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Viability of *Lactobacillus acidophilus* in synbiotic guava mousses and its survival under *in vitro* simulated gastrointestinal conditions

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ABSTRACT

The effects of refrigeration, freezing and substitution of milk fat by inulin and whey protein concentrate (WPC) on *Lactobacillus acidophilus* La-5 viability and resistance to gastric and enteric simulated conditions in synbiotic guava mousses effects were investigated. Refrigerated mousses supplemented with WPC presented the best probiotic viability, ranging from 7.77 to 6.24 log cfu/g during 28 days of storage. The highest probiotic populations, above 7.45 log cfu/g, were observed for all frozen mousses during 112 days of storage. Decreased *L. acidophilus* survival during the *in vitro* gastrointestinal simulation was observed both for refrigerated and frozen mousses. Nonetheless, for the refrigerated mousses, the addition of inulin enhanced the probiotic survival during the *in vitro* assays in the first week of storage. *L. acidophilus* survival in simulated gastrointestinal fluids was also improved through freezing. The frozen storage may be used to provide increased shelf-life for synbiotic guava mousses. Even though the protective effect of inulin and WPC on the probiotic microorganism tested was shown to be more specific for the refrigerated products, the partial replacement of milk fat by these ingredients may also help, as it improves the nutritional value of mousses in both storage conditions.

1. Introduction

In an effort to expand the probiotic product range, intensive research endeavours have focussed on protecting the viability of probiotic cultures during product manufacture and storage and during the gastrointestinal transit (Ross et al., 2005; Corrêa et al., 2008). A number of approaches have been investigated for enhancing the survival of probiotic bacteria in acid and enteric conditions, including physical protection conferred by an adequate choice of the food system (Schillinger et al., 2005; Corcoran et al., 2007).

Resistance to gastric acid and physiological concentrations of bile is among the *in vitro* tests that are frequently suggested for the evaluation of the probiotic potential of a bacterial strain (Schillinger et al., 2005; Mathara et al., 2008). Even though these assays are not fully adequate to predict the functionality of the strain in the human body (Vizoso Pinto et al., 2006), the study of the tolerance to gastrointestinal conditions conducted with probiotic bacteria incorporated in the final product seems to be helpful in the selection of an adequate food matrix that contributes for probiotic survival in the gastrointestinal tract (GIT) (Schillinger et al., 2005).

Inulin-type fructans may exert a protective effect as prebiotic food ingredients, improving the survival and activity of probiotic bacteria during the storage of probiotic foods, as well as the passage through the GIT (Donkor et al., 2007). Other ingredients like whey protein concentrate (WPC) may also present similar protective effects, increasing the maintenance during the shelf-life and the resistance against the pHs changes and the enzymes secreted during passage through the GIT, allowing the probiotic bacteria to reach the intestine in a higher viable cell concentration (Kos et al., 2000; Akalın et al., 2007).

The current consumers' interest towards reduced or low-fat products that contribute to decreased risks of chronic-degenerative diseases encourages the development of probiotic low-fat foods containing inulin and/or WPC, once both ingredients can be employed as fat replacers, due to their excellent properties as emulsifiers and texture agents (Akalın et al., 2007; Franck, 2008). Additionally, the physiological, immunological and functional benefits involving the consumption of foods containing either inulin-type fructans or whey proteins have been extensively reported by several authors (Lobo et al., 2007; Luhovyy et al., 2007; Pérez-Cano et al., 2007; Kolida and Gibson, 2008).

Aerated dairy creams and desserts like mousses are emerging as interesting food systems to study the effects of the incorporation of probiotic cultures and functional ingredients like prebiotics (Aragon-Alegro et al., 2007) and whey proteins (Emam-Djome et al., 2008). Although the industrial production of mousse is delicate, requiring knowledge about the formation and stabilization of foam, and the use of emulsifiers and stabilizers (Cardarelli et al., 2008a), this food

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Table 1
Simplex-centroid experimental design employed in the present study.

Trials	Proportion of each ingredient in the mixture (x_1, x_2, x_3)	Amounts of each ingredient (g) in 100 g of mousse		
		Milk fat (x_1)	Inulin (x_2)	WPC (x_3)
T1 – control	(1, 0, 0)	4	0	0
T2	(0, 1, 0)	0	4	0
T3	(0, 0, 1)	0	0	4
T4	(½,½, 0)	2	2	0
T5	(½, 0, ½)	2	0	2
T6	(0, ½, ½)	0	2	2
T7	(⅓, ⅓, ⅓)	1.33	1.33	1.33

product permits the addition of several flavouring agents and can be stored under refrigeration or freezing (Buriti et al., 2007b).

Chocolate and fruit juices or pulps are the main flavouring agents employed for the production of mousse (Aragon-Alegro et al., 2007; Buriti et al., 2007b). In Brazil, guava (*Psidium guajava*), a typical fruit of tropical and subtropical regions, seems to be an excellent flavouring agent for dairy desserts, due to its large production and consumption (Zietemann and Roberto, 2007). With regard to the emulsification and foaming processes, inulin and WPC may be useful for the manufacture of probiotic mousse with low-fat content. The additional cryoprotectant effect of inulin, leading to changes in the nucleation patterns and reducing the ice-crystal sizes (Franck, 2008), also contributes for the maintenance of the probiotic microorganism viability during the frozen storage.

The aim of this study was to investigate the effect of refrigeration, freezing and substitution of milk fat by inulin and WPC on the viability and resistance to simulated gastric and enteric conditions of a commercial probiotic strain of *Lactobacillus acidophilus* added in synbiotic guava mousses.

2. Material and methods

2.1. Experimental design and guava mousses manufacture

Seven pilot-scale guava mousse-making trials, denoted T1–T7, were prepared according to Table 1, using a *simplex-centroid* design, replacing the milk fat from milk cream (x_1) by inulin (x_2) and WPC (x_3) in different proportions. Combinations of the ingredients: commercial sterilized milk cream (25% fat, Nestlé, Araçatuba, Brazil), prebiotic fibre inulin (98.5% inulin on whole matter, Beneo® HP-Gel, Orafti, Oreye, Belgium) and WPC (81% whey protein, WPC 80, Kienast & Kratschmer, Santo André, Brazil) were used. All trials were performed using the probiotic culture of *L. acidophilus* La-5 (Christian Hansen, Hørsholm, Denmark) and the prebiotic fibre oligofructose (FOS) (Beneo® P95, Orafti). The proportion of 6% FOS in all trials was chosen, according to the amounts of fructans considered effective to confer prebiotic benefits (Kolida and Gibson, 2008) to compose a synbiotic food. The complete list of ingredients used for the production of the different guava mousses and the total solids provided by the sum of all components are described in Table 2.

All guava mousses were produced in amounts to obtain 4 kg of the final product with commercial skimmed milk (Paulista, Divisão de Beneficiamento da Danone, Guaratinguetá, Brazil, ultra high temperature [UHT]), skimmed powdered milk (Molico, Nestlé, Araçatuba, Brazil), sucrose (União, Coopersucar-União, Limeira, Brazil), pasteurised and frozen guava pulp (*Psidium guajava*; Icefruit-Maisa, Icefruit Comércio de Alimentos, Tatuí, Brazil), lactic acid (85% food-grade solution, Purac Sínteses, Rio de Janeiro, Brazil) and emulsifier (Cremodan® Mousse 30-B, Danisco, Cotia, Brazil).

For mousse manufacture, skimmed powdered milk, FOS and WPC (for T3, T5–T7) were incorporated previously in the skimmed milk one day before the production, so as to dissolve these powdered ingredients completely, and maintained under refrigeration at 4 °C, until mixture with further ingredients. One portion of around 40 ml of this pre-mixture was sterilized and employed, in the following day, for the fermentation of the probiotic culture at 37 °C for 150 min. Meanwhile, the further ingredients were mixed and heated during approximately 10 min in order to achieve 85 °C in a pilot-scale universal mixture machine (Geiger UMMSK-12, Geiger, Pinhais, Brazil). Next, the temperature was reduced to 40 °C for the addition of the enriched fermented milk with the *L. acidophilus* culture. Then, the mass was cooled to 10–15 °C for aeration in a planetary beater (Amadio model 20, Irmãos Amadio, São Paulo, Brazil). In this process, the mass achieved about 80–85% of its initial volume, as established in a previous study (Buriti et al., 2007b). Subsequently, portions of 25 g of mousses were packaged in appropriate polypropylene plastic pots for food products (68 mm of diameter, 32 mm of height, 55 ml of total volume, Tries Aditivos Plásticos, São Paulo, Brazil) with a manual dispenser (Intelimaq model IQ81-A, Intelimaq Máquinas Inteligentes, São Paulo, Brazil) and sealed with a metallic cover with varnish in a sealer (Delgo Nr. 1968, Delgo Metalúrgica, Cotia, Brazil). The batches of mousses were divided into two halves: one half was stored under refrigeration (4 ± 1 °C) during 28 days and the other one was stored under freezing (−18 ± 3 °C) for up to 112 days. The shelf-life of refrigerated and frozen products was established based on previous studies conducted in Brazil with refrigerated probiotic dairy desserts

Table 2
Ingredients used for the production of the guava mousse trials studied (T1–T7), according to the experimental design described in Table 1, including the total solids provided by the sum of all components.

Ingredients (%)	Mousses						
	T1	T2	T3	T4	T5	T6	T7
Skimmed milk	47.25	59.25	59.25	53.25	53.25	59.25	55.25
Sterilized milk cream* (25% fat)	16.0	0	0	8.0	8.0	0	5.33
Inulin**	0	4.0	0	2.0	0	2.0	1.33
WPC***	0	0	4.0	0	2.0	2.0	1.33
FOS	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Skimmed powdered milk	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Pasteurised and frozen guava pulp	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Sucrose	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Emulsifier/stabilizer	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Lactic acid	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lactobacillus acidophilus	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total solids	35.1	35.1	35.1	35.1	35.1	35.1	35.1
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* 6.2% non-fat milk solids; 68.8% moisture.
** 98.5% inulin on whole matter.
*** 81.0% protein; 6% fat; 3% ash; 5% lactose; 4% moisture on whole matter.