a success. The broad potential interest in functional dairy products is an important market advantage. Functional dairy products that affect conditions such as osteoporosis, heart disease and cancer are attractive specifically to adults, while products with claims on tooth health, bone health and immunity

appeal to adults and children in a similar way. The possible range of sensory characteristics with dairy ingredients also allows the production of diverse textures and aromas, adding another benefit.

Current knowledge on probiotics support a number of potential health benefits. They help to maintain good balance and composition of intestinal flora increasing the ability to resist pathogens invasion and maintain the host's well being. Reduction of blood pressure, cholesterol and/or triglycerides levels, reduction of lactose intolerance problems, immune system enhancement, anti carcinogenic activity and improve nutrients utilization are well described in literature. The use of probiotics for preventing and treating illnesses related to gastrointestinal, respiratory and urogenital tracts have been studied. They have been widely used in therapeutic applications as constipation, diarrhea control, bowel syndrome, control of inflammatory processes, prevention of eczema, osteoporosis and food allergy (Aureli et al., 2011; Ranadheera et al., 2010; Rastall et al., 2000; Vasiljevic and Shah, 2008).

The most common probiotic strains used in dairy foods belong to *Lactobacillus* (*L. acidophilus*, *L. johnsonii*, *L. gasseri*, *L. crispatus*, *L. casei/paracasei*, *L. rhamnosus*, *L. reuteri*, *L. plantarum*) and *Bifidobacterium* (*Bifidobacterium lactis*, *B. bifidum*, *B. infantis*, *B. breve*, *B. animalis*, *B. adolescentis*) genera (Saxelin, 2008).

In Europe EFSA is responsible for the evaluation procedure that accepts or rejects applications for health and nutrition claims on food and beverages (EU Regulation 1924/2006). In recent years this European authority has rejected probiotic health claims adducing that there is no sufficient scientific evidence for the declared beneficial effects. This situation obliged food companies from probiotic industry to perform new clinical studies trying to generate solid scientific evidence for specific probiotic strains and health benefits for submission to the EFSA approval. Consumers still identify probiotic dairy products as healthy despite of this situation.

According to Shortt et al. (2003), the dairy industry is in an excellent position to develop and exploit the functional food market. These products are significant players in the functional food market; for example, they were estimated to account for approximately 60% of functional food sales in Europe by 2000. In 2008, consumers market for probiotic foods was over 1.4 billion Euros in Western Europe, and their annual sales growth was forecast at 7-8% for a 5 year period (Saxelin, 2008). Developing new technologies and new functional dairy products is nowadays relevant.

This chapter focuses on the development of innovative probiotic dairy products considering limiting factors for the survival of probiotics, techniques for the addition and protection of these microorganisms, the quality modifications of final products, the application of sensory analysis and finally how to determine probiotic populations in dairy products.

2. Limiting factors for the survival of probiotics

The food industry has an important market created by the incorporation of probiotic microorganisms into products. However, the addition of this kind of cultures in a food

product could be difficult because of the bacteria conditions required in order to survive or to grow in food. Some authors have suggested that more research regarding the challenges that represent incorporating a probiotic culture is necessary because most of the information available is focused on health benefits of the probiotics (Champagne et al., 2005). Evaluation of technological traits such as growth and survival in milk-based media and during product manufacture and shelf life can be important considerations for the selection of strains for food applications (Stanton et al., 2003).

Successful marketing of probiotic products require a minimal amount of viable probiotic cells guaranteed throughout shelf life. To obtain the beneficial effects associated with this type of food, the bacteria must remain viable and in a proper concentration when the host consumes the product. This fact could determine the shelf life of the developed product, because the survival of the probiotics depends on many factors in the food (Talwaker and Kailasapathy, 2004).

Champagne et al. (2005) list seven factors that culture distributors and food manufacturers need to consider in order to add probiotics successfully into products. These factors include: type and form of the culture, the amount of bacteria required to obtain a beneficial effect, toxicity, production process effect on viability, the determination of probiotic cells used in the product, stability during storage and possible changes in sensory properties of the food.

To use a probiotic strain compatible with food production processes technologies is ideal. This means that the elaboration, distribution and commercialization of the product should not have any effect in the viability of bacteria. For example, in the specific case of dairy products, the probiotic should have the capacity to grow in milk (or dairy) but also have a low metabolic activity at low temperatures, in order to guarantee the proper amount of bacteria in the product with no significant changes in quality during shelf life. However, probiotic bacteria generally do not grow well in milk and are adversely affected by storage conditions in some dairy products (Champagne, 2008).

The compatibility and adaptability between the selected strain(s) and the food used as carrier is fundamental, and may represent a significant technological challenge since many probiotic microorganisms are sensitive to the concentration of oxygen, carbon dioxide and salt, high and freezing temperatures and acidic environments (Corrales et al., 2007; Cruz et al., 2009a; Fortin et al., 2011; Talwaker and Kailasapathy, 2004).

Since many dairy products are fermented, it is common to found levels of acidity that may affect the probiotics viability. Numerous studies have reported large losses in viability during storage of fermented milk, yogurt and alike (dairy products known as acid). It is believed that the pH is actually a critical stress factor in the probiotics viability through storage, although there are variations between species and strains for the survival in acidic environments (Roy, 2005). Donkor et al. (2006) evaluated the effect of the acidity of yogurt on the viability of some *Lactobacilli* and *Bifidobacteria* strains. They concluded that *Lactobacilli* strains showed a good cellular stability maintaining constant concentration throughout the storage period regardless of final pH. On the other hand, the cell counts of *Bifidobacteria*

decreased by one log cycle at the end of the storage period, due to the high production of organic acids.

Boza et al. (2010) studied the effect of adding *Lactobacillus paracasei* subsp. *paracasei* to a semi hard cheese. Figure 1 presents the pH variation found in cheese during ripening at controlled conditions of 12°C and 85% RH. An important initial decrease is observed (day 0 to 13), pH values tend then to stabilize during cheese ageing process.

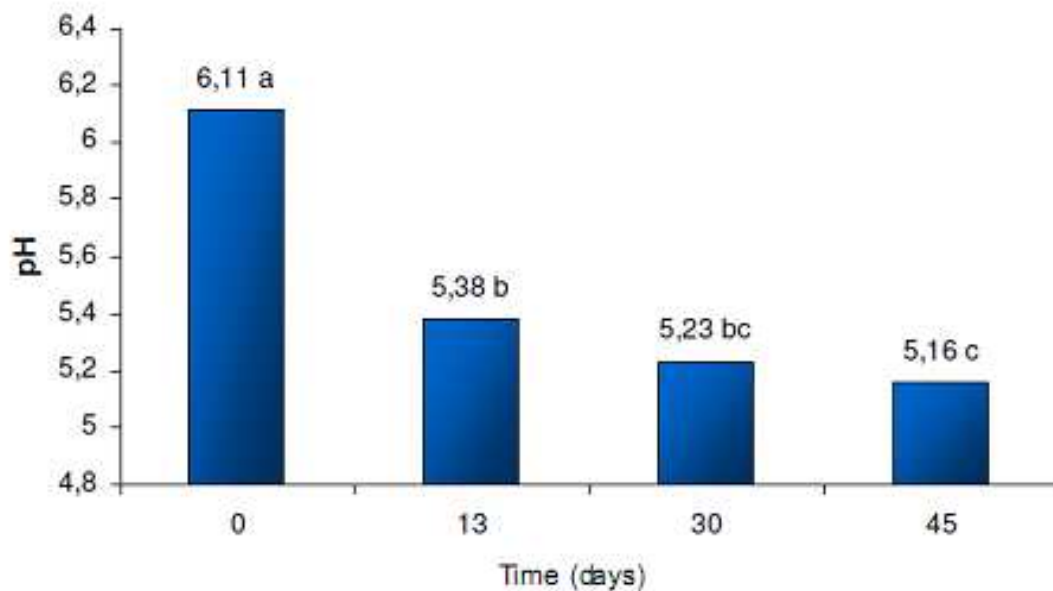


Figure 1. Values of pH for semi hard cheese with *Lactobacillus paracasei* subsp. *paracasei* aged for different periods at 12°C and 85% RH [18]. Different letters in the columns indicate significant differences ($P<0.05$).

Corriols (2004) studied the survival of *Bifidobacterium lactis* in a light sour cream (12% fat, w/w) during 40 days at 5°C. In this study, product behavior considering pH of a regular sour cream inoculated with a starter culture mix of *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactococcus lactis* subsp. *diacetylactis* and a probiotic sour cream (starter culture + *Bifidobacterium lactis*) was performed. Table 2 presents pH values for probiotic light sour cream during storage time at 4°C. Evaluating pH at day 8, 15 and 22 showed that there was no significant difference ($P>0.05$) in these values.

Storage time (days)	pH
0	4.51 a
8	4.37 b
15	4.36 b
22	4.39 b

Table 2. Variation of pH for 12% fat (w/w) sour cream with *B. lactis* during refrigerated storage at 4°C. Average of 5 measurements of three independent experiments. Values followed by the same letter within a column are not significant at $P<0.05$.

Since there was a slight product post-acidification (see table 2) *B. lactis* survival was possible as acidity could be a cause of probiotics viability loss in fermented products. No significant difference ($P>0.05$) was found in probiotic and regular sour cream pH values. Finally, this study showed that it was possible to preserve a probiotic population around 7×10^6 CFU/g after 40 days of storage indicating that this cheese could be considered a functional product along its shelf life. Author reported an increase of 12% on final cost of probiotic light sour cream when compared to regular product.

It is also important to note the relationship between probiotics and other fermenting microorganisms, as there may be synergistic or antagonistic effects between them (Heller, 1998). During the manufacture of cheese or yogurt, addition of the starters and probiotic cultures usually result in a slower growth of the probiotic strains. This is possibly because the starter cultures produce substances that inhibit not only pathogens and spoilage microorganisms but also probiotics, and because of the rapid growth of starter cultures, the nutrients availability for probiotics decreases (Roy, 2005). Champagne et al. (2005) mentioned that very few strategies have been proposed to reduce the starters' negative effects on the probiotic cultures, and that the most common is reducing starter dose (entirely or partially). However, precautions must be taken when lowering the dose of the starter microorganisms, because probiotics can also show a negative effect on these cultures and this would slow their activity.

Environments with a rich concentration of oxygen due to transportation systems and stirring or whipping procedures are also commonly found in dairy processing, especially in ice creams and some types of yogurts and fermented milks. The exposure of cultures to dissolved oxygen causes the accumulation of toxic metabolites such as superoxide, hydroxyl radicals and hydrogen peroxide, which eventually lead to cell death of the probiotic microorganisms that partially or completely lack of an electrons transport system. Regarding this oxygen toxic effect on probiotics, there are variations between species. For example, *Bifidobacterium* spp., strictly anaerobic in nature, is generally considered more vulnerable than strains of *Lactobacillus acidophilus* (Talwaker and Kailasapathy, 2004).

Another important issue concerning the addition of probiotic strains into food is temperature. Heating temperatures below 45°C are usually compatible with the cultures, although this depends on the time and the specific strain. Processes that include heating steps above 45°C result in destruction of at least a portion of the probiotic population (Roy, 2005).

On the other hand, low temperatures are generally used to delay the chemical reactions and growth of microorganisms found in foods, therefore a lower temperature implies greater bacterial inhibition growth. A temperature low enough will inhibit the growth of all microorganisms including probiotics. Because of their nature, dairy products, fermented or not, require low storage temperature for preservation, and this fact determines the survival and development of probiotics in these products. It is believed that freezing also leads to a considerable reduction in the number of viable microorganisms in food, although this reduction would depend on the freezing rate and the specific strain tolerance to low temperature.

Corrales et al. (2007) evaluated the effect of the dynamic freezing operation on the viability of two different probiotic strains, *Lactobacillus acidophilus* and *Bifidobacterium lactis*, during ice cream production. It was found that the reduction rate of both strains during this operation was not significant ($P>0.05$), but throughout the whole process of elaboration of the ice cream (dynamic freezing and then hardening at -30°C) there was a significant reduction on both populations.

Other unit operations like pressing and draining could also affect the bacterial counts in the products. The effect of pressing and draining in a cheese probiotic cells is obviously a loss of these cells in the whey, so the final concentration in the pressed cheese is difficult to control (Heller, 1998). Segura (2005) evaluated the effect of the pressing operation in a Turrialba cheese (typical Costarican fresh cheese, $>60\%$ water, w/w) added with *Bifidobacterium lactis*. Probiotic population was determined before and after the pressing operation, and significant differences were found ($P<0.05$). A loss of approximately two logarithms on probiotic population was reported after the pressing operation.

Despite the above results, it is believed that cheese could be a very good vehicle for delivering probiotic strains into the organism, since cheese has a stable structure and usually a high fat content (case of aged cheeses), factors that can help bacteria to survive during product storage and transit on the gastro-intestinal tract.

When comparing with yogurt, the problem for cheese (especially semi-hard and hard cheese) acting as carrier for probiotics results from the high fat and salt content and the relatively low recommended daily intake. Also the concentration of probiotics in cheese should be about four to five times higher than in yogurt. However, this does not apply to fresh cheese, which can easily be adjusted to low fat and salt contents, and for which recommended daily intake is rather high (Cruz et al., 2009a).

Figure 2 shows the growth of a strain of *L. paracasei* subsp. *paracasei* in a semi hard cheese during a ripening period of 45 days at 12°C and 85% RH (Boza et al., 2010). Probiotic population increased during the ripening period reaching interesting levels according with the high levels population goal.

Figure 3 shows the stationary behavior of the same bacteria viability in the ripened cheese kept under refrigeration for 49 days. It should be noted that strains of *Lactobacillus paracasei* have been isolated from naturally ripened cheeses and recognized as non starter lactic acid bacteria (Lynch et al., 1999), indicating that the matrix of the cheese is a good substrate for the growth of this bacterium.

The trend in cheeses, as in yogurt and fermented milks, is that probiotic bacteria populations remain stable or loose viability during ripening and storage (Kılıc et al., 2009; Ong et al., 2006; Songisepp et al., 2004; Vinderola et al., 2000; Yilmaztekin et al., 2004). There are also studies that have shown the growth of some probiotics in cheese during ripening periods or storage under refrigerated conditions (Boza et al., 2010; Buriti et al., 2005; Gardiner et al., 2002; Gardiner et al., 1998; Segura, 2005). However, growth and survival of probiotic microorganisms in ripened cheeses are believed to depend on many factors (like

ripening temperature and the probiotic strain interactions with other microorganisms found in cheese) hence hard to generalize.

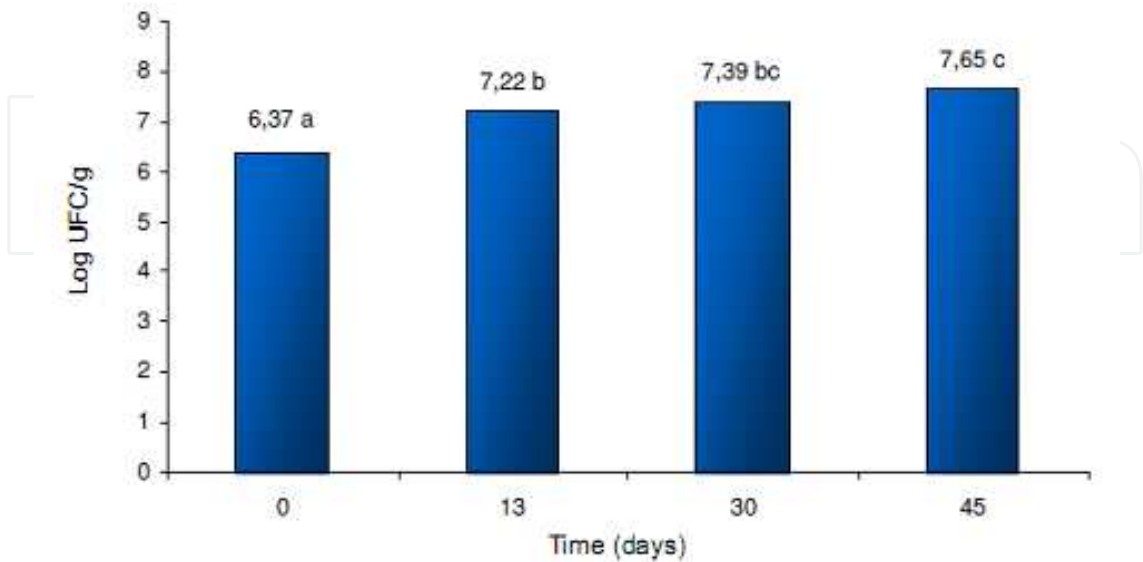


Figure 2. Logarithm of the number of colony forming units of *Lactobacillus paracasei* subsp. *paracasei* per gram of semi hard cheese for different time periods at 12°C and 85% RH. Different letters in the columns indicate significant differences ($P<0,05$).

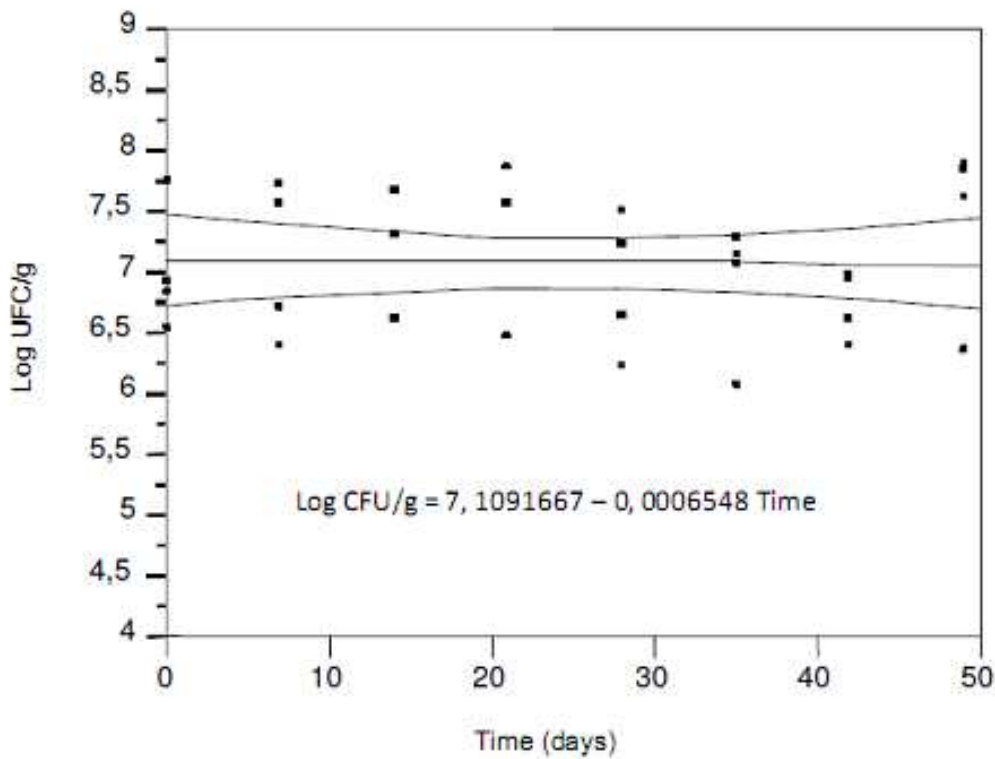


Figure 3. Logarithm of the number of colony forming units of *Lactobacillus paracasei* subsp. *paracasei* per gram of semi hard cheese vacuum packed and stored for 49 days at 5°C (Boza et al., 2010).

Indulgence products like ice-creams are potential probiotic vehicles as well, with the advantage of being appreciated by people belonging to all age groups and social levels (Cruz et al., 2009b). However, in these products, due to low storage temperatures and high concentration of dissolved oxygen, it is difficult for probiotic microorganisms to increase their number. The study conducted by Corrales et al. (2007) determined the behavior of two different probiotic strains, *L. acidophilus* and *B. lactis*, in ice cream throughout 85 days of storage at -30°C . Figure 4 (a and b) shows the behavior of probiotic strains.

The author found that freeze storage conditions affected significantly ($P < 0.05$) the viability of the two microorganisms, and reported losses of 0.76 and 1.10 logarithmic units for *L. acidophilus* and *B. lactis* respectively. Functional shelf life (plate counts $> 10^6$ CFU/g) was found to be 90 days. An increase of 28% in variable costs was calculated for the product.

Salem et al. (2005) manufactured ice cream with different strains of *Lactobacilli* and *Bifidobacteria*. The probiotic ice cream was evaluated for cultures survival during 12 weeks of frozen storage at -26°C . Initial freezing of ice cream mix followed by hardening caused a reduction of less than one log cycle in viable counts of probiotics. The viable counts decreased during frozen storage by 2.23, 1.68, 1.54, 1.23 and 1.77 log for *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus reuteri*, *Lactobacillus gasseri* and *Lactobacillus rhamnosus*, respectively. Although there was a decrease in the number of viable cells, the investigators considered the ice cream as a probiotic food during 12 weeks of storage, since the viable population remained above the recommended minimum limit of 1×10^6 CFU /g.

Feraz and colleagues (2012) investigated the survival of *L. acidophilus* in ice cream with different overrun levels during a 60 day storage period. All the ice creams presented a minimum count of 1×10^6 CFU/g at the end of 60 days of frozen storage.

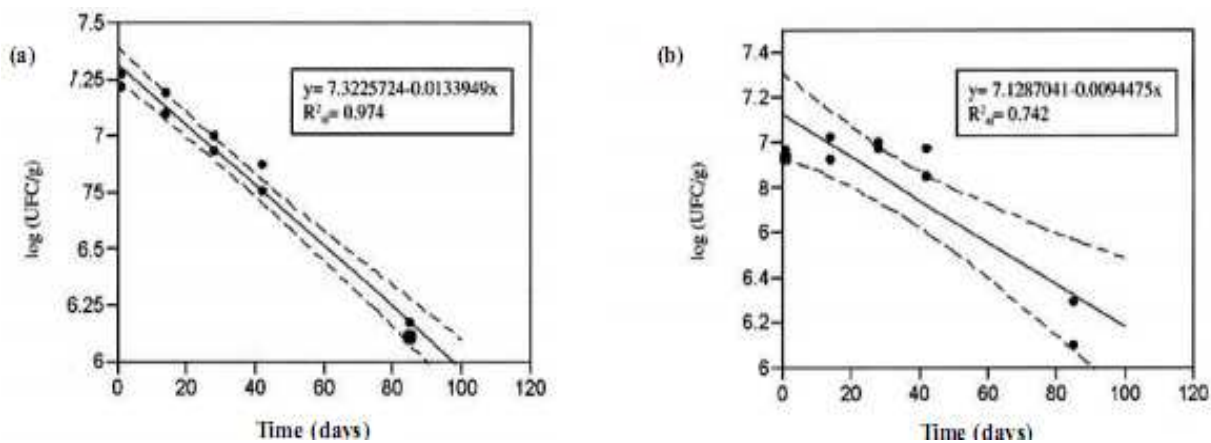


Figure 4. Behavior of *Lactobacillus acidophilus* (a) and *Bifidobacterium lactis* (b) during ice cream storage at -30°C (Corrales et al., 2007).

3. Techniques for the addition and protection of probiotics in dairy products

Controlled growth of probiotic bacteria in a dairy product during ripening or fermentation periods are desirable and interesting from a productive and economic point of view. This

ideal situation may allow food producers to use a lower initial dose of inoculum, or may help to replace the microorganisms that could have been eliminated or destroyed during a specific step of the production process like thermal treatment, dynamic freezing or draining.

It has been already explained that probiotics generally do not grow well in milk, and in fact, as mentioned before, the populations of many probiotic bacteria are not even stable during storage of dairy products. However, it is possible to find variations among strains of the same species, and the current trend is the development of new dairy products by using new ingredients that favor the growth of these microorganisms, such as yeasts, tomato juice, rice and soy milk (Champagne et al., 2005; Liu and Tsao, 2009).

Champagne (2008) suggests some ways to address stability problems, and these include: strain selection, ingredients selection (flavours, enzymes, fruits or vegetables, prebiotics) and packaging. All these techniques can be used to innovate and develop new products. Other techniques may include the microencapsulation with lipid materials, alginate and prebiotics (Akhiar, 2010; Siuta-Cruce and Goulet, 2001), the addition of antioxidants such as ascorbate and L-Cysteine, and the elimination from the environment of strains producing hydrogen peroxide (Champagne et al. 2005).

It was mentioned (Cruz et al., 2009a) that one strategy for enhancing bacterial tolerance toward stresses such as temperature, pH or bile salts is prior exposure to sub-lethal levels of the given stress. Cruz et al. (2009a) proposed as alternative to avoid destruction by heat the addition of the probiotic after pasteurization, microencapsulation, pre-adaptation of cells to stress and changing technologies by a slight decrease in temperature.

In order to use probiotic bacteria with proven health benefits in the manufacture of dairy products, sometimes the process has to be modified and adapted for the strains, due to their high sensitivity. According to Cruz et al. (2009a) there are two options for the addition of probiotic bacteria during cheese processing which can directly affect the survival rate of these microorganisms: probiotic bacteria can be added before the fermentation (together with the starter culture), or after it.

Daigle et al. (1999) produced Cheddar cheese from microfiltered milk standardized with cream and fermented with *Bifidobacterium infantis*. In this case, bifidobacteria showed good survival ($> 3 \times 10^6$ CFU/g) on cheese packaged under vacuum and kept at 4°C for 84 days. Cheddar cheese was also successfully produced with a spray dried adjunct of powder milk containing a strain of *Lactobacillus paracasei*. Data obtained demonstrated that probiotic spray-dried powder is a good option of probiotic addition to dairy products (Daigle et al, 1999).

Other research group (Songisepp et al., 2004) added *Lactobacillus fermentum* ME-3, which has been shown to possess antimicrobial and antioxidative properties, to a "Pikantne" cheese which is a semi-soft Estonian cheese with an open texture. They tested two different methods: adding the probiotic combination with the starter culture and adding the probiotic on the drained curd. The cheese produced using the first method showed better sensory characteristics and therefore was chosen to carry out stability tests of probiotic during ripening and storage. The results showed that the strain used was well

suited to the process (levels of 5×10^7 CFU/g on ripened cheese) and maintained its probiotic effects.

Lactobacillus casei cells were immobilized on fruit pieces (apple and pear) and used them in the production of Feta cheese (Kourkoutas et al., 2005). Cheese was also produced with free cells of *L. casei*. At the end of the ripening period the authors concluded that the immobilized cells remained viable in the fruit, and in higher counts than in the cheese. Therefore, it is believed that these pieces of fruit were an effective support for the incorporation of probiotics in this type of product.

Ong and other researchers (2006) added combinations of *Lactobacillus acidophilus*, *L. casei* and *Bifidobacterium longum*; and *L. acidophilus*, *L. paracasei* and *B. Lactis* to Cheddar cheese. In this case cheese was produce following a standard procedure, in which milk, after being standardized was tempered to 31°C before inoculation with cheese starter culture and probiotic bacteria. All probiotic adjuncts survived manufacturing process and maintained their viability until the end of the ripening process.

Segura (2005) elaborated a probiotic fresh cheese (>60% water), adding *Bifidobacterium lactis* either to the milk before fermentation or to the curd (mixed with salt). It was found that a large number of bacteria were lost in subsequent operations such as pressing, but this phenomenon was lower when the probiotic culture was added to the curd (see Table 3).

Boza et al. (2010) modified the traditional process of semi hard cheese to avoid larger losses of probiotic in the whey. They added a strain of *Lactobacillus paracasei* mixed with salt after a preliminary pressing of the curd, wherein a major portion of whey was removed, obtaining a cheese with a viable probiotic cell number greater than 1×10^6 CFU/g.

Logarithm of the population of <i>B. lactis</i>			
Inoculation technique	Before pressing the curd	After pressing the curd	Variation in the logarithm of the probiotic population
Addition after pasteurization	8.51 ^{a1}	2.95 ^{b1}	5.56
Addition to the curd	9.81 ^{a2}	6.09 ^{b2}	3.72

^{a, b}... Different letters between columns indicate significant differences ($P < 0.05$).

^{1, 2}... Different numbers between rows indicate significant differences ($P < 0.05$).

Table 3. *Bifidobacterium lactis* population logarithmic variation before and after the pressing stage of a fresh cheese using two inoculation techniques.

Evaluation of the effect of inoculation time of the probiotics on viable counts of five bacteria in curds and whey during Cheddar cheese manufacture was performed (Fortin et al., 2011). These authors found that inoculation of probiotics in milk before renneting resulted in almost half the cell losses in whey compared with the addition just before the cheddarization step, and they also discovered that addition of probiotics in milk improved

their subsequent stability by about 1 log over the 20 days storage period as compared with cells added at cheddarization. Specifically, significantly higher populations of *Bifidobacteria* in curds were detected when the probiotic culture was added to milk. They found that although the quantity of whey generated during cheddarization is much lower than that obtained after the first cutting, the population of probiotics in the whey was ten times higher than after the first cutting when probiotics were added to milk. The authors proposed that cells were not as well entrapped in the curd mass at cheddarization than at renneting.

Arguedas (2010) added *L. paracasei* subesp. *paracasei* in a Philadelphia type cheese (24% fat, w/w) and evaluated their survival behavior during 40 days at 5°C. This author found that it was possible to reach a population around 7×10^6 CFU/g after 40 days of storage, and this cheese could be considered a functional product along the shelf life. Considering that during the Philadelphia type cheese production there is a pasteurization step followed by homogenization and fermentation, probiotic culture was added during the stirring step just before packaging. Figure 5 presents the modified production process. The author reported an increase of 11% on the final cost of the probiotic cream cheese when compared with the regular product.

When producing ice cream with probiotics, cultures may be added in two ways, considering that they are of the DVS (Direct Vat Set) type for direct addition to the product during its manufacture: either adding them directly to the pasteurized mix or using the milk as a substrate for fermentation, producing frozen yoghurt ice cream (Cruz et al., 2009b).

Corrales et al. (2007) developed a process of ice cream adding *Bifidobacterium lactis* and *Lactobacillus acidophilus*. Figure 6 presents the followed steps for the product preparation. The frozen bacteria was dispersed in 1 L of pasteurized milk (2% fat content), and then added the milk to the ice cream mix with constant stirring.

In a similar way, free and encapsulated cells of *L. casei* and *B. lactis* were added to ice cream to evaluate the effect of microencapsulation and resistant starch on the probiotic survival (Homayouni et al., 2008). In general, the results indicated that encapsulation can significantly increase the survival rate of probiotic bacteria on ice cream over an extended shelf-life.

Functional ice creams have been produced by mixing fortified milk fermented with probiotic strains with an ice cream mix, followed by freezing (Salem et al., 2005). Probiotic ice cream has been also produced by the addition of probiotic yogurt to the mix prior the dynamic freezing-step (Soukoulis et al., 2010).

More recently, the effect of different overrun levels on probiotics survival on ice cream has been studied by Ferraz et al. (2012), incorporating *Lactobacillus acidophilus* into a vanilla flavored product. *L. acidophilus* was added to the mix with constant stirring just before freezing. Ice creams were processed with overruns of 45%, 60%, and 90%. Although all presented a minimum count of 1×10^6 CFU/g at the end of 60 days of frozen storage, higher overrun levels negatively influenced cell viability, being reported a decrease of 2 log units for the 90% overrun treatment. The authors suggest that lower overrun levels should be

adopted during the manufacture of ice cream with probiotics in order to maintain its functional status through the shelf life.

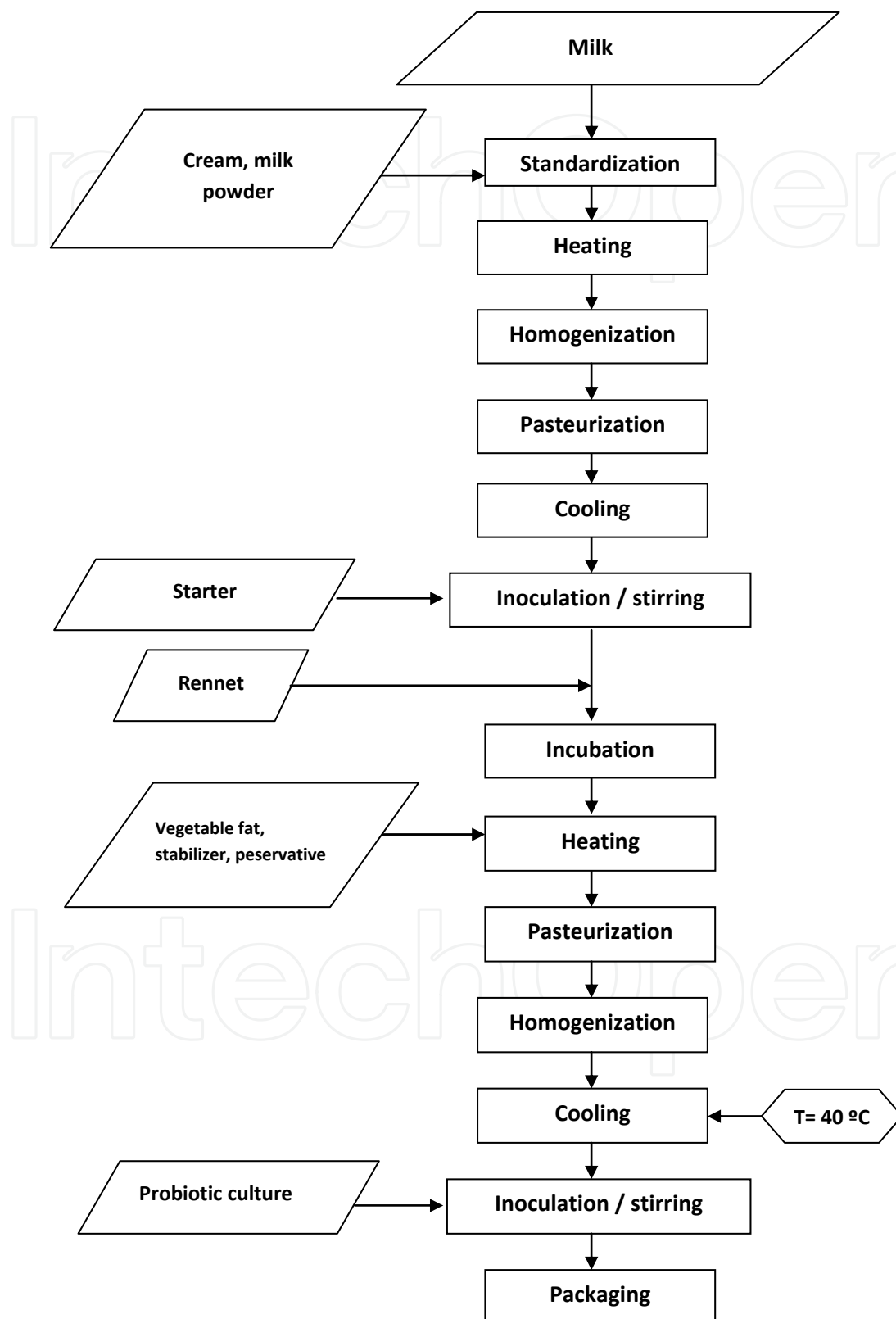


Figure 5. Production flow chart for Philadelphia type cheese with probiotics.

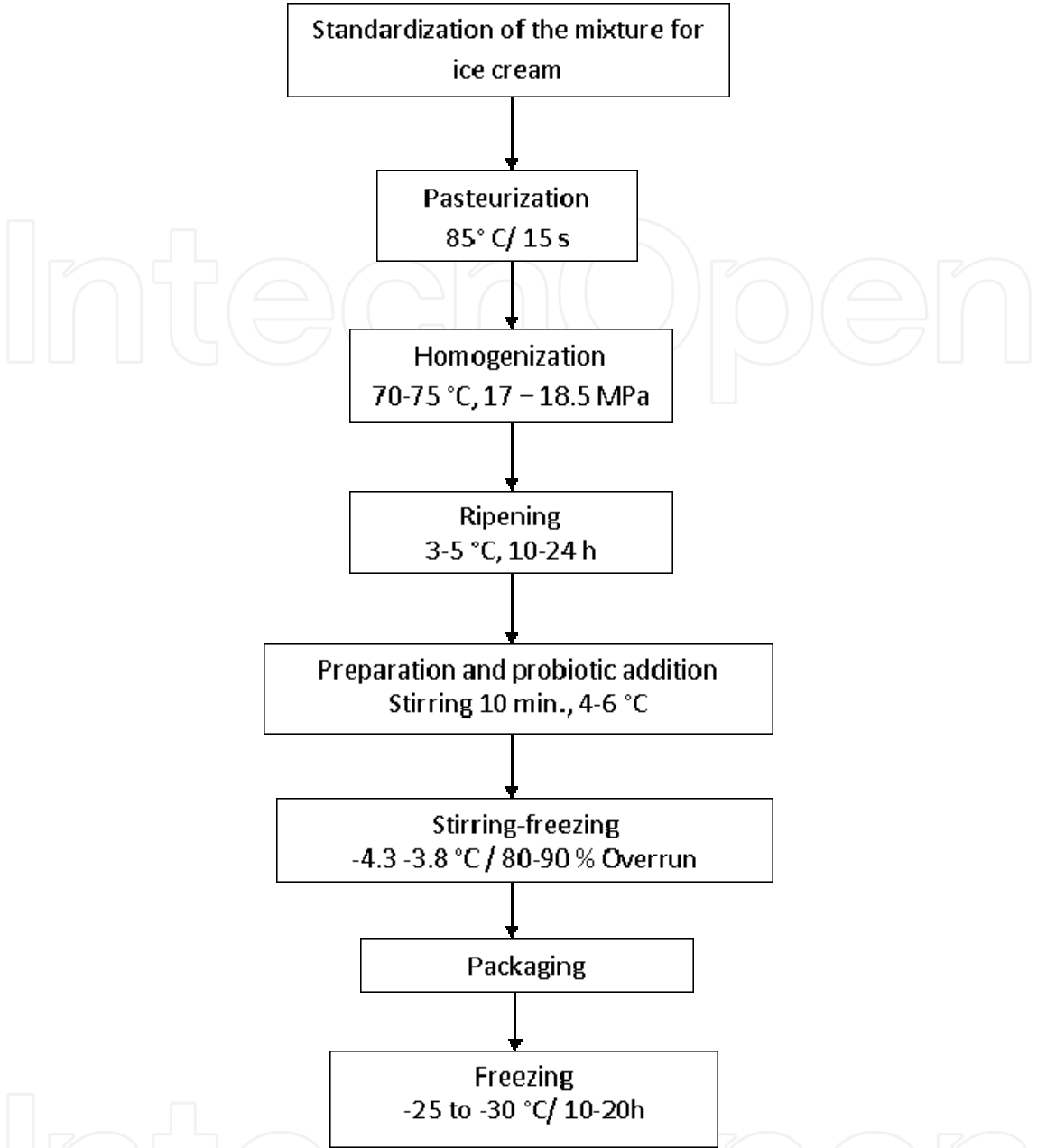


Figure 6. Production flow chart for ice cream with probiotics.

4. Quality modifications of products and sensory analysis

The products chosen for probiotic incorporation must be carefully studied, since the addition and/or multiplication of probiotic microorganisms could produce undesirable characteristics in the products (Dias and Mix, 2008; Komatsu et al., 2008). For many products the addition of probiotics may represent changes that significantly impact its physico-chemical properties, due to the metabolic activity of these living microorganisms and/or changes made on standard food processing procedures. Hence, careful selection of strains is necessary to minimize quality losses caused by alterations to flavor and texture of foods.

According to Champagne et al. (2005) many studies have shown that for some products the addition of probiotics do not lead to significant differences in the sensory properties, although changes in chemical composition and texture may occur these do not necessary have a relevant effect on flavor for some foods (depending on the extent of probiotic growth). This seems to be the case for fermented cheeses.

Natural cheeses are known for their complex microbial ecosystem which is in a constant state of flux as the cheese ages (Dias and mix, 2008). In general, a probiotic cheese should have the same acceptance as a conventional cheese: the incorporation of probiotic bacteria should not imply a loss of quality of the product. In this context, the level of proteolysis and lipolysis must be the same or even greater than cheese which does not have this functional status (Cruz et al., 2009a).

Buriti et al. (2005) evaluated the effect of *Lactobacillus acidophilus* on the instrumental texture profile and related properties of Minas fresh cheese (>65% water, w/w) during storage at 5°C up to 21 days. Parameters measured included hardness, elasticity, cohesiveness, chewiness and gumminess. Four cheese-making trials (T) were prepared, two supplemented with a mesophilic type O culture (T1, T2) and two with lactic acid (T3, T4). *L. acidophilus* was added in T2 and T3. Probiotic cheeses T3 were firmer by the end of storage, due to higher values of pH and hardness, and according to the authors also had better results in the sensory evaluation (preference-ranking test). Differences detected were attributed to the starter, rather than to *L. acidophilus*. In this study percentage of syneresis and the proteolytic index were also determined after the different storage times, finding no relevant differences.

For this same type of cheese, it was proved that the use of a probiotic culture (containing *L. acidophilus*, *B. animalis* and *S. thermophilus*) complementary to lactic acid, aiming to substitute traditionally employed culture for Minas cheese production, is advantageous (Buriti et al., 2007). Cheeses with added probiotic culture showed to be less brittle and with more favorable sensory characteristics than those made with the traditional lactic acid culture. Researchers conducted an instrumental texture profile analysis of cheeses and a preference-ranking test.

In other study the influence of probiotic bacteria on proteolytic patterns and production of organic acid during ripening period of 6 months on Cheddar cheese at 4°C was evaluated (Ong et al., 2006). No significant differences ($P>0.05$) were observed in composition (fat, protein, moisture, salt content), but acetic acid concentration was higher in probiotic cheeses. The assessment of proteolysis during ripening showed no significant differences in the level of water-soluble nitrogen (primary proteolysis), but the concentration of free amino acids were significantly higher in probiotic cheeses (secondary proteolysis).

More recently, the survival and influence on sensory characteristics of probiotic strains of *Lactobacillus fermentum* and *Lactobacillus plantarum*, all derived from human faces, were investigated in Turkish Beyaz cheese production. Quantification of volatile aroma components by gas chromatography was performed as well as sensory evaluation. The results showed that tested probiotic culture mix was successfully used in cheese production without adversely affecting cheese quality during ripening. The chemical composition and

sensory quality of probiotic cheeses were also comparable with traditional cheeses (Kılıc et al., 2009).

Arguedas (2010) analyzed the effect of adding *L. paracasei* subsp. *paracasei* in a Philadelphia type cheese (24% fat, w/w) on product texture during the shelf life. Table 4 shows the results obtained on hardness, cohesivity, adhesivity and gumminess (instrumental analysis) at day 2 and 44 for samples of regular and probiotic cheese at refrigerated storage (5°C).

There was no significant difference ($P > 0.05$) in any parameter between regular and probiotic cream cheese although there was a variation as a function of time on hardness, cohesivity and gumminess for the samples analyzed. In general, these three parameters decreased along storage probably due to syneresis. Since there was no interaction between the time effect and the type of product effect, the decrease on these parameters is not related with the probiotic presence.

Treatment		Hardness (N)	Cohesivity	Adhesivity (erg)	Gumminess (N)
With probiotics	2 days	7,9970	0,3194	-141475,0	2,5964
	44 days	5,6058	0,2115	-120637,5	1,1735
Without probiotics	2 days	6,5627	0,2584	-139880,0	1,6967
	44 days	6,0673	0,2285	-115408,3	1,3882

Table 4. Philadelphia type cheese texture average values obtained during refrigerated storage at days 2 and 44 (Arguedas, 2010).

There was no significant difference ($P > 0.05$) in any parameter between regular and probiotic cream cheese although there was a variation as a function of time on hardness, cohesivity and gumminess for the samples analyzed. In general, these three parameters decreased along storage probably due to syneresis. Since there was no interaction between the time effect and the type of product effect, decreased on these parameters is not related with the probiotic presence.

Consumers rated taste liking degree for cheese during refrigerated storage (5°C) at days 2, 16, 30 and 44. Figure 7 shows the average results for probiotic Philadelphia cheese type during this period of time. No significant differences ($P > 0.05$) were found along shelf life considering taste liking degree for Philadelphia cheese type with *Lactobacillus paracasei* subsp. *paracasei*. Average liking degree was 6.5.

Ice cream and ice milk appear to be good products for the delivery of probiotic bacteria. When the cream blend is prepared by adding a fermented milk, the resulting flavor of the product can be affected (Champagne et al., 2005; Cruz et al., 2009b). However, when small quantities of concentrated cultures are introduced, the sensory properties are not affected. Strain or species do seem to be important, since ice creams manufactured with *L. reuteri* cultures have shown to be “more sour” than those made from corresponding cultures of *L. acidophilus*, *L. rhamnosus*, or *B. bifidum* (Champagne et al., 2005). Also, products like non-fermented probiotic ice-cream will not normally present problems resulting from the

microbial metabolism, since they are stored at very low temperatures, minimizing the probiotic microorganisms' biochemical reactions (Cruz et al., 2009b).

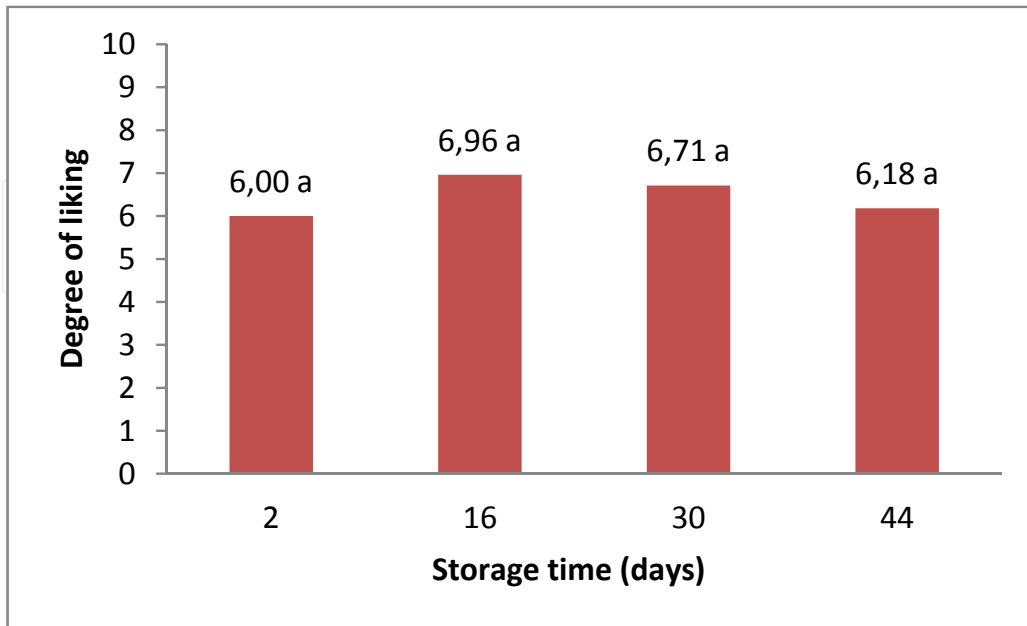


Figure 7. Consumers average taste liking degree of Philadelphia cheese type with *Lactobacillus paracasei* subsp. *paracasei* during storage (Arguedas, 2010). Different letters in the columns indicate significant differences ($P < 0,05$).

Corrales et al. (2007) conducted a sensory evaluation of the ice cream flavor, using the duo-trio differentiation technique with 30 semi-trained panelists. It was found that 17 of the 30 semi-trained panelists were able to detect the sample that was equal to the pattern, indicating that no significant difference ($P > 0.05$) was found in the ice creams flavor with and without probiotics. This result supports the conclusion that the consumer did not detect changes in the flavor of ice cream, contributing to the product acceptance.

According to Soukoulis et al. (2010), probiotic ice cream is a functional frozen dairy dessert with particular sensory characteristics combining the flavor and taste of fermented milks with the texture of ice cream. In their study, the effects of compositional parameters (hydrocolloids type and amount, yogurt and milk fat content) on texture and flavor of a probiotic ice cream were evaluated. In such a product, the use of hydrocolloids like xanthan gum and low acidified formulations are recommended to improved creamy sensation, high textural quality and enhanced flavor. They found that based on hedonic and descriptive evaluation, consumers' acceptability of probiotic ice cream is mainly affected by ten sensory drivers including "sweet", "sour", "astringent", "vanilla flavor", "gummy", "coarse", "watery", "creamy", and "foamy".

The effect of several probiotic strains on the sensory acceptance of ice cream was evaluated by Salem et al. (2005). Probiotic ice cream was manufactured by mixing fortified milk fermented with probiotic strains with an ice cream mix. They found that all the ice cream samples received a high score in the sensory evaluation. Ice cream containing *Lactobacillus reuteri* was judged to be sourer and reached a higher score for "probiotic" flavor.

Two types of synbiotic ice cream containing 1% (w/w) resistant starch with free and encapsulated *Lactobacillus casei* and *Bifidobacterium lactis* were manufactured by Homayouni et al. (2008). The synbiotic ice cream samples were sensory assessed by 32 panelists. According to the authors, total evaluations in term of color, texture and taste of all samples were positive and did not have any marked off-flavor during the storage period. None of the ice creams were judged to be crumbly, weak, fluffy or sandy.

Finally, Ferraz et al. (2012) supplemented a vanilla ice cream with *Lactobacillus acidophilus* at different overrun levels (45%, 60%, and 90%). They did not report an influence for any overrun level ($P>0.05$) on acceptability regarding appearance, aroma, and taste of the ice creams.

Performing sensory evaluation is certainly an important step in probiotic dairy products development before the launch of the product into the market. As new products with probiotics may change some characteristics studying the behavior of trained panelists and consumers toward the developed product is a key factor and might represent a powerful tool to recover information that could support a product launch.

Another central issue in new probiotic products is to guarantee enough microorganism population in order to allow consumers to experience the beneficial effects described before. Probiotic quantification with an appropriate technique is a must in the product process development.

5. Probiotic quantification techniques

Proper selection of an analytical method for the probiotic microorganism's enumeration in food is critical since confirmation of whether the product has the minimum required amount of bacteria to provide the health benefits associated will depend on the result obtained.

The choice of culture medium and methodology for selective enumeration of commercial probiotic strains in combination with starters depends strongly on the product matrix, the target group and the taxonomic diversity of the bacterial background flora in the product (Van de Castele et al., 2006). There is a wide variety of analysis methods that consider all these aspects and are extensively documented by various authors.

Several media have been suggested for the enumeration of probiotic bacteria alone or in combination in commercial cultures or products (Vinderola and Reinheimer, 2000). MRS agar is the media most commonly used and is normally supplemented with different sugars as maltose or glucose and with antibiotics solutions such as dicloxacillin, clindamycin, vancomycin, nalidixic acid, among many others. It is also common to add inhibitory agents as LiCl, NaCl, acids, bile salts and sorbitol. Supplements selection is made depending on the microorganism of interest and strains that wanted to be inhibited, for this purpose combination of both is very common. RCA agar with different antibiotics and salts is likewise used.

For *Bifidobacterium* sp. count, an incubation of plates under anaerobic conditions is required while *Lactobacillus* sp. strains can be recovered both aerobically and anaerobically. Therefore one criterion for selecting the correct method is not only the strain of interest oxygen requirement but also accompanying flora characteristics. Similarly, temperature and incubation time varies between methods. Most of probiotic cultures are recovered at 37°C but increasing incubation temperature at 43°C is often used to inhibit mesophilic flora. Incubation times typically range from three to six days.

An important aspect to consider is that probiotic microorganisms viable cells amount should be kept at the minimum accepted level in order to be considered as a functional food during its entire shelf life. Therefore, in new product development probiotic bacteria count should be performed in fresh product and throughout shelf life. In many cases, shelf life of such products is determined as a function of time in which availability of minimum required concentration of probiotics can be guaranteed.

In the scientific literature, populations of 10^6 - 10^7 CFU/g in the final product are established as therapeutic quantities of probiotic cultures in processed foods (Talwaker et al., 2004), reaching 10^8 - 10^9 CFU, provided by a daily consumption of 100 g or 100 ml of food, hence benefiting human health (Jayamanne and Adams, 2006). For example, in Brazil, the present legislation states that the minimum viable quantity of probiotic culture should be between 10^8 and 10^9 CFU per daily portion of product and that the probiotic population should be stated on the product label (Brazilian Agency of Sanitary Surveillance, 2012).

6. Conclusion

The use of products like yogurt, fermented milks, different cheeses and ice cream as probiotic food carrier opened a valuable alternative for dairy industry. To meet consumers demand for probiotic foods in different countries, different types of products are needed. Research has demonstrated that it is possible to incorporate successfully probiotics reaching the recommended amounts in order for consumers to experience the described health benefits. It is also possible to reach a reasonable shelf life according to the expected product characteristics.

From a technological point of view adding probiotics into dairy products could represent a difficult task depending on the type of product or microorganisms. Knowledge of all unit operations involved in processing and adaptations in traditional dairy process are helpful. Preliminary test to follow product and bacteria behavior provide useful information and sometimes it is necessary to change process parameters or inoculation step.

Proper techniques for population determination must be used to follow probiotic behavior during production and storage time and correctly predict shelf life. Performing physico-chemical analysis is decisive since characterization of product gives important information of probiotic effects and finally appropriate sensory techniques help to determine if attributes may have an influence on consumer acceptance. Since final product quality modifications could occur it is important to perform sensorial test with trained, semi-trained judges or

directly with consumers at this stage. Results obtained in a product developing process are indeed specific for the product, microorganism or mixture of microorganisms and technology involved. It is not possible to generalize them to other products, strains or elaboration techniques.

Developing successful functional dairy food requires to be supported by scientific research. Product development in this field should consider knowing the consumer expectations, the technological process, the appropriate analyzing techniques and marketing. Nutrition advantages of dairy products need to be emphasized and information should be focused on consumers but also need to consider health care professionals.

Industry needs relevant regulation of physiological claims and health claims and nowadays some companies are performing clinical studies with particular strains to prove specific benefits but it is clear that production of functional dairy foods following the rules of medicine production is hardly of interest.

Considering the healthy population there may be potential to develop targeted products for different age groups. In the reduction of risk and treatments of various diseases, probiotics have resulting in promising benefits. However, it is important to understand the mechanisms behind the effects on our well-being. Information regarding the interaction between bacteria and dairy is focused on growth and survival of probiotics during production, storage and gastric transit therefore more research is needed to determine the effect of food substrate on metabolic activities of probiotics associated with their beneficial properties.

Author details

Esteban Boza-Méndez, Rebeca López-Calvo and Marianela Cortés-Muñoz*
National Research Center of Food Science and Technology (CITA),
University of Costa Rica, San José, Costa Rica

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* Corresponding Author

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