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Flávia C. A. Buriti ^a & Susana M. I. Saad ^b

^a Embrapa Caprinos e Ovinos, Empresa Brasileira de Pesquisa Agropecuária, Estrada Sobral-Groaíras, Km 4, P. O. Box 145, 62011-970, Sobral, CE, Brazil

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^b Departamento de Tecnologia Bioquímico-Farmacêutica, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, 05508-000, São Paulo, SP, Brazil

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Flávia C. A. Buriti ^a and Susana M. I. Saad ^b

^a Embrapa Caprinos e Ovinos, Empresa Brasileira de Pesquisa Agropecuária, Estrada Sobral-Groaíras, Km 4, P. O. Box 145, 62011-970, Sobral, CE, Brazil

^b Departamento de Tecnologia Bioquímico-Farmacêutica, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, 05508-000, São Paulo, SP, Brazil

Address correspondence to Prof. Dr. Susana M. I. Saad, Departamento de Tecnologia Bioquímico-Farmacêutica, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, 05508-000, São Paulo, SP, Brazil. Phone.: +55 11 30912378; Fax: +55 11 38156386. E-mail: susaad@usp.br.

Abstract

Nowadays, food companies are endeavouring to differentiate their products through creative segmentation and positioning strategies based on superior functionality and quality. Some kinds of dairy desserts have shown a great market potential, as a function of consumers interested in healthier and functional products with fine taste and mouthfeel. In this context, chilled dairy desserts are emerging as attractive options for the incorporation of probiotic cultures and prebiotic ingredients, as seen in the recent launches from the food industry, as well as in the

growing number of scientific studies dealing with this subject published in the last years. The main aspects involved in the development of probiotic and/or prebiotic dairy desserts for storage under refrigerated conditions are presented in this review.

Key words: dairy desserts, food development, functional foods, prebiotics, probiotics, refrigerated storage.

INTRODUCTION

Dairy desserts are widely consumed worldwide by several groups of consumers, including children and elderly people, at different meals, environments and occasions. This large consumption is mainly influenced by the nutritional and sensory characteristics of this kind of food (Tárrega and Costell, 2007; Ares et al., 2009b). Over the last decade there has been an increase in the marketing activity regarding these products and, nowadays, a broad range of ready-to-eat milk based desserts is available to the consumer (Verbeken et al., 2006; Cardarelli et al., 2008; Depypere et al., 2009).

According to the statistical data available worldwide, the total production of fresh dairy products (yoghurts, milk drinks, and dairy desserts) totalised 21 million tonnes in 2007, compared to 11 million tonnes in 1997 (IDF, 2008). Recently, the market value of chilled desserts was estimated at over a billion pounds (€1.2 billion) (Anonymous, 2010). In Brazil, the market for dairy desserts engendered BRL 225 million (around €100 million) in 2008, with a high growth prospective (Fator Brasil, 2009).

With increasing levels of competition across health and wellness categories, companies have endeavoured to further differentiate their products through creative segmentation and positioning strategies based on superior functionality (Ares et al., 2008a; Raud, 2008; Bogue et al., 2009). Some kinds of dairy desserts have shown a great market potential, as a function of consumer behaviour, interested in healthier and functional products (Dyminski et al., 2000; Pinto et al., 2003; Aragon-Alegro et al., 2007). Moreover, the consumption trend of indulgence products also represents a great market potential, even though it has required adjustment for

reducing fat and calories content of consumer's favourite desserts without compromising taste and mouthfeel (Maltete, 2008; FIESP/ITAL, 2010).

Due to these reasons, dairy desserts are interesting options for the incorporation of functional ingredients such as probiotic and prebiotic ones (Cardarelli et al., 2008). These factors can explain the recent launches of dairy desserts included in well established brands of probiotic products among consumers (Faron, 2010; Assad et al., 2010).

The success of dairy probiotics can partly be explained by their general positive image among consumers. As a matter of fact, consumers are more and more aware that food has a direct contribution to their health (Sangeetha et al., 2005; Siró et al., 2008). In a study conducted by Ares et al. (2008b), milk-based desserts were considered as 'credible carriers of functional messages' by consumers interested in functional food. Thus, the addition of probiotic cultures and prebiotic ingredients to milk-based desserts, besides enhancing the products image and value, is especially attractive to those consumers interested in healthy food.

With regards to probiotic cultures and/or prebiotic ingredients, it is important to understand these concepts. Probiotics and prebiotics are physiologically active components that have an important role in gut fermentation by influencing the microbiota composition and fermentation metabolites, and consequently by contributing to local and systemic effects in humans (Cardarelli et al., 2007; Saulnier et al., 2009). Probiotics are defined as live microorganisms, that, when administered in adequate amounts, confer a health benefit on the host. (FAO/WHO, 2001). Prebiotics are selectively fermented ingredients that allow specific changes in the composition and/or gastrointestinal microbiota activity that confer benefits upon host well-being and health (Gibson et al., 2004; Roberfroid, 2007). Due to their potential synergy, foods containing a

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combination of probiotic bacteria and prebiotics ingredients are referred to as synbiotics (Arvanitoyannis and Van Houwelingen-Koukaliaroglou, 2005; Swennen et al., 2006). Kolida and Gibson (2011) describe a further condition necessary to be fulfilled for synbiotic foods: the prebiotic must selectively support the growth of the probiotic component.

In view of the opportunities for this category of food products to carry functional components, this article reviews important studies published in the last years concerning the development of probiotic and/or prebiotic dairy desserts for storage under refrigerated conditions and presents the main challenges involved in this field.

MAIN ASPECTS INVOLVED IN THE PREPARATION OF PROBIOTIC AND/OR PREBIOTIC MILK-BASED DESSERTS

Regulatory aspects

One the most important aspects of probiotics regarding concern to consumers and regulators is that of safety (Farnworth, 2008). Members of the genera *Lactobacillus* and *Bifidobacterium* are mainly used as probiotics, which have an extensive safety record for use in the generally healthy population (Douglas and Sanders, 2008; Ross et al., 2010; Whelan and Myers, 2010).

As for other probiotic products, the formulation ingredients of dairy desserts and their processing steps should not result in loss of probiotic microorganism viability during their production and shelf-life or reduction in sensorial quality. Standards requiring a minimum probiotic dose of 6-7 log colony-forming units (cfu) per g in dairy products have been introduced

by several food organizations worldwide (Talwalkar and Kailasapathy, 2004a; Akalın and Erışır, 2008).

According to Stanton et al. (2001) and Farnworth (2008), the Fermented Milks and Lactic Acid Bacteria Beverages Association stipulated at least 7 log cfu viable bacteria per g or mL product to be considered probiotic in Japan. The Canadian Food Inspection Agency (CFIA) establishes a minimum level of 9 log cfu per daily serving portion of the microorganism for the use of term "probiotic" or other similar designations and should be accompanied by specific, validated statements about the benefits or effects of the probiotic (Health Canada, 2009; CFIA, 2010a). The reference amount considering a ready-to-serve form of fresh dairy desserts is 100 g (CFIA, 2010b). In accordance with Brazilian regulatory standards, probiotic microorganisms should be present in concentrations higher than 8-9 log cfu per daily serving portion of the product ready to consume during the entire shelf-life (ANVISA, 2008). Brazilian standards recommend the daily serving portion of 120 g, equivalent to ½ tea-cup, for milk derived desserts (ANVISA, 2003).

Up to the present moment, fructooligosaccharides (FOS). inulin and galactooligosaccharides (GOS) are the most studied ingredients for which health benefits possibly associated with the prebiotic effects were repeatedly demonstrated through experimental and human trials (Roberfroid et al., 2010). According to FAO (2007), these and other chemical groups commonly used as prebiotics (soya-oligosaccharides, xylooligosaccharides, pyrodextrins, isomaltooligosaccharides, and lactulose) have a long history of safe use. However, even for a same component, regulations may differ from one country to another and, at the same time, legislation is very limited regarding control of the use of the word "prebiotic" on food products,

since some of these ingredients still need more studies to prove their real benefits (Dwyer, 2007; FAO, 2007).

As reported by Wells et al. (2008), FOS, GOS, and lactulose are prebiotic ingredients recognized in Europe. Claims regarding the prebiotic potential of other food ingredients and their scientific substantiation are under consideration by the European Food Safety Authority (EFSA) to comply the Regulation (EC) No 1924/2006 on Nutrition and Health Claims Made on Foods (Corrigendum, 2007; Stowell, 2009).

In Brazil, FOS and inulin are the only ingredients allowed to claim a prebiotic effect (ANVISA, 2008). Additionally, according to Brazilian legislation, the allowed claim for prebiotic and/or probiotic foods is restricted to the contribution to intestinal microbiota balance only. If this effect is supposed to be declared in milk-based desserts, the prebiotic ingredient should be present at concentrations of, at least, 3 g in the daily recommended serving portion of product ready to consume (ANVISA, 2008).

In fact, considering only the ability to inulin-type fructans to increase faecal bifidobacteria in humans, daily doses of 4 to 5 g for at least 2 weeks showed to be effective (Roberfroid et al., 2010). For the purpose of nutritional labelling, Roberfroid (1999) recommended that inulin-type fructans, as well as all of the non-digestible oligosaccharides that are largely or completely fermented in colon, be given a caloric value of 1.5 (6.3) kcal/g (kJ/g).

An overview of gel formation

A gel is defined as an insoluble semi-rigid structure of a solid dispersed in a liquid. Soluble polymers become insoluble to form a semisolid structure (gel), due to the association of the

polymer molecules with the solution where it is present. Gel formation from polysaccharides is affected by many factors, such as the food processing steps, other ingredients, heating, temperature, and pH. These factors also contribute to the gel strength and other rheological properties (Kim et al., 2001).

Most of the milk-based desserts are composed of ingredients that interact with milk proteins and contribute to their stability and consistency, such as starch and/or several hydrocolloid types (Dello Staffolo et al., 2007). Native and modified starches from several sources, mainly from maize, rice, and tapioca, are widely used in the production of probiotic and/or prebiotic milk-based desserts, due to their thickener and gelling properties (Helland et al., 2004; Tárrega and Costell, 2006b; Corrêa et al., 2008; Magariños et al., 2008; González-Tomás et al., 2009b). Starch forms are composed of amylose and amylopectin molecules (Kötting et al., 2010). Amylose molecules contain linear chains formed by the polymerization of D-glucose monomers linked by α -(1 \rightarrow 4) bonds, which correspond to 15% to 20% of the total starch. Amylopectin is the major starch component, also formed by an α -(1 \rightarrow 4)-D-glucose structure, with ramifications via α -(1 \rightarrow 6) bonds in its main chain (Sajilata et al., 2006; Perera et al., 2010). The amylose/amylopectin ratio varies according to the source of the starch and its maturity, but it is about 1:3 for most general starches (Knill and Kennedy, 2005). When starch is heated to about 50°C, in the presence of water, the amylose in the granule swells; the crystalline structure of the amylopectin disintegrates and the granule ruptures. The polysaccharide chains take up a random configuration, causing swelling of the starch and thickening of the surrounding matrix, in a process that leads to gel formation (Sajilata et al., 2006).

According to Liu et al. (2009), the starch gelatinization refers to the destruction of the crystalline structure in starch granules, which is an irreversible process that includes granular swelling, native crystalline melting and molecular solubilisation. During gelatinization, the part formed by the amylose is not completely dissolved, forming crystallin aggregates linked by hydrogen bonds. The formed gel may occasionally release water in a process called syneresis. Differently from amylose, amylopectin gels are more stable and less susceptible to retrogradation, due to its branched structure (Rapaille and Vanhemelrijck, 1998).

Under certain process conditions, such as temperature, pH, or pressure, native starches might show undesirable properties. Other limitations for native starch applications include low resistance to high shear rates, high susceptibility to retrogradation, and syneresis (Bertolini, 2010; Huber & BeMiler, 2010). Conversely, modified starches show higher thermomechanical resistance and more stability than native starches, producing dairy desserts with more texture and without syneresis (Tárrega and Costell, 2006a). For instance, the introducing of small amounts of ionic or hydrophobic groups onto the molecules alters positively the properties of starch, including solution viscosity, association behaviour, and shelf life stability in final products (Xie et al., 2005). Acetylation, hydroxypropylation, and cross-linking are common methods employed for the production of modified starches, which contribute significantly to the stabilization of food systems under refrigerated conditions (Aziz et al., 2004; Luallen, 2002).

Hydrocolloids are food additives whose function is to thicken, stabilize, enlarge, add viscosity, elasticity, and provide the desirable texture to the food product (Maruyama et al., 2006; Brownlee, 2011). In most practical applications of hydrocolloids, they are primarily polysaccharides, even though some proteins might be used for this purpose (Burey et al., 2008).

Usually, these polysaccharides are gums composed by long-chain and high molecular weight polymers, which are water-soluble and capable of forming gels. Thus, the dessert composition (particularly its protein content), its pH, and the processing conditions employed during its production should be considered to select the most appropriate hydrocolloid. Moreover, hydrocolloids are sensitive to shear and thermal treatment and are affected by the system acidity, which may partly change its technological properties (Rapaille and Vanhemelrijck, 1998).

Therefore, the food industry uses combinations of different gums, according to a specific product and its processing conditions. Some examples of gums important for food production include carrageenans (mainly κ and 1-carrageenans), galactomannans (guar, locust bean and tara gums), pectin, and xanthan (Maruyama et al., 2006; Cerqueira et al., 2009; Bayarri et al., 2010). Collagen proteins are also used as stabilizing components in the production of milk-based desserts. Gelatine is composed of animal protein derived from the collagen obtained by acid or alkaline extraction from pigskins, cowhides or bones. Gelatine is applied as foaming and stabilizing agents in the foam structure of mousses and other aerated creams, as well as gelling agents for puddings (Rapaille and Vanhemelrijck, 1998).

Prebiotic ingredients may also present gelling properties in dairy desserts. Contrary to what is observed regarding the addition of several probiotic microorganisms, the processing of dairy dessert with inulin, FOS and/or GOS has the advantage that these prebiotics show good stability during the usual food processes, mainly during heat treatments. At room temperature, solubility of inulin-type fructans reduces with an increased degree of polymerization (DP); therefore high temperature might be required for solubilising long-chain inulin during food product processing. Nonetheless, the β-bonds between the fructose units in FOS may be at least partially hydrolysed

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in very acidic conditions (Kim et al., 2001; Franck, 2002; 2008; Macfarlane et al., 2008). Others factors related to the technological properties of these prebiotic ingredients will be discussed in another section of this review.

Addition of probiotic microorganisms in the production of refrigerated milk-based desserts

The way the strain is prepared can have a significant effect to a successful introduction of probiotics in a food product (Champagne et al., 2005). Although a considerable proportion of probiotic cultures existing in the market is available in the lyophilized condition DVS type (direct vat set) for direct addition to the product, most studies on milk-based desserts report previous strain activation prior to adding it to the product (Helland et al., 2004; Buriti et al., 2007; Corrêa et al., 2008; Magariños et al., 2008). Despite the existence of growth factors for probiotic bacteria in the food matrix itself (sucrose, available proteins, and peptides), the probiotic metabolism is reduced at refrigeration temperatures. Hence, the use of these components is limited during shelf life, when compared to their use during fermentation, for instance. Consequently, the microorganism activation is seen as an important step in the dessert processing. In fact, it is important for probiotic bacteria to be added in sufficient amounts to provide health benefits to the consumers, which requires inoculation of 7-8 log cfu/g, as possible losses during shelf life should be forecasted.

Helland et al. (2004) used *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* Bb12, *L. acidophilus* NCIMB 701748 (1748), and *Lactobacillus rhamnosus* GG strains for pudding production. Firstly, each microorganism was cultivated for 2 consecutive days through the inoculation (1%) in MRS medium, followed by the incubation at 37°C. Cell concentrates were

obtained by centrifugation of this fermented medium and later washing of cells pellets with potassium phosphate 0.05 M (pH 7.0) solution. After centrifugation, the pellets were resuspended in 100 mL of Ringers solution containing 10% sucrose and stored at -80°C. After cooling (37°C) of previously cooked (>90°C/ 20 min) and sterilized (121°C/ 15 min) pudding mixtures, isolated or combined cultures were added aiming to obtain an initial concentration of 7 log cfu/g. After adding the cultures, the pudding mixtures were incubated at 37°C for 12 h and then cooled and stored at temperatures between 4 and 6°C. After this 12-hour fermentation period, increased probiotic microorganisms populations were observed, ranging from 8 to 9.1 log cfu/g in the milk-based puddings.

For the production of milk-based mousses with fruit juice or pulp, Buriti et al. (2007) employed 20 mL of denatured milk for fermentation of a *L. acidophilus* La-5 culture (0.1 to 0.2g culture /kg of product) at 37°C for 150 minutes. This culture was added to the mixture used for mousses preparation after its pasteurizing and cooling at 40°C. The concentration of probiotic microorganism in the final product (day 1) ranged between 6.5 and 7 log cfu/g.

A similar strategy was used by Corrêa et al. (2008). In this study, *Bifidobacterium lactis* BL-04 or *Lactobacillus paracasei subsp. paracasei* LBC 82 (0.05g each) were inoculated into 20 mL of milk, kept at 37°C for 120 minutes for individual or co-culture addition while preparing coconut flan, reaching populations up to 6 to 7 log cfu/g in the final product stored at 5°C.

In Magariños's et al. (2008) study, milk-based desserts were produced from *Lactobacillus* casei Shirota and *B. animalis* Bb12. Two grams of each culture were individually added to 60 mL of milk containing 0.05% L-Cys-HCl, 2% glucose, and 1% yeast extract. *B. animalis* Bb12 and *L. casei* Shirota were incubated at 38°C and at 32°C, respectively, until reaching pH 5.0

before use. The required incubation time to obtain that pH in the inoculate was 1.25 ± 0.05 h and 3.12 ± 0.10 h for *L. casei* Shirota and *B. animalis* Bb12, respectively. The inoculated concentrations were of 9.17 and 9.54 log cfu/g for *L. casei* Shirota and *B. animalis* Bb12, respectively. After inoculate dilution in the final product, populations of both microorganisms were reduced to 8 log cfu/g in the final product, maintaining this population for 14 storage days at 5° C.

PROBIOTIC DESSERTS

Physical-chemical features

The physical-chemical features of probiotic desserts usually depend on the employed strain and if this strain will be used on an isolated or combined basis. Ingredients used in the formulation may also interfere with the probiotic bacteria metabolism, mainly affecting the pH, as a consequence of organic acid production. Helland et al. (2004) followed up the development of organic and volatile compounds in milk-based probiotic puddings stored at 4 to 6°C for 21 days. At the end of the storage period, the *L. rhamnosus* GG strain was responsible for the highest production of lactic acid (close to 10000 mg/kg), citric acid (1819 mg/kg), acetoin (109.4 mg/kg), and ethanol (9.1 mg/kg). The smallest production of lactic acid obtained at the 21st day was attributed to puddings produced with the *L. acidophilus* 1748 strain (close to 5000 mg/kg), equivalent to half of the content produced by *L. rhamnosus* GG. When used separately, *L. acidophilus* La-5 showed the smallest production of citric acid (1447 mg/kg) in milk-based puddings. The authors also observed that the use of *L. acidophilus* La-5 in a co-culture with *B. animalis* Bb12 resulted in the lowest acetoin (33.6 mg/kg) and ethanol (3.5 mg/kg) contents.

Concerning volatile compounds, for all cultures used in pudding production, a reduction in the acetaldehyde content and an increased diacetyl content were noticed. Puddings produced with the *L. acidophilus* 1748 and the *L. rhamnosus* GG strains showed, respectively, the highest (above 2 mg/kg) and the lowest (below 0.5 mg/kg) acetaldehyde contents by the end of the storage period. On the other hand, a pudding prepared with *L. rhamnosus* GG showed a very high diacetyl content on the 21st day (18 mg/kg), when compared to the other formulations (between 2 and 5 mg/kg). In that study, however, the addition of polydextrose (6.0%) did not influence the production of the evaluated compounds and the pH reduction during the storage of puddings prepared with most of those probiotic strains.

On the other hand, Aragon-Alegro et al. (2007) observed that the simultaneous addition of the prebiotic ingredient inulin (5.01%) and of *L. paracasei* LBC 82 to chocolate mousses caused a more significant pH reduction during a 28-day period at 4±1°C (from 6.21 to 5.37), when compared to control-mousses (from 6.22 to 6.01) and mousses containing only *L. paracasei* (from 6.26 to 5.67). Coconut flan, one of the most traditional Brazilian desserts, was studied by Corrêa et al (2008) as a vehicle for *L. paracasei* LBC 82 and *B. lactis* BL 04 strains, isolated or in co-culture. The authors noticed that the use of these strains in co-culture resulted in a higher pH value during the 28 storage days at 5°C (reduction from 6.8 to 6.4), when compared to the one observed for the individual use of *L. paracasei* (reduction from 6.6 to 6.0) only. The study results regarding the use of probiotics in co-culture were quite similar to those observed by Magariños et al. (2008) for milk-based desserts containing *B. animalis* Bb12 and *L. casei* Shirota, with initial pH of 6.8, later reduced to 6.52 at the 21st storage day at 5°C, as well.

Influence of food ingredients, additives and oxygen on probiotic viability

Food ingredients and additives that contribute to specific flavour features, appearance, and consistency are essential in milk-based desserts preparation. Those ingredients and additives comprise sweeteners, fruit, natural and artificial colourings and flavouring agents, thickeners, stabilizers, acidifying agents, among others. These components effects should not interfere with the viability of probiotic bacteria during the products storage. Thus, in order to achieve the sensorial properties desired during the development of a new product, the food technologist ought to consider the tolerance of probiotic microorganisms to the ingredient or additive which will contribute to that product feature (Vinderola et al., 2002a; Buriti et al., 2007; Komatsu et al., 2008).

The influence of several food ingredients and additives, widely used in the production of milk-based products, on viability of bifidobacteria probiotic strains, *L. acidophilus*, and *L. casei* group strains (*L. casei*, *L. paracasei* and *L. rhamnosus*) were tested by Vinderola et al. (2002a). The authors observed that sucrose, commercial flavourings of strawberry, vanilla, and banana, besides a flavouring-colouring commercial mixture of peach, inhibited the tested cultures when used at high concentrations. Bifidobacteria strains were inhibited with 15 to 20% sucrose concentrations. Natural colourings, including carmine, curcuma/bixin, and bixin did not affect the growth of the studied bacteria. Other flavouring-colorant commercial mixtures, like strawberry and vanilla, showed an important inhibition potential in concentrations usually used by the food industry, mainly for bifidobacteria and *L. acidophilus*. The *L. acidophilus* CNRZ 1881 strain was inhibited by strawberry, pineapple, and kiwi juices. Strawberry juice also

inhibited *Bifidobacterium longum* A1 strain. On the other land, when fruit juices were neutralized, they did not affect these strains viability.

Inhibition of probiotic bacteria by high sugar concentrations is due to the adverse osmotic effect and low a_w (Shah and Ravula, 2000). With regard to the fruit juices, the pH and the composition of organic acids, besides other factors, may influence the viability of probiotic bacteria (Kailasapathy et al., 2008; Nualkaekul and Charalampopoulos, 2011). According to Nualkaekul and Charalampopoulos (2011), the pH homeostasis between the intracellular and the extracellular environment of lactic acid bacteria is maintained through the action of a proton translocating ATPase, which requires energy for the extrusion of protons from the cytoplasm. In this way, other essential cellular functions are deprived of ATP at low pH, and cell viability cannot be maintained. Moreover, the authors considered that fruit organic acids are commonly used as preservatives due to their antimicrobial properties; therefore, the probability of a negative effect on probiotic survival might exist.

Regarding the inhibitory effect of flavouring agents, antimicrobial activity is possibly due to the presence of essential oil, reported as capable of inducing cell lysis, and of phenolic compounds, such as eugenol, cinnamic acid, carvacrol and thymol (Inouye et al., 2001; Gutierrez et al., 2009). Nevertheless, Sagdic et al. (in press) reported that supplementation of ice-creams with pomegranate peel extract, peppermint essential oil, ellagic acid, gallic acid, or grape seed extract did not affect the survival of *Lactobacillus casei* Shirota.

In a study conducted with milk-based mousses (Buriti et al., 2007), the addition of passion fruit as concentrated juice or pasteurized frozen pulp reduced *L. acidophilus* La-5 viability in 4.7 log cycles in 21 days of refrigerated storage at 4°C. On the other hand, the reduction on viability

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of the same microorganism was only of 1 log cycle with the addition of pasteurized frozen guava to the refrigerated mousses studied in the same period. The fruits acid effect on the *L. acidophilus* viability in that study was discarded, differently from what was observed by Vinderola et al. (2002a), since acceptable values of the probiotic microorganisms population were maintained (above 6 log cfu/g), even with the addition of lactic acid to mousses produced with guava pulp. Hence, the behaviour variation among the *L. acidophilus* strain employed observed for each mousse formulation was attributed to the different compounds present in the two fruits tested.

Oxygen sensitivity is also considered an important problem in the manufacture and storage of probiotic foods, particularly for highly aerated products containing bifidobacteria (Bolduc et al., 2006; Kawasaki et al., 2006). This fact are in part due to anaerobic or microaerophilic nature of these microorganisms lacking effective oxygen scavenging cellular mechanisms such as catalase production. Toxic oxygen metabolites may accumulate in the cell leading to cell death from oxidative damage (Talwalkar and Kailasapathy, 2004b). As a result, a loss of probiotic viability during manufacture and storage, and a detrimental survival throughout the gastrointestinal tract might take place (Grosso and Fávaro-Trindade, 2004; Kawasaki et al., 2006).

To protect probiotic bacteria from the deleterious effects of oxygen toxicity, many strategies have been evaluated and showed to be effective in dairy products. Some methods reported recommend the use of special high oxygen consuming strains, of ascorbic acid as an oxygen scavenger in specific products, of cysteine as a redox-potential reducing agent, of microencapsulation, besides the use of less permeable to oxygen packaging material, and

oxidative stress adaptation (Hsiao et al., 2004; Talwalkar and Kailasapathy, 2004b; Bolduc et al., 2006; Güler-Akın and Akın, 2007).

Stability of probiotic microorganisms

Different combinations of strains allow the production of dairy products with target technological features and potential nutritional and health benefits. However, microbial interactions, either beneficial (protocooperation) or unfavourable (antagonism) among these cultures may generate undesirable changes in the composition of the bacterial microbiota during the manufacture and cold storage of these products (Vinderola et al., 2002b; 2008). Thus, adequate combinations of probiotic strains should be tested specifically for the product to be used as a vehicle for this combination of microorganisms, as well as the proportion among the different strains should be evaluated during all steps, since its preparation until the end of the storage period (Tamime et al., 2005; Komatsu et al., 2008)

In a study with coconut flan, Corrêa et al. (2008) observed that the average *B. lactis* populations, when used separately or in co-culture with *L. paracasei*, were always maintained above 7.1 log cfu/g during a 28-day storage period. The authors observed a significant variation in *B. lactis* populations, whether or not in the presence of *L. paracasei* (p<0.05). However, this variation was very discrete when the microorganism growth was being considered (around 0.2 cycles log). On the other hand, *L. paracasei* populations were smaller, in the presence or absence of *B. lactis*, in the beginning of storage, 6.60 to 6.42 log cfu/g, respectively. Nevertheless, *L. paracasei* populations increased significantly (p<0.05) for up to 2 cycles log throughout the 28 days storage in the absence of *B. lactis*. During the same storage period, *L. paracasei* populations

also increased significantly (p<0.05) when in co-culture with *B. lactis*, even though variation was very low, not reaching 1 cycle log. The authors concluded that, in spite of the fact that there was no favourable interaction between the two probiotic strains, the microorganisms viability for all studied desserts reached the criteria established for a probiotic food product, resulting in potentially functional products.

On the other hand, a favourable interaction between the probiotic strains (*L. acidophilus* La-5 and *B. animalis* Bb 12) was reported by Helland et al. (2004) in different pudding formulations stored for 21 days between 4 and 6°C. In the milk-based puddings, the probiotic microorganisms populations were very high, close to or higher than 8 log cfu/g, for these and other strains (including *L. acidophilus* 1748 and *L. rhamnosus* GG); *L. acidophilus* La-5 showed the best viability (close to 9 log cfu/g), when added in co-culture with *B. animalis* Bb12.

Protective effect of food ingredients on probiotic bacteria

Considering the several factors mentioned above, which are able to impair culture survival, the development of probiotic desserts ought to aim at probiotic viability preservation during the products' storage. A number of novel technologies are now emerging, which are able to improve the viability of human intestinal strains for probiotic applications, which means that it might be possible to exploit many 'sensitive' cultures which hitherto have been difficult to propagate and maintain at high viability. Various protective compounds may also improve viability of probiotic cultures during manufacture. Examples include glucose to energize cells on exposure to acid and cryoprotectants, such as inulin, to improve survivability during freeze drying. So, the use of

ingredients that result in a protective effect towards these microorganisms should be encouraged (Ross et al., 2005).

Inulin and FOS are prebiotic ingredients widely used by the food industry, due to their extensively documented physiologic and functional benefits (Delzenne and Neyrinck, 2008; Klinder et al., 2008; Roberfroid, 2008; Lobo et al., 2009). These prebiotics are commonly used in the production of milk-based desserts (Franck, 2008). In probiotic and/or synbiotic food products, both inulin and FOS may exert a protecting effect towards added microorganisms, increasing the survival and activity of probiotic bacteria during storage, as well as during their way through the gastrointestinal tract (GIT) (Donkor et al., 2007; Allgeyer et al., 2010; Rodrigues et al., 2011).

Buriti et al. (2010b) observed that total or partial replacement of milk fat employed in the formulation of probiotic guava mousses by the prebiotic ingredient inulin increased the *L. acidophilus* La-5 survival during exposure of this product to *in vitro* simulated gastric and enteric conditions. In that study, *in vitro* gastrointestinal conditions were simulated for 6 h through the addition of the enzymes pepsin (3 g/L), lipase (0.9 mg/L), pancreatin (1 g/L), and bile (10 g/L) and with the variation of pH from 1.6 ± 0.3 up to 7.2 ± 0.3 . Samples were maintained at 37° C throughout the assay period. On the first storage day, all mousses presented *L. acidophilus* populations higher than 7.6 log cfu/g in the beginning of the assay. However, the authors observed that, after 6 h, the population of the probiotic microorganism was below the detection limits for the counting procedure employed (<1.24 log cfu/g) in the control mousse with higher milk fat content. Differently, the samples in which the milk fat was partially or totally replaced by inulin showed significant increased probiotic survival (p<0.05) after 6h of assay.

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On the other hand, even though achieving very favourable populations of *L. paracasei* subsp. *paracasei* LBC 82 in chocolate mousses produced with or without the addition of inulin (above 7 log cfu/g, during 28 storage days at 4°C), Aragon-Alegro et al. (2007) did not observe any influence of this prebiotic ingredient on *L. paracasei* viability.

Other polysaccharides, like polydextrose, may be used as nutritional sources by probiotic bacteria, increasing their viability in food products. However, Helland et al. (2004) did not observe any viability changes in 4 probiotic strains (*L. acidophilus* La-5 and 1748, *B. animalis* Bb12, and *L. rhamnosus* GG), when used in different pudding formulations, whether or not in the presence of polydextrose, during 21 days of storage between 4 and 6°C.

Protein-based ingredients, such as whey protein concentrates (WPC), also show protecting effects towards probiotic bacteria in food products, increasing the microorganisms viability during the product shelf life (Janer et al., 2004; Akalın et al., 2007).

While still evaluating guava mousses, Buriti et al. (2010b) observed that using WPC for partially or totally replacing the added milk fat resulted in a higher *L. acidophilus* population after 28 days of storage under refrigeration (4±1°C), above 6 log cfu/g. On the other hand, for mousses that did not contain any WPC in their formulations, there was a reduction of at least 2 cycles log by the end of storage. Thus, it was possible to obtain healthier products (with reduced fat content) and enough probiotic viability for beneficial *in vivo* effects, assured until the end of its shelf life when using WPC.

PROBIOTIC AND PREBIOTIC MILK BASED DESSERTS

Sensory features

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New ingredients and cultures may change the sensorial product properties and influence the overall acceptability by consumers (Cardarelli et al., 2008). These changes occur mainly through the production of organic compounds and subsequent reduction in pH caused by probiotic microorganisms (as previously demonstrated), changing the product flavour and aroma. Also, texture and appearance changes caused by the use of prebiotic fibres or ingredients, in order to increase culture viability during storage might take place (Helland et al., 2004; Komatsu et al., 2008).

In general, consumers are not willing to accept functional foods that taste worse than conventional foods (Ares et al., 2009a). Thus, the study of sensory features of a product with added probiotic culture or prebiotic ingredient and the consumer reaction to these changes are important steps in the product development (Corrêa et al., 2008). On the other hand, probiotic bacteria, particularly those from the *Lactobacillus casei* group (*L. casei*, *L. paracasei*, and *L. rhamnosus* species), and prebiotic ingredients are of great interest for the food industry to improve food quality (Buriti and Saad, 2007; Franck 2008). Therefore, they are of useful application for dessert production.

Influence of probiotic cultures on acceptance

Very favourable results were obtained by Corrêa et al. (2008). In these authors' study, coconut flans supplemented with probiotic cultures were also assessed regarding sensory features, when compared to a control (without any culture addition). The authors found that the presence of *L. paracasei* LBC 82 and of *B. lactis* BL-04 did not lead to any significant differences regarding the dessert acceptability throughout 21 days of storage (p<0.05). Similarly,

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desserts supplemented with either probiotic cultures, isolated or in a co-culture, did not show any significant differences between each other and the control (p<0.05). On the other hand, the authors reported that, in spite of not having found any significant interaction among the tested strains, regarding the dessert acceptability, there was a tendency for the reduction of acceptability scores by some panellists, when both microorganisms were present in the formulation. Despite this tendency, acceptability scores remained high, above 7.0 on day 21 of storage. Thus, excellent sensory features were kept in all the probiotic dessert formulations.

L. paracasei LBC 82 incorporation in chocolate mousses, without or with inulin, denoted probiotic and synbiotic, respectively, demonstrated to be equally promising (Aragon-Alegro et al., 2007). The authors compared probiotic and synbiotic mousses, along with a control, using preference-ranking test with non-trained panellists. In this assessment, the mousses did not differ significantly among each other (p>0.05), even though a tendency for better results (major preference) was observed for the probiotic formulation.

However, consecutive significant reductions (p<0.05) in the acceptability of probiotic milk-based desserts (containing *B. animalis* Bb12 and *L. casei* Shirota), served with cranberry sauce (added at the moment of the test) were observed by Magariños et al. (2008), after 14 and 21 storage days of storage. According to the authors, the lowest acceptability scores were observed after 21 days, which was correlated to the smallest viability of those microorganisms during storage (below 7.5 log cfu/g).

Influence of prebiotic ingredients on flavour, texture and acceptance

Desserts stand out among the main food categories in which prebiotic ingredients are included. Besides contributing for several beneficial health effects, prebiotic ingredients may be used in milk-based desserts as body and texture agents, stabilizers, and fat and/or sugar substitute (Franck, 2002; 2008).

Oligofructose, a short-chain length oligosaccharide obtained by partial enzymatic hydrolysis of inulin, has a sweetness of about 35%, in comparison with sucrose. Its sweetening profile closely approaches that of sugar, the taste is very clean and without any lingering effect. This ingredient is used in dessert manufacturing, especially successful when combined with fruits, improving mouth perception of aroma and flavour. Moreover, oligofructose may reduce aftertaste in diet desserts, which is due to the presence of intense sweeteners, such as aspartame, acesulfame K, and sucralose. The prebiotic fibre inulin has a bland neutral taste, without any off-flavour or aftertaste. Due to its specific gelling properties, this fibre is the most used prebiotic in the production of desserts. Inulin is used to improve emulsion stability and foam structure in aerated desserts. In dairy mousses (chocolate, fruit, yoghurt, or fresh cheese-based), for example, the incorporation of a low proportion (1–4%) of inulin improves the process-ability for a longer storage period and upgrades the quality, when compared to conventional mousses (Roberfroid, 2005; Franck, 2008; Manthey and Xu, 2010).

The instrumental texture of chocolate mousses supplemented with *L. paracasei* LBC 82, whether or not supplemented with inulin, was assessed in the study conducted by Cardarelli et al. (2008). The authors observed that the addition of inulin (at 5.01%) contributed for higher firmness (5.24 N) and higher absolute adhesiveness (0.956 N) values of those mousses after 28

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days of storage at 4±1°C, confirming the technological property of increased aerated structure stability, attributed to this ingredient.

At the same time, inulin has a smooth fatty mouthfeel and may contribute for a well-balanced flavour release (Franck, 2008). In further studies conducted with chocolate mousses added of *L. paracasei* and inulin, Cardarelli et al. (2008) assessed these desserts through a difference-from-control test with trained tasters, focusing on the attributes of colour, aroma, texture, flavour, and consistence. In that study, mousses with *L. paracasei* and inulin differed from the control in all studied attributes (p<0.05). These differences were considered as favourable and advantageous, especially when concerning texture and flavour.

Inulin as fat substitute in low-fat milk-based desserts

Due to its nutritional and technological properties, the prebiotic fibre inulin has been frequently used as a substitute for fat in food (Villegas and Costell, 2007).

In low-fat dairy desserts, the addition of inulin in low proportions (2–3%) imparts a better-balanced round flavour and a creamier mouthfeel (Franck, 2008). However, the inulin effect on texture and flavour perception in low fat products will depend on a number of factors, and not only on the amount of this ingredient added with the purpose of replacing whole milk fat or milk cream. Several studies demonstrated that the success of inulin as a fat substitute in low-fat dairy desserts is influenced by the balance of inulin concentration with the other ingredients of the formulation, such as whey protein concentrate (Buriti et al., 2010a), polysaccharides – hydroxypopylated waxy maize starch (Tárrega and Costell, 2006b), tapioca starch (González-Tomás et al., 2009b; Arcia et al., 2010), λ-carrageenan (Tárrega et al., 2010) or even milk fat

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(González-Tomás et al., 2009a,b; Buriti et al., 2010a; Torres et al., 2010). Also, the selection of an inulin with a proper DP may determine the best results regarding texture and sensory features of the final product (González-Tomás et al., 2009a,b; Torres et al., 2010; Tárrega et al., 2011; Bayarri et al., 2011).

Native and long-chain inulin develop a gel structure formed by a network of crystalline particles in concentrated aqueous solutions. In general, crystallization rate and gel firmness increase with inulin concentration, shear treatment, and with the presence of seeding crystals after preparation, which depends on the thermal treatment (Torres et al., 2010). Nonetheless, long-chain inulin is more thermally stable, less soluble and more viscous than the native product, and may be used as a fat substitute, with an efficiency that is practically double than that of native inulin (Villegas and Costell, 2007; Tárrega et al., 2011). The property of long-chain inulin to act as fat mimetic or fat replacer is related to its ability to form microcrystals that interact with each other forming small aggregates, which occlude a great amount of water, creating a fine and creamy texture that provides a mouth sensation similar to that of fat (Villegas and Costell, 2007; Meyer et al., In Press).

Tárrega and Costell (2006b) evaluated the influence of adding 6% long-chain inulin (DP ≥ 23) on rheological and sensory features of fat-free vanilla-flavoured milk-based desserts containing different proportions of starch (2.5%, 3.5%, and 4%). Products containing the same starch proportions, but no inulin, were manufactured with whole milk and skimmed milk. The authors observed that the ability of inulin as fat-replacer was remarkable only in desserts with low starch concentrations (2.5% and 3.5%). A possible explanation provided by the authors for those results was that inulin did not interact synergistically with starch and that, under these

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conditions, since both compete for the water present in the formulation, inulin could act as a diluent. In samples with lower starch concentrations (2.5% and 3.25%), there was enough water in the system, so that inulin did not influence the starch granule swelling process. For samples with higher starch concentrations (4%), with part of the water bound to the inulin chains, swelling of the starch granules was limited, the volume fraction of swollen particles was lower, with a consequent reduction in the system viscosity.

Buriti et al. (2010a) assessed the substitution of milk fat by long-chain inulin (DP > 23) and/or WPC in guava mousses containing the probiotic strain L. acidophilus La-5 and the prebiotic fibre oligofructose, stored at $4\pm1^{\circ}$ C for 28 days. In this study, similar instrumental values of firmness and cohesiveness were observed for mousses produced with milk fat, inulin or WPC employed separately, in the proportion of 4% each. On the other hand, simultaneous addition of inulin and WPC, both at 2%, resulted in firmer products (4.76 N) and with reduced cohesiveness (0.39). This way, opposite to what was observed by Tárrega and Costell (2006b) for desserts containing inulin and starch, there was an interaction between inulin and WPC (another ingredient also used as fat substitute), which resulted in mousses with very different features, when compared to the original product.

The addition of a long-chain length inulin (DP \geq 23) at 7.5% in formulations of custard desserts prepared with whole and skimmed milk influenced upon the rheological features of these products during storage at $4\pm1^{\circ}$ C, as reported by Torres et al. (2010). According to the authors, depending on the amount of fat present in the milk used in the production of dairy dessert, the degree in which long-chain inulin influences upon rheology of samples may vary.

In the study of González-Tomás et al. (2009a), starch-based dairy desserts prepared with skimmed milk and long-chain inulin exhibited similar rheological behaviour and sensory characteristics to whole-milk samples without inulin, regarding creaminess and consistency intensity. Nonetheless, the authors reported significant increase of roughness intensity by the addition of long-chain inulin (p<0.05), which was not verified when short-chain or native inulin were used instead. The authors attributed this negative effect on sensory characteristics of samples to the probable presence of crystal aggregates of long-chain inulin with different particle sizes.

Information about the features of the inulin aggregates in starch-based dairy desserts obtained from Low Temperature Scanning Electron Microscopy (Cryo-SEM) and Light microscopy was given by Meyer et al. (In Press). In cryo-SEM micrographs, samples prepared with skimmed-milk and long-chain inulin presented some aggregates, characterised by a rough outline with pointed edges, embedded in the matrix formed by a network of milk proteins, where starch granules were immersed. Using light microscopy, some large particles or aggregates and more very fine particles were also found in these samples. In this way, the authors confirmed the previous assumption of González-Tomás et al. (2009a) that the aggregation of inulin crystals took place in the continuous phase of low-fat starch-based dairy desserts with long-chain inulin.

Therefore, all these studies involving the addition of inulin in low-fat dairy desserts show the importance of evaluating a suitable combination of ingredients in formulations, so that undesired changes which mischaracterize these products are avoid.

CONCLUSIONS

In general, the use of probiotic cultures and prebiotic ingredients in milk-based desserts has been very promising and the growing number of studies conducted in this area shows very favourable results. Most studies show that probiotic desserts present enough populations of viable cultures for their *in vivo* beneficial effects during their shelf lives. Efforts have been attained, aiming to obtain even higher stability of these microorganisms during storage, without resulting in changes in these products' sensory features. At the same time, the fibre inulin has been the most used ingredient for the purpose of developing prebiotic milk-based desserts, due to its health benefits and its advantageous technological properties for this food line. Researches in this sector, as well as the development of new dessert formulations tend to grow within the next years.

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