International Journal of Dairy Technology SDT SOCIETY OF DAIRY TECHNOLOGY

doi: 10.1111/j.1471-0307.2011.00739.x

ORIGINAL RESEARCH

Effect of long-chain inulin on the texture profile and survival of *Lactobacillus paracasei* ssp. *paracasei* in set yoghurts during refrigerated storage

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*The effects of adding inulin at 20 g/L as a fat replacer and probiotic bacteria on the physicochemical and textural characteristics of yoghurt were studied. The ability of long-chain inulin to improve the probiotic (*Lactobacillus paracasei *ssp.* paracasei*) bacteria viability in yoghurt was investigated. The addition of inulin made the texture (firmness, cohesiveness, adhesiveness and gumminess) of skimmed yoghurt similar to that of whole yoghurt, demonstrating the role of inulin as a fat replacer. However inulin increased syneresis and did not influence the viability of probiotic bacteria in the yoghurts. The addition of probiotic bacteria in yoghurts improved syneresis and increased firmness and gumminess.*

Keywords Inulin, Probiotic, Prebiotic, Yoghurt, Fat replacer.

INTRODUCTION

Probiotics are live microorganisms that confer benefits to the host when consumed in adequate amounts (FAO/WHO 2001). Despite the importance of viability of these bacteria, studies conducted have shown poor viability of probiotic bacteria in yoghurts (Lourens-Hattingh and Viljoen 2001).

Inulin could have a protective effect on probiotic cultures, including increased survival and activity of the cultures during storage of the product (Akalin *et al.* 2004; Aryana and Mcgrew 2007; Donkor *et al.* 2007). This effect was ascribed to inulin be a substrate available for metabolism; to the release in the milk of additional nutrients like aminoacids, or to the ability of inulin to protect the bacterial cells from damage caused by the environment (Makras *et al.* 2005; Oliveira *et al.* 2011a). The growth and viability of probiotic cultures in the presence of inulin varies with the degree of polymerisation of the inulin and is strain specific (Makras *et al.* 2005).

Inulin has been used as a fat replacer in the industry and has shown positive effects on the rheology and storage stability of dairy products (Guven *et al.* 2005). The addition of inulin has a greater influence on the texture of the products, because it is incorporated into the product matrix, conferring and enforcing already existent bonding between different components of the food and, when used as a fat replacer, is directly responsible for the softness and creaminess of the food when ingested (Cruz *et al.* 2010). The addition of probiotic bacteria can improve technological characteristics of fermented dairy products, like stability and texture, mainly because certain strains, such as *Lactobacillus paracasei,* are able to produce exopolysaccharides (EPS), which can act as thickeners, stabilisers, emulsifiers and gelling and water-binding agents in the food (Duboc and Mollet 2001; Oliveira *et al.* 2011b). Few studies have evaluated the effect of adding inulin and/or probiotic cultures on the instrumental textural parameters of yoghurt (La Torre *et al.* 2003; Brennan and Tudorica 2008; Oliveira *et al.* 2011b).

The aim of this study was to investigate the ability of long-chain inulin to improve the probiotic (*Lactobacillus paracasei* ssp. *paracasei)* viability and the influence of inulin on the textural characteristics of yoghurt when used as a fat replacer. In addition, the effects of storage (4 °C for 28 days) and probiotic addition on the viability of the starter and probiotic cultures and on the physicochemical and textural characteristics of yoghurt were studied.

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Correction added after online publication 15 November 2011: chemical composition changed from (g 100/g) to (g/100g).

MATERIALS AND METHODS

Materials

Whole and skimmed milk powder (SMP) (Nestlé, Araçatuba, São Paulo, Brazil), the long-chain inulin (Rafitiline HP, Orafti, Oreye, Belgium, degree of polymerisation of 23) and the probiotic culture (*Lactobacillus paracasei* ssp. *paracasei,* L. casei-01, Chr. Hansen; Valinhos, São Paulo, Brazil) were used in the experiment. Lyophilised mixed cultures containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (YC X-11, Chr. Hansen; Valinhos) were used as starter culture.

Preparation of yoghurts' formulations

Five formulations were prepared: whole; normal skimmed; prebiotic; probiotic; and synbiotic, as shown in Table 1.

Whole milk powder (whole formulation) or SMP (normal skimmed, prebiotic, probiotic and synbiotic formulations) was reconstituted to 13 g/L with distilled water. To adjust the solid content in the milk, in formulations with no inulin addition (whole, normal skimmed and probiotic) 35 g/L of SMP were added. In formulations with inulin (prebiotic and synbiotic) 15 g/L of SMP and 20 g/L of inulin were added (Akalin *et al.* 2004). The mixtures were heated to 85 °C for 30 min and were cooled to 42 °C (Akalin *et al.* 2004). Then, 0.1 U/L of the starter culture was used to inoculate the milk mixtures. Probiotic and synbiotic formulations were also added with 0.1 g/L of the probiotic *Lactobacillus paracasei* ssp. *paracasei.* The yoghurt base was put into 80-mL plastic cups and incubated at 42 °C until it reached pH 4.5. The yoghurts were stored at 4 °C for 28 days (Donkor *et al.* 2007).

Chemical composition

Analysis of the moisture, protein, fat, ash and lactose (Fehling method) contents was performed according to AOAC (1995) and the inulin concentration was determined using a Fructan HK enzymatic kit (Megazyme International Ireland Ltd, Wicklow, Ireland).

Evaluation of post-acidification characteristics

Yoghurts were analysed after 1, 7, 14, 21 and 28 days of storage. At each sampling day, microbiological analysis and measurement of syneresis (using duplicate samples) and physicochemical and texture analysis (using triplicate samples) were carried out.

Table 1 Experimental design of yoghurt formulations

Formulations	*Type of milk powder*	*Skimmed milk powder (g/L)*	*Long-chain inulin (g/L)*	Lactobacillus paracasei *ssp.* paracasei *(g/L)*
Whole	Whole	35	–	–
Normal skimmed	Skimmed	35	–	–
Prebiotic	Skimmed	15	20	–
Probiotic	Skimmed	35	–	0.1
Synbiotic	Skimmed	15	20	0.1

pH and titratable acidity

The pH of the yoghurts was determined using a calibrated digital pH meter (Tecnal Tec 3MP; Piracicaba, São Paulo, Brazil). The titratable acidity was determined according to the AOAC method (1995).

Texture profile analysis (TPA)

TPA [firmness (*N*), cohesiveness (dimensionless), adhesiveness (*Ns*) and gumminess (*N*)] was carried out using a TA-XT2i Universal Texture Analyzer (Stable Microsystems, Godalming, UK). Formulations, in the original containers, were compressed at a depth of 8 mm using a type P 25/L acrylic cylinder probe with compression rate of 2 mm/s and a force of 0.10 N for 0.5 s.

Syneresis

The amount of whey released in the formulations was measured according to the method of Aryana (2003). Three cups of yoghurt at 8 °C were inverted onto a fine mesh screen placed on top of a funnel. The quantity of whey collected after 2 h of drainage at 21 °C was used as an index of the formulation's water-holding capacity (whey volume per 100 g of sample).

Microbiological analysis

After homogenisation, 1 mL of each formulation was diluted with 9 mL of sterile 0.1% (w/v) peptone water (Oxoid, Basingstoke, UK), mixed with a vortex mixer and subsequently serially diluted. Viable yoghurt starter culture and *Lactobacillus paracasei* ssp. *paracasei* numbers were determined using the pour plate technique. The counts of *Streptococcus thermophilus* were determined on M17- lactose agar (Himedia, Mumbai, India) after incubating the plates aerobically at 45 °C for 24 h (Tabasco *et al.* 2007). MRS agar (Himedia) adjusted to pH 5.2 and anaerobic incubation at 45 °C for 72 h was used for the determination of *Lactobacillus delbrueckii* ssp. *bulgaricus* counts (Tharmaraj and Shah 2003). Counts of *Lactobacillus paracasei* ssp. *paracasei* were determined on MRS vancomycin agar and anaerobic incubation at 37 °C for 72 h (Tharmaraj and Shah 2003).

Experimental design and statistical analysis

The complete experiment was replicated three times using a completely randomised design. For the physicochemical, texture and microbiological characteristics, analyses were done using a split-plot design, in which the main treatment was the formulation and secondary treatment was the storage duration. Results were evaluated by analysis of variance (ANOVA) and significant differences among means were determined using the ''*t*'' test ($P \leq 0.05$). For the analysis of chemical composition, results were evaluated by analysis of variance (ANOVA) and

significant differences among means were determined using Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Chemical composition

The chemical composition (g/100g) of the formulated yoghurts (day 1) fell within the following ranges: total solids (15.88–16.11); protein (4.6–5.66); fat (0.1 or 3.54); ash (1.001–1.309) (data not shown) and lactose (5.84–8.23) (Table 2). Total solids levels were similar ($P > 0.05$) among formulations. Whole formulation contained a higher fat content (3.54%) than all non-fat formulations (normal skimmed, prebiotic, probiotic and synbiotic; 0.1%). Total protein, lactose and ash levels were higher in normal skimmed and probiotic formulations, because of the higher quantity of SMP added in these formulations when the solid content was adjusted. The lowest values for these parameters were in whole formulation due to the lower levels of these components on whole milk powder. The chemical compositions of the yoghurts are consistent with those of Guven *et al.* (2005) and Khan *et al.* (2008).

Post-acidification characteristics

The formulation x storage time interaction was not significant ($P > 0.05$) for any characteristic evaluated, indicating that formulations had similar behaviour in respect of the storage time.

Physicochemical characteristics

The physicochemical characteristics of yoghurts during storage are shown in Table 2. During refrigerated storage, decreases in pH and lactose content and an increase in titratable acidity and syneresis ($P \leq 0.05$) of the yoghurts were observed. The continuous fermentation of lactose by lactic bacteria causes lactic acid production and higher acidity in the products (Lourens-Hattingh and Viljoen 2001), which results in greater contraction of the casein micelle matrix, increasing the expulsion of whey (Achanta *et al.* 2007).

Table 2 Physicochemical characteristics of yoghurts during storage at 4 °C[a]

Physicochemical characteristics	*Storage time (Days)*	*Formulation*[b]				
		Whole	*Normal skimmed*	*Prebiotic*	*Probiotic*	*Synbiotic*
Lactose content (g/100g)	1	5.84 ± 0.04^{Ad}	8.22 ± 0.06^{Aa}	7.01 ± 0.05^{Ac}	7.03 ± 0.54^{Ab}	7.07 ± 0.02^{Ac}
	7	5.88 ± 0.10^{Bd}	7.96 ± 0.12^{Ba}	6.90 ± 0.21^{Bc}	6.94 ± 0.19^{Bb}	6.80 ± 0.06^{Bc}
	14	$5.83 \pm 0.05^{B,Cd}$	$7.81 \pm 0.12^{B,Ca}$	$6.74 \pm 0.10^{B,Cc}$	$7.01 \pm 0.01^{B,Cb}$	$6.70 \pm 0.10^{B,Cc}$
	21	5.80 ± 0.10^{Cd}	7.78 ± 0.13^{Ca}	6.63 ± 0.15^{Cc}	6.91 ± 0.13^{Cb}	6.78 ± 0.08^{Cc}
	28	5.50 ± 0.09^{Dd}	7.60 ± 0.15^{Da}	6.45 ± 0.14^{Dc}	6.96 ± 0.19^{Db}	$6.58 \pm 0.05^{D,c}$
pH	1	4.47 ± 0.01^{Aa}	4.44 ± 0.01^{Ac}	4.45 ± 0.01^{Ab}	4.44 ± 0.01^{Ac}	4.45 ± 0.01^{Ab}
	7	4.40 ± 0.01^{Ba}	4.37 ± 0.01^{Bc}	4.38 ± 0.01^{Bb}	4.37 ± 0.01^{Bc}	4.38 ± 0.01^{Bb}
	14	4.35 ± 0.01^{Ca}	4.33 ± 0.01^{Cc}	4.33 ± 0.01^{Cb}	4.32 ± 0.01^{Cc}	4.33 ± 0.01^{Cb}
	21	4.28 ± 0.01^{Da}	4.26 ± 0.01^{Dc}	4.26 ± 0.01^{Db}	4.25 ± 0.01^{Dc}	4.26 ± 0.01^{Db}
	28	4.26 ± 0.01^{Ea}	4.24 ± 0.01^{Ec}	4.24 ± 0.00^{Eb}	4.23 ± 0.01^{Ec}	4.24 ± 0.01^{Eb}
Titratable acidity (% lactic acid)	1	0.99 ± 0.07^{Bc}	1.23 ± 0.06^{Ba}	1.07 ± 0.03^{Bb}	1.25 ± 0.03^{Ba}	1.10 ± 0.03^{Bb}
	7	1.04 ± 0.08^{Bc}	1.23 ± 0.04^{Ba}	1.10 ± 0.05^{Bb}	1.30 ± 0.01^{Ba}	1.10 ± 0.01^{Bb}
	14	1.07 ± 0.13^{Ac}	1.28 ± 0.03^{Aa}	1.11 ± 0.03^{Ab}	1.31 ± 0.03^{Aa}	1.14 ± 0.06^{Ab}
	21	1.09 ± 0.12^{Ac}	1.27 ± 0.05^{Aa}	1.13 ± 0.02^{Ab}	1.31 ± 0.03^{Aa}	1.13 ± 0.03^{Ab}
	28	1.08 ± 0.09^{Ac}	1.27 ± 0.04^{Aa}	1.15 ± 0.03^{Ab}	1.31 ± 0.04^{Aa}	1.16 ± 0.03^{Ab}
Syneresis (mL/100g)	1	25.24 ± 1.83^{Db}	23.41 ± 1.67^{Dc}	29.07 ± 3.31^{Da}	20.76 ± 1.23^{Dd}	24.42 ± 2.44^{Db}
	7	28.84 ± 1.24^{Cb}	23.54 ± 0.28^{Cc}	29.98 ± 2.09^{Ca}	22.38 ± 1.66^{Cd}	27.39 ± 2.86^{Cb}
	14	28.47 ± 0.20^{Bb}	25.82 ± 0.74^{Bc}	32.53 ± 2.30^{Ba}	24.34 ± 4.29^{Bd}	28.89 ± 3.01^{Bb}
	21	28.91 ± 2.16^{Bb}	26.02 ± 1.71^{Bc}	33.30 ± 3.69^{Ba}	22.99 ± 1.55^{Bd}	31.70 ± 1.68^{Bb}
	28	34.48 ± 2.65^{Ab}	26.94 ± 0.53^{Ac}	33.92 ± 3.30^{Aa}	25.87 ± 0.56^{Ad}	31.14 ± 2.21^{Ab}
Inulin content (g/100g)	1	–	–	2.01 ± 0.01^{Aa}	–	2.00 ± 0.01^{Aa}
	7	–	–	2.00 ± 0.00^{Aa}	–	2.00 ± 0.00^{Aa}
	14	–	–	2.00 ± 0.00^{Aa}	–	2.00 ± 0.00^{Aa}
	21	–	–	1.98 ± 0.00^{Ba}	–	1.98 ± 0.01^{Ba}
	28	–	–	1.96 ± 0.01^{Ca}	–	1.96 ± 0.01^{Ca}

[a]Means ± SD in the same column with different capital letters superscripts indicating significant difference at $P \leq 0.05$ for each formulation affected by storage. Means ± SD in the same row with different small letters superscripts indicating significant difference at $P \leq 0.05$ among yoghurt formulations for the same day of storage ($n = 9$, excepted for syneresis $n = 6$).

[b]Formulation: whole; normal skimmed; prebiotic (skimmed + inulin); probiotic (skimmed + *Lactobacillus paracasei* ssp. *paracasei*); synbiotic (skimmed + inulin + *Lactobacillus paracasei* ssp. *paracasei*).

The prebiotic yoghurt had a lower acidity than the normal skimmed yoghurt and a higher acidity than the whole yoghurt ($P \leq 0.05$). The lower acidification of inulin supplemented products compared to the normal skimmed product can increase the shelf life of low fat yoghurts, because the shelf life of yoghurt is often limited by excessive acidification during storage (Akalin *et al.* 2007). The addition of probiotic bacteria did not affect the pH or acidity, as demonstrated by the similar results for the probiotic and the normal skimmed yoghurts with respect to these parameters.

The differences observed among formulations with respect to acidity and pH can be related to the different chemical compositions of the formulations. The protein can interfere with the pH due to the buffering capacity of proteins (Akalin *et al.* 2007). The lactose is the preferred substrate used by microorganisms, leading to the formation of organic acids (Lourens-Hattingh and Viljoen 2001).

The extent of syneresis was affected ($P \leq 0.05$) by the addition of inulin and probiotic bacteria. Probiotic yoghurt underwent less ($P \leq 0.05$) syneresis than any other formulation, which could be related to probable EPS production. The EPS produced by a probiotic culture acts as a food stabiliser that contributes to the yoghurt gel structure, preventing fracture and wheying off (Kailasapathy 2006). Schiavão-Souza *et al.* (2007) observed that the probiotic strain used in this study (*L. casei*-01, Chr. Hansen) was able to produce EPS. A good-quality yoghurt should retain water without undergoing syneresis, therefore, the use of probiotic culture in this study helped to maintain the gel structure of the yoghurts.

The prebiotic yoghurt expelled more whey than other formulations ($P \leq 0.05$). The presence of a long chain polysaccharide likely interfered with a development of a 3-dimensional structure of casein, leading to a weaker gel incapable of retaining water (Lucey *et al.* 1998). The results suggest the need to seek alternatives to prevent this major defect when inulin is used, for example, simultaneous supplementation with probiotic bacteria (as in synbiotic formulation), milk solids or stabilisers.

There was no difference ($P > 0.05$) between formulations with added inulin (prebiotic and synbiotic) with respect to inulin content, which indicates that there was no metabolism of inulin by the probiotic culture during the fermentation and storage periods. There was an average reduction of 2.4% in the inulin content ($P \leq 0.05$) during refrigerated storage, which could be related to acid hydrolysis. In an acidic environment, inulin can be hydrolyzed, resulting in the loss of functional properties (Orafti – Active Food Ingredients 1999).

Ingestion of 4 g of inulin per day is considered effective in stimulating the growth of bifidobacteria in the colonic microbiota (Manning and Gibson 2004). Based on this guideline, 200 g/day of the yoghurts (prebiotic and synbiotic) formulated in this study would be enough to exert significant effects.

Texture characteristics

The texture profiles of yoghurt formulations during storage are shown in Table 3. During storage, the gumminess values remain constant and firmness and adhesiveness of the yoghurt varied, although the values for the 1st and 28th days of storage were not different. On the 28th day, the cohesiveness was lower than on the 1st day. Stability of the texture parameters during storage is desirable, because when these parameters are stable, a product that is a few weeks old will be similar to a newly manufactured product.

The addition of probiotic bacteria resulted in firmer and gummy products ($P \leq 0.05$) and had no effect on the adhesiveness and cohesiveness ($P > 0.05$) of the yoghurts, comparing probiotic formulation with the normal skimmed formulation. EPS probably produced by probiotic cultures could increase the viscosity, water retention and interaction with other components of milk, resulting in increased rigidity of the casein matrix in the final product (Duboc and Mollet 2001). The increased firmness is related to an improvement of the texture of the yoghurt and makes the yoghurt less susceptible to rearrangements within its network and consequently less susceptible to shrinkage and serum expulsion (Brennan and Tudorica 2008; Oliveira *et al.* 2011b).

The firmness is a critical texture characteristic of yoghurts, however, other texture parameters, such as cohesiveness, adhesiveness and gumminess are important for set-type yoghurts (Domagala *et al.* 2006), as these products should be spoonable, firm and free from slimy or deadhead textures (Tamime and Robinson 1999).

Maintaining the cohesiveness and adhesiveness indicated that the addition of probiotic culture did not affect the extent to which the gel could be deformed before its rupture nor the force that would be needed to remove the yoghurt adhered to the spoon or mouth during eating the product (Kumar and Mishra 2003). Adhesive and cohesive yoghurts may be pulled into threads or strings and may have a greater degree of stickiness in the mouth, influencing the consistency and texture negatively (Kailasapathy 2006). With the unchanged levels of cohesiveness, multiplication of firmness and cohesiveness, namely gumminess, also increased. The values for the texture profile parameters of yoghurts are consistent with those of La Torre *et al.* (2003) and Kumar and Mishra (2003) for bovine and buffalo milk yoghurt, respectively.

The prebiotic yoghurt showed values of firmness, cohesiveness, adhesiveness and gumminess similar to those of the whole product and lower than those of the normal skimmed product, except cohesiveness. This indicated that inulin could be used as a fat substitute. Inulin molecules, like fat globules, are dispersed within the casein micelles, interfering with protein matrix formation and resulting in the formation of a softer gel (Paseephol *et al.* 2008). Skimmed products, in turn, have a compact protein matrix with fewer spaces, and are therefore firmer (Brennan and Tudorica 2008).

Bacteria survival

The starter (Figures 1a,b) and probiotic (Figure 1c) counts were similar on the 1st and 28th days ($P > 0.05$), indicating the

Table 3 Texture profile of yoghurt formulations during storage at 4 °C[a]

Texture profile parameters	*Storage time (Days)*	*Formulation*[b]				
		Whole	*Normal skimmed*	*Prebiotic*	*Probiotic*	*Synbiotic*
Firmness (*N*)	1	$1.82 \pm 0.59^{Bb,c}$	1.87 ± 0.32^{Bb}	1.84 ± 0.16^{Bc}	2.52 ± 0.16^{Ba}	1.99 ± 0.33^{Bb}
	7	$1.90 \pm 0.71^{Ab,c}$	2.25 ± 0.22^{Ab}	1.87 ± 0.17^{Ac}	2.51 ± 0.31^{Aa}	2.09 ± 0.22^{Ab}
	14	$1.92 \pm 0.29^{Ab,c}$	2.20 ± 0.07^{Ab}	1.88 ± 0.17^{Ac}	2.64 ± 0.28^{Aa}	2.05 ± 0.20^{Ab}
	21	$2.21 \pm 0.28^{A,Bb,c}$	$2.09 \pm 0.19^{A,Bb}$	$1.77 \pm 0.14^{A,Bc}$	$2.29 \pm 0.12^{A,Ba}$	$2.00 \pm 0.28^{A,Bb}$
	28	$2.10 \pm 0.13^{A,Bb,c}$	$2.06 \pm 0.35^{A,Bb}$	$1.88 \pm 0.21^{A,Bc}$	$2.41 \pm 0.39^{A,Ba}$	$2.01 \pm 0.25^{A,Bb}$
Cohesiveness	1	0.41 ± 0.03^{Aa}	0.42 ± 0.02^{Aa}	0.40 ± 0.01^{Aa}	0.41 ± 0.02^{Aa}	0.41 ± 0.02^{Aa}
	7	0.41 ± 0.05^{Aa}	0.41 ± 0.01^{Aa}	0.40 ± 0.04^{Aa}	0.42 ± 0.04^{Aa}	0.40 ± 0.02^{Aa}
	14	0.39 ± 0.01^{Ba}	0.40 ± 0.02^{Ba}	0.40 ± 0.01^{Ba}	0.39 ± 0.02^{Ba}	0.38 ± 0.01^{Ba}
	21	0.40 ± 0.01^{Aa}	0.43 ± 0.03^{Aa}	0.42 ± 0.03^{Aa}	0.40 ± 0.01^{Aa}	0.41 ± 0.04^{Aa}
	28	0.39 ± 0.01^{Ba}	0.40 ± 0.04^{Ba}	0.39 ± 0.01^{Ba}	0.40 ± 0.04^{Ba}	0.40 ± 0.04^{Ba}
Adhesiveness (*Ns*)	1	1.15 ± 0.41^{Bb}	1.08 ± 0.13^{Ba}	1.03 ± 0.08^{Bb}	1.24 ± 0.37^{Ba}	0.99 ± 0.09^{Bb}
	7	$0.94 \pm 0.23^{A,Bb}$	$1.19 \pm 0.28^{A,Ba}$	$1.06 \pm 0.18^{A,Bb}$	$1.32 \pm 0.25^{A,Ba}$	$1.09 \pm 0.20^{A,Bb}$
	14	1.05 ± 0.21^{Ab}	1.57 ± 0.16^{Aa}	1.07 ± 0.14^{Ab}	1.30 ± 0.34^{Aa}	1.09 ± 0.15^{Ab}
	21	$1.19 \pm 0.23^{A,Bb}$	$1.33 \pm 0.20^{A,Ba}$	$0.97 \pm 0.07^{A,Bb}$	$1.18 \pm 0.19^{A,Ba}$	$1.06 \pm 0.18^{A,Bb}$
	28	$1.18 \pm 0.17^{A,Bb}$	$1.32 \pm 0.49^{A,Ba}$	$1.14 \pm 0.19^{A,Bb}$	$1.24 \pm 0.24^{A,Ba}$	$1.10 \pm 0.21^{A,Bb}$
Gumminess (*N*)	1	0.79 ± 0.22^{Ac}	0.78 ± 0.08^{Ab}	0.75 ± 0.05^{Ac}	1.07 ± 0.05^{Aa}	0.79 ± 0.14^{Ac}
	7	0.72 ± 0.24^{Ac}	0.85 ± 0.12^{Ab}	0.73 ± 0.07^{Ac}	1.02 ± 0.13^{Aa}	0.78 ± 0.07^{Ac}
	14	0.78 ± 0.12^{Ac}	1.00 ± 0.05^{Ab}	0.73 ± 0.06^{Ac}	1.08 ± 0.15^{Aa}	0.76 ± 0.06^{Ac}
	21	0.76 ± 0.30^{Ac}	0.89 ± 0.06^{Ab}	0.76 ± 0.04^{Ac}	0.93 ± 0.04^{Aa}	0.82 ± 0.07^{Ac}
	28	0.81 ± 0.04^{Ac}	0.86 ± 0.16^{Ab}	0.75 ± 0.12^{Ac}	0.96 ± 0.15^{Aa}	0.78 ± 0.04^{Ac}

[a]Means ± SD in the same column with different capital letters superscripts indicating significant difference at $P \leq 0.05$ for each formulation affected by storage. Means ± SD in the same row with different small letters superscripts indicating significant difference at $P \leq 0.05$ among yoghurt formulations for the same day of storage ($n = 9$).

[b]Formulation: whole; normal skimmed; prebiotic (skimmed + inulin); probiotic (skimmed + *Lactobacillus paracasei* ssp. *paracasei*); synbiotic (skimmed + inulin + *Lactobacillus paracasei* ssp. *paracasei*).

maintenance of viability under experimental storage conditions. This maintenance is important for compliance with food safety laws and to classify yoghurts as probiotic foods.

Probiotic and synbiotic formulations contained greater than 10^7 CFU/mL of *Lactobacillus paracasei* ssp. *paracasei* at storage time (Figure 1c), therefore, the yoghurts contained higher levels than those considered to have a probiotic effect (10^6 CFU/g) (Donkor *et al.* 2007). These results indicate that there was good compatibility between the probiotic and the starter cultures and that it may be possible to use the health and functional claims attributed to probiotics.

There were no differences in the viability of *Streptococcus thermophilus* among yoghurt formulations ($P > 0.05$) (Figure 1a), indicating that the addition of inulin or probiotic bacteria did not interfere in the growth of this microorganism. The average counts of *Streptococcus thermophilus* during storage were higher than 9 log CFU/mL, which is consistent with other studies (Akalin *et al.* 2007).

Normal skimmed and probiotic formulations had higher counts of *Lactobacillus delbrueckii* ssp. *bulgaricus*, with no differences between those two formulations ($P > 0.05$), while whole yoghurt had the lowest counts (Figure 1b). These differences could be related to the lactose content of the formulations. *Lactobacillus delbrueckii* ssp. *bulgaricus* is the bacteria primarily responsible for the acidification of fermented milk products, thus, it is possible that formulations with more lactose content, which therefore, have more substrate to be degraded into organic acids, had better conditions for the development of this bacteria and thus had higher counts.

The average counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* during storage were lower than 6 log CFU/mL, which is low when compared to the results of other studies (Akalin *et al.* 2004). However, it has been demonstrated that lower counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* are advantageous for the viability of probiotic organisms as *Lactobacillus delbrueckii* ssp. *bulgaricus* lowers the pH during storage to a level that may affect negatively probiotic organisms (Kailasapathy 2006).

The addition of inulin did not affect the viability of the probiotic bacteria ($P > 0.05$), as demonstrated by the similar counts of the probiotic culture in probiotic and synbiotic formulations (Figure 1c). The growth and viability of probiotic cultures in the presence of inulin varies with the degree of polymerisation, being more efficient for short chains (Makras *et al.* 2005). Furthermore, the ability to ferment this carbohydrate is strain-specific; *Lactobacillus paracasei* ssp. *paracasei* is a mesophilic microorganism, and the use of milk powder creates an environment rich in lactose, the preferred substrate of lactic bacteria (Makras *et al.* 2005; Akalin *et al.* 2007). Thus, the degree of

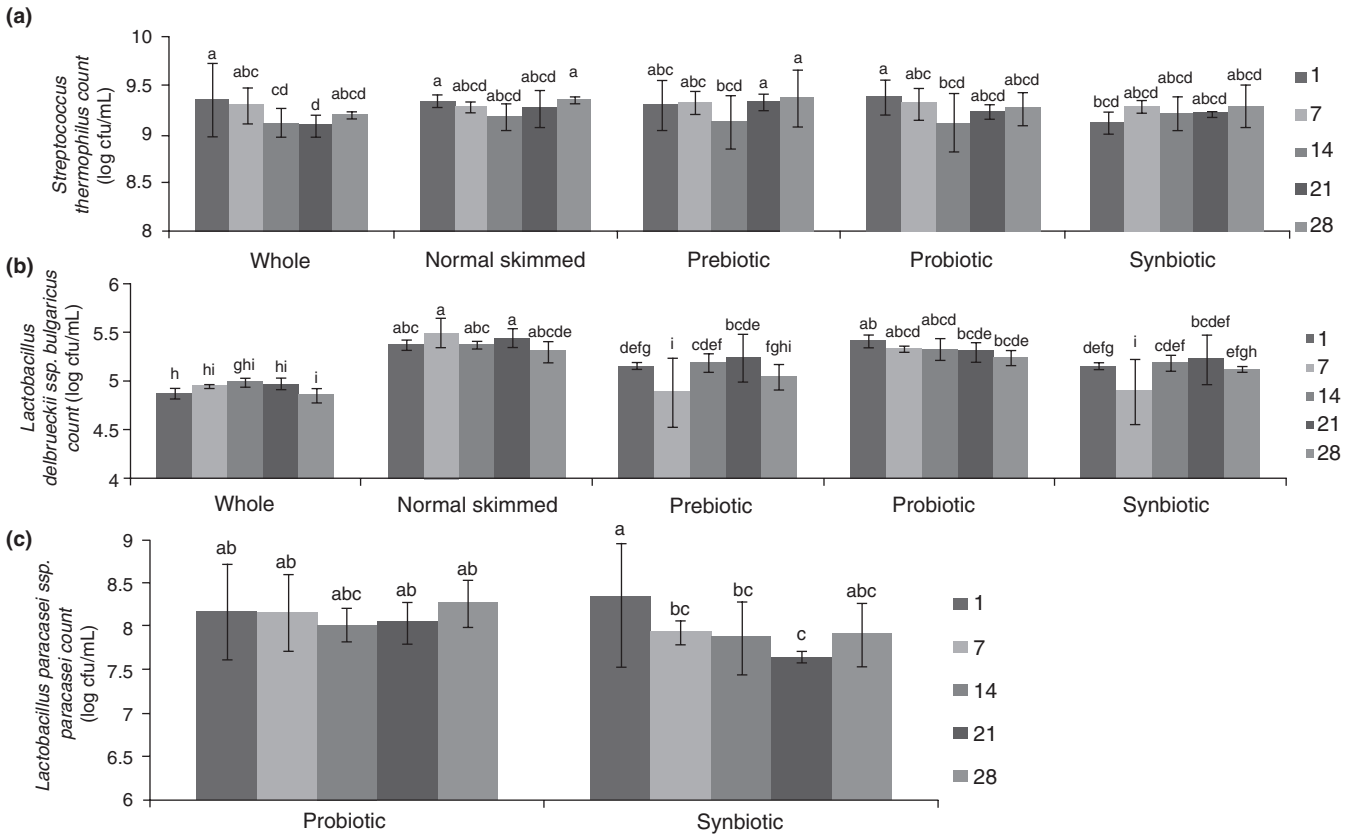

Figure 1 Viability (log CFU per millilitre) of the starter and probiotic bacteria in yoghurt formulations (whole, normal skimmed, prebiotic, probiotic and synbiotic) during refrigerated storage (4 °C). Storage time (days): 1 (■), 7 (■), 14 (■), 21 (■) and 28 (■). (a) Counts of *Streptococcus thermophilus;* (b) Counts of *Lactobacillus delbrueckii* ssp. *bulgaricus;* (c) Counts of *Lactobacillus paracasei* ssp. *paracasei.* Error bars represent standard deviation (n = 6).

polymerisation of inulin (average of 23), the low storage temperature (4 °C) and the use of powdered milk, probably contributed to lack of effect of inulin on the viability of *Lactobacillus paracasei* ssp. *paracasei* in this study.

CONCLUSIONS

Long-chain inulin shows good storage stability in the yoghurt acidic environment and could be used as a fat replacer, as it gave the skimmed yoghurt an instrumental texture profile similar to that of the whole yoghurt. However the addition of inulin increased yoghurt syneresis, suggesting the need to seek methods to prevent this major defect when inulin is used. The addition of *Lactobacillus paracasei* ssp. *paracasei* assisted in water retention in the gel and increased the firmness and gumminess of skimmed yoghurt. Thus, the addition of *Lactobacillus paracasei* ssp. *paracasei* can improve important characteristics of set-type yoghurts. Long-chain inulin did not influence *Lactobacillus paracasei* ssp. *paracasei* probiotic bacteria viability during either the fermentation or storage of yoghurt. Therefore, it is not recommended to use inulin with high degree of polymerisation as a way to improve the viability of the probiotic strain used in this study in yoghurts.

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