



Influence of milk type and addition of passion fruit peel powder on fermentation kinetics, texture profile and bacterial viability in probiotic yoghurts

A.P. do Espírito Santo^{a,b}, P. Perego^b, A. Converti^b, M.N. Oliveira^{a,*}

^a Department of Biochemical and Pharmaceutical Technology, São Paulo University, Av Prof Lineu Prestes, 580, Bl 16, 05508-900 São Paulo, Brazil

^b Department of Chemical and Process Engineering, University of Genoa, Via Opera Pia, 15, 16145 Genoa, Italy

ARTICLE INFO

Article history:

Received 31 August 2011
Received in revised form
27 January 2012
Accepted 28 January 2012

Keywords:

Probiotics
Passion fruit fiber
Yoghurt
Texture

ABSTRACT

The effect of the addition of passion fruit peel powder (PFPP) on the fermentation kinetics and texture parameters, post-acidification and bacteria counts of probiotic yoghurts made with two milk types were evaluated during 28 days of storage at 4 °C. Milks were fermented by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (CY340), and one strain of probiotic bacteria: *Lactobacillus acidophilus* (L10 and NCFM), *Bifidobacterium animalis* subsp. *lactis* (BI04 and HN019). The addition of PFPP reduced significantly fermentation time of skim milk co-fermented by the strains L10, NCFM and HN019. At the end of 28-day shelf-life, counts of *B. lactis* BI04 were about 1 Log CFU mL⁻¹ higher in whole yoghurt fermented with PFPP regarding its control but, in general, the addition of PFPP had less influence on counts than the milk type itself. The titratable acidity in yoghurts with PFPP was significantly higher than in their respective controls, and in skim yoghurts higher than in the whole ones. The PFPP increased firmness, consistency (except for the NCFM strain of *L. acidophilus*) and cohesiveness of all skim yoghurts. The results point out the suitability of using passion fruit by-product in the formulation of both skim and whole probiotic yoghurts.

© 2012 Elsevier Ltd. Open access under the [Elsevier OA license](http://creativecommons.org/licenses/by/3.0/).

1. Introduction

The passion fruit has origin in tropical countries of America, and Brazil is its greatest producer and consumer, exporting the fruit mainly to United Kingdom, France, Belgium, German and the Netherlands (EMBRAPA, 2010). The cultivation of yellow passion fruit (*Passiflora edulis* var. *flavicarpa* Deg., Passifloraceae) has been preferred for industrial juice production that generates large quantities of by-product composed by seeds and shells representing more than half of the total fruit weight (Salgado, Bombarde, Mansi, Piedade, & Meletti, 2010).

Functional properties such as anti-hypertensive, hypocholesterolemic and reduction of blood glucose level, have been attributed to the passion fruit peel (Chau & Huang, 2005; Janebro et al., 2008; Salgado et al., 2010; Zibadi et al., 2007). Beyond the content of 10–20 g of pectin, a soluble fiber which is known for its prebiotic action, the passion fruit peel is composed of approximately 1.5 g of protein, 0.8 g of lipids, 8.7 g of ash, 56 g of carbohydrates per 100 g of dry matter and is also a source of iron, calcium, phosphorus and niacin (Cordova, Gama, Winter,

Kaskantzis Neto, & Freitas, 2005; Yapo & Koffi, 2008). Therefore, it should not be regarded just as an industrial waste, since it can be used for the development of new functional products such as the probiotic ones.

Both dietary fiber and probiotics are reported to relieve constipation and reduce the incidence of colon cancer (Farnworth, 2008; Kaur & Gupta, 2002). In addition, some dietetic fibers from fruit have been recommended as ingredient to probiotic dairy foods because of their beneficial effect on the viability of these bacteria (Espírito-Santo et al., 2010; Kourkoutas et al., 2006; Sendra et al., 2008). However, from the technological point of view the addition of fruit dietetic fiber into a food product with a smooth texture such as yoghurt is a challenge. Both the fermentation and the fragile equilibrium of yoghurt structure can be affected by any fiber added into the milk as well as by the milk type itself (Kumar & Mishra, 2003; Sendra et al., 2008; Sodin, Remeuf, Haddad, & Corrieu, 2004; Staffolo, Bertola, Martino, & Bevilacqua, 2004). The analysis of the texture profile of yoghurt-like products offers some advantages such as reduced test time and quantification of structural breakdown, being a useful technique to evaluate the protein gel strength (Kumar & Mishra, 2003).

The influence of the milk type and the addition of total dietetic fiber from fruits on kinetics and textural properties of fermented milk products still have been underexploited. This study aimed at

* Corresponding author. Tel.: +55 11 3091 3690; fax: +55 11 3815 6386.
E-mail address: monolive@usp.br (M.N. Oliveira).

evaluating the effect of the milk type and of the powder obtained from passion fruit by-product on the kinetic and texture parameters, post-acidification and microorganism counts of probiotic yoghurts during four weeks of cold storage.

2. Materials and methods

2.1. Total dietary fiber preparation

Passion fruit by-product was obtained from an industry of fruit pulp located in the city of Jundiaí, São Paulo State, Brazil. The peels of passion fruit were dried in oven under air flow at 60 °C until constant weight. The dry peels were reduced to fine powder in a Bimby processor (model TM 31, Vorwerk®, Wuppertal, Germany). In order to make the mixture of the fiber powder into the reconstituted milk easier, the particle size was standardized to less than 42 µm, measured through sieves (Granustest, São Paulo, Brazil). The passion fruit peel powder (PFPP) was stored in clapped glass bottles and kept under refrigeration at 4 °C until use.

2.2. Milk preparation

Skimmed milk Molico® and whole milk Ninho® powders (Nestlé, Araçatuba, Brazil) were both reconstituted to 12 g 100 mL⁻¹ of distilled water and each one was divided into two milk samples. In order to define the highest amount of the passion fruit peel powder that caused the minimum volume of whey separation by the end of fermentation, previous fermentation tests were made with the two types of milk in graduated 50 mL Falcon tubes with addition of the powder varying from 0.5 to 1.0 g 100 mL⁻¹ of milk. As result, 0.7 g of PFPP in 100 mL of milk was added into the two types of milk. Samples without the PFPP were used as control. All milk bases were heat treated at 85 °C for 15 min under agitation in a water bath and then divided into sterile Schott® flasks (500 mL), cooled in an ice bath, and stored at 4 °C for 24 h.

2.3. Microbial cultures

We used in this study a freeze-dried starter yoghurt culture (CY340. DSM, Moorebank, NSW, Australia) – composed of *Streptococcus thermophilus* (St) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) – and four probiotics, namely two strains of *Lactobacillus acidophilus* (L10. DSM, and NCFM. Danisco, Madison, WI, USA) and two strains of *Bifidobacterium animalis* subsp. *lactis* (B104 and HN019. Danisco). The lyophilized cultures were diluted in sterilized milk and divided into aliquots into Eppendorf® flasks and frozen at –20 °C. Each inoculum was prepared by thawing the cultures and diluting them into 50 mL of sterilized skim or whole milk, according to the milk base to be fermented. Each Schott® flask containing 500 mL of reconstituted milk was inoculated with 1 mL of yoghurt starter co-culture with an average count of 8.2 Log CFU mL⁻¹ of St and 5.4 Log CFU mL⁻¹ of Lb and 1 mL of probiotic culture with counts around 6.4 Log CFU mL⁻¹ ($P > 0.05$).

2.4. Experimental procedure

Eight different PFPP-enriched yoghurts were prepared using the four probiotic strains in the two different milk bases, plus eight controls without passion fruit peel powder.

After inoculation, the flasks with the samples were transferred to water bath equipment assembled to a CINAC (Cynetique d'acidification, Ysebaert, Frépillon, France) system (Spinnler & Corrieu, 1989), which allows the continuous measurement and recording of pH and the measurement of the four kinetic parameters

considered in this study: (a) the maximum acidification rate (V_{max}), expressed in 10⁻³ pH units per min, (b) the time to reach the maximum acidification rate ($T_{V_{max}}$), (c) the time to reach pH 5.0 ($T_{pH5.0}$), near to the isoelectric point of casein and (d) the time to complete the fermentation ($T_{pH4.5}$), all expressed in hours. Two independent batch fermentations were carried out in duplicate on different days at 42 °C up to pH 4.5.

Once the desired pH was reached, the fermentation time ($T_{pH4.5}$) was recorded and the flasks were cooled to 20 °C in an ice bath. The coagulum was then broken by means of a perforated disk on a stainless steel rod that was moved upwards and downwards for 2 min. The stirred yoghurt was put into 50 mL polypropylene cups, thermally sealed and stored at 4 °C.

2.5. Total solids, post-acidification and titratable acidity

Determination of total solids in milk bases and titratable acidity in yoghurts were made according to AOAC (1995). The post-acidification was determined as pH after 1, 14 and 28 days of cold storage using a pH meter, model Q-400M1 (Quimis, São Paulo, Brazil). The results were expressed as the means of four replicates.

2.6. Microbiological analyses

Bacterial enumerations were carried out after 1, 14 and 28 days of cold storage in four replicates of each batch. Samples (1 mL) were diluted with 0.1 g 100 g⁻¹ sterile peptone water (9 mL). Afterward, serial dilutions were carried out, and bacteria were counted, applying the pour plate technique (Kodaka, Mizuuchi, Teramura, & Nirazuka, 2005). All media were obtained from Oxoid (Basingstoke, UK). In co-cultures, *S. thermophilus* colonies were enumerated in M17 agar, while those of *L. delbrueckii* subsp. *bulgaricus* in MRS (pH 5.4), both under aerobic incubation at 37 °C for 48 h. The probiotic microorganisms were incubated at 37 °C for 72 h under anaerobic conditions provided by AnaeroGen (Oxoid). Enumerations of *L. acidophilus* were carried out in MRS (pH 6.2) plus 10 µL mL⁻¹ clindamycin (50 µg mL⁻¹), and *B. animalis* subsp. *lactis* in Reinforced Clostridial Agar plus 100 µL mL⁻¹ of dicloxacillin (2 mg mL⁻¹). Antibiotics were employed to allow selective growth of the probiotic bacteria. M17 and MRS media (pH 5.4) were prepared according to Jordano, Serrano, Torres, and Salmeron (1992) and Dave and Shah (1996), and MRS plus clindamycin according to Lankaputhra and Shah (1996). Cell concentration was expressed as Log CFU mL⁻¹ of yoghurt.

2.7. Texture profile

Texture measurements were carried out as described by Damin, Minowa, Alcantara, and Oliveira (2008). Firmness was determined at 4–6 °C by penetration tests made with a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, England) on 50 g packed samples. The probe was a 25 mm diameter acrylic cylinder, moved at a pretest speed of 5 mm s⁻¹ and a test speed of 1 mm s⁻¹ through 10 mm within the sample. The results were expressed as the average of three measurements. Texture properties such as firmness, consistency and cohesiveness were considered. As yoghurt presents a pseudoplastic behavior and exhibits partial thixotropy, firmness was measured as the force required to break the structure formed after the cessation of stirring and during the cold storage of the yoghurt (Ramchandran & Shah, 2009; Rawson & Marshall, 1997), consistency as the property by which a material (in this case the yoghurt) resists to a change in shape (Deman, 1983) and cohesiveness as the extent to which the yoghurt could be deformed before it ruptures (Rawson & Marshall, 1997).

2.8. Statistical analyses

The parameters of experimental yogurts were assessed by General Linear Model ANOVA by using Statistica 8.0® software (Statsoft, Tulsa, OK, USA). Different groups were compared by the Tukey test at $P < 0.05$, and statistically significant differences among them were indicated by different letters.

3. Results and discussion

3.1. Total solid, pH and kinetics parameters of acidification

The content of total solids of both whole and skim heat treated milk bases without PFPP was around $13.04 \pm 0.12 \text{ g } 100 \text{ g}^{-1}$, while with PFPP was $14.01 \pm 0.09 \text{ g } 100 \text{ g}^{-1}$. As expected, the presence of PFPP increased significantly the total solids content of milk bases (by approximately 1%, $P < 0.05$). The PFPP addition reduced significantly the initial pH of the milk bases which was 6.42 ± 0.07 and 6.58 ± 0.09 in milks with and without PFPP respectively ($P < 0.05$).

As Table 1 shows, the maximum rate of acidification (V_{\max}) was significantly reduced ($P < 0.05$) by the addition of passion fruit peel powder in both milk types, which can probably be ascribed to the presence of substances with buffering capacity in the passion fruit peel, such as organic acids and phenolic compounds (Zibadi & Watson, 2004). Furthermore, it was observed that control skim yoghurts co-fermented by *Bifidobacterium* strains exhibited higher V_{\max} than the control whole yoghurts co-fermented by the same strains ($P < 0.05$). Nevertheless, the time to reach the maximum acidification rate (T_{\max}) was significantly reduced by the presence of the PFPP only in whole milk bases and in skim ones co-fermented by lactobacilli. The passion fruit peel powder had no effect on the time to reach pH 5.0 ($T_{\text{pH}5.0}$) except for the skim yoghurt co-fermented by *L. acidophilus* NCFM, in which the PFPP reduced this parameter. Moreover, the time to complete fermentation ($T_{\text{pH}5.0}$) in skim control yoghurts co-fermented by *Lactobacillus* strains was longer than in whole ones ($P < 0.05$), thereby indicating a clear effect of the milk type (Table 1).

The fermentation lasted from 4.3 to 5.5 h in whole yoghurts and from 5.3 to 6.8 h in skim yoghurts. Considering the milk type, in general the fermentation was quicker in whole milk than in skim milk ($P < 0.05$), while the addition of passion fruit peel powder significantly accelerated the fermentation in all skim yoghurts, except that performed by *Bifidobacterium lactis* B104. On the other

hand, the fiber had no statistically significant effect on $T_{\text{pH}4.5}$ in whole yoghurts ($P > 0.05$). The largest reduction of $T_{\text{pH}4.5}$ (1 h) due to the passion fruit peel powder addition was observed in skim yoghurt fermented by *L. acidophilus* NCFM ($P < 0.05$), although no statistically significant difference ($P > 0.05$), was noticed in the whole yoghurts fermented by the same probiotic strain.

According to Varghese and Mishra (2008), the buffering capacity is directly proportional to the total solids (TS) content of the fermented product, which can lead to longer fermentation time. This observation, which is certainly valid for TS increasing with milk derivatives, does not seem to be applicable to TS increase induced by passion fruit peel powder addition that in some cases even accelerated the fermentation (Table 1). On the other hand, Almeida, Tamime, and Oliveira (2009) ascribed the different acidification profiles of different LABs to their peculiar capacity to assimilate nutritive compounds of the milk, which could explain the differences in the kinetic parameters observed amongst the various yoghurts. According to McCann, Fabre, and Day (2011), the carrot cell wall addition was clearly the responsible for the reduction in 1 h of the fermentation time of yoghurt fermented by St and Lb. However, in the present study, the correlation analyses indicates that multiple factors, such as the lipid content of the milk, the culture composition and the presence of PFPP can affect the acidification parameters of probiotic yoghurts.

3.2. Post-acidification and titratable acidity

The results of post-acidification (pH) and titratable acidity during the shelf-life of the yogurts are presented in Table 2. After one day of cold storage, the pH of yoghurts ranged from 4.37 to 4.50, and the largest differences between the yoghurts with passion fruit peel powder and the controls were detected in skim yoghurts fermented by *L. acidophilus* L10 (4.42 PFPP yoghurt and 4.50 control) and *B. lactis* B104 (4.42 PFPP yoghurt and 4.48 control) ($P < 0.05$). Titratable acidity varied from 0.64 to 0.74 mg lactic acid g^{-1} in whole yoghurts and from 0.87 to 1.07 mg lactic acid g^{-1} in skim yoghurts. The increase in this parameter induced by the addition of passion fruit peel powder was statistically significant in all yoghurts ($P < 0.05$), but the whole ones co-fermented by *B. lactis* strains.

After 14 days of shelf-life the pH of all yoghurts decreased significantly ($P < 0.05$) and ranged from 4.21 to 4.38 amongst the whole yoghurts and from 4.26 to 4.38 amongst the skim ones. On the other hand, after 28 days, it was observed a slight but significant

Table 1

Kinetic parameters of acidification of plain and skim milks fermented by starter co-culture associated with different probiotic strains with and without passion fruit fiber.

Milk type	Treatment	Probiotic strain	V_{\max} ($10^{-3} \text{ upH min}^{-1}$)	T_{\max} (h)	$T_{\text{pH}5.0}$ (h)	$T_{\text{pH}4.5}$ (h)
Skim	Control	<i>L. acidophilus</i> L10	18.36 ± 0.01^d	2.6 ± 0.01^d	3.35 ± 0.01^{de}	6.9 ± 0.29^h
		<i>L. acidophilus</i> NCFM	16.69 ± 0.02^c	2.6 ± 0.01^d	3.42 ± 0.00^e	6.6 ± 0.22^{gh}
		<i>B. lactis</i> B104	22.35 ± 0.03^e	2.3 ± 0.00^{bcd}	2.88 ± 0.02^{ab}	5.3 ± 0.19^{cd}
		<i>B. lactis</i> HN019	22.91 ± 0.02^e	2.3 ± 0.00^{cd}	$3.02 \pm 0.01^{a-d}$	6.1 ± 0.24^{fg}
	With passion fruit fiber	<i>L. acidophilus</i> L10	13.93 ± 0.01^a	1.9 ± 0.00^{abc}	3.07 ± 0.03^{bcd}	6.1 ± 0.19^{fg}
		<i>L. acidophilus</i> NCFM	14.49 ± 0.01^a	1.9 ± 0.02^{abc}	$3.05 \pm 0.02^{a-d}$	5.6 ± 0.19^{ef}
		<i>B. lactis</i> B104	14.02 ± 0.02^a	2.2 ± 0.01^{bcd}	$3.02 \pm 0.02^{a-d}$	5.8 ± 0.17^{fg}
		<i>B. lactis</i> HN019	14.11 ± 0.02^a	2.6 ± 0.01^d	2.98 ± 0.03^{abc}	5.4 ± 0.17^{cde}
Whole	Control	<i>L. acidophilus</i> L10	17.91 ± 0.02^d	2.3 ± 0.01^{cd}	3.03 ± 0.03^{abc}	5.2 ± 0.16^{bcd}
		<i>L. acidophilus</i> NCFM	16.63 ± 0.02^{bc}	2.3 ± 0.00^{bcd}	2.8 ± 0.04^{ab}	4.4 ± 0.23^{ab}
		<i>B. lactis</i> B104	18.73 ± 0.01^d	2.3 ± 0.00^{bcd}	2.87 ± 0.00^{ab}	5.0 ± 0.20^{abc}
		<i>B. lactis</i> HN019	17.87 ± 0.01^d	2.3 ± 0.04^{bcd}	2.73 ± 0.03^a	4.3 ± 0.25^a
	With passion fruit fiber	<i>L. acidophilus</i> L10	15.66 ± 0.01^b	1.8 ± 0.01^a	3.27 ± 0.01^{cde}	5.5 ± 0.29^{def}
		<i>L. acidophilus</i> NCFM	14.10 ± 0.01^a	1.8 ± 0.00^a	$3.03 \pm 0.00^{a-d}$	4.8 ± 0.18^{abc}
		<i>B. lactis</i> B104	13.90 ± 0.02^a	1.8 ± 0.03^a	2.92 ± 0.03^{ab}	5.0 ± 0.27^{abc}
		<i>B. lactis</i> HN019	14.57 ± 0.03^a	1.8 ± 0.00^a	2.77 ± 0.05^{ab}	4.9 ± 0.21^{abc}

Means ($N = 4$) \pm standard deviation with different letters in the same column are significantly different ($P < 0.05$). V_{\max} , maximum rate of acidification; T_{\max} , time to reach V_{\max} ; $T_{\text{pH}5.0}$, time to reach pH 5.0; $T_{\text{pH}4.5}$, end time of fermentation.

Table 2
Post-acidification (pH) and titratable acidity during shelf-life of control and passion fruit fiber yogurts.

Yoghurts			pH ^a			Titratable acidity (% lactic acid) ^a			
Milk type	Treatment	Probiotic strain	d 1	d 14	d 28	d 1	d 14	d 28	
Whole	Control	La L10	4.50 ^x	4.38 ^{opqr}	4.40 ^{pqrs}	0.87 ^{ij}	0.95 ^k	0.96 ^k	
		La NCFM	4.43 ^{stu}	4.34 ^{klm}	4.40 ^{pqrs}	0.90 ^l	0.98 ^{kl}	1.02 ^{lmn}	
		BI04	4.48 ^{vx}	4.32 ^{hijk}	4.34 ^{lmn}	0.96 ^k	0.98 ^{kl}	1.18 st	
		BI HN019	4.43 ^{tu}	4.28 ^{efg}	4.39 ^{pqr}	0.95 ^k	0.99 ^{klm}	1.14 ^{rs}	
	With passion fruit fiber	La L10	4.43 ^{stu}	4.26 ^{bcd}	4.33 ^{hijk}	0.97 ^k	1.09 ^{pq}	1.10 ^{pq}	
		La NCFM	4.45 ^{tuv}	4.30 ^{ghi}	4.34 ^{klm}	0.97 ^k	1.04 ^{no}	1.08 ^{op}	
		BI04	4.42 ^{stu}	4.29 ^{efg}	4.34 ^{klm}	1.07 ^{nop}	1.10 ^{pq}	1.22 ^{tu}	
		BI HN019	4.47 ^{uvx}	4.30 ^{fgh}	4.32 ^{hij}	1.04 ^{mn}	1.08 ^{op}	1.25 ^u	
	Whole	Control	La L10	4.39 ^{opqr}	4.23 ^{ab}	4.23 ^{abc}	0.66 ^a	0.77 ^{efg}	0.74 ^{de}
			La NCFM	4.42 ^{rst}	4.21 ^a	4.22 ^{ab}	0.65 ^a	0.78 ^{gh}	0.76 ^{ef}
			BI04	4.37 ^{lmn}	4.24 ^{abc}	4.22 ^{ab}	0.67 ^{ab}	0.76 ^{cd}	0.76 ^{cd}
			BI HN019	4.44 ^{tuw}	4.28 ^{efg}	4.26 ^{cde}	0.64 ^a	0.72 ^{cd}	0.73 ^{cd}
With passion fruit fiber		La L10	4.37 ^{lmno}	4.29 ^{fgh}	4.32 ^{fghi}	0.68 ^a	0.74 ^{de}	0.74 ^{de}	
		La NCFM	4.46 ^{uv}	4.35 ^{lmno}	4.38 ^{nop}	0.65 ^{ab}	0.74 ^{de}	0.70 ^{bc}	
		BI04	4.41 ^{rst}	4.32 ^{fghi}	4.31 ^{ghij}	0.75 ^{def}	0.80 ^{hi}	0.78 ^{ghi}	
		BI HN019	4.42 ^{stu}	4.34 ^{klm}	4.33 ^{ijkl}	0.72 ^{cd}	0.80 ^{hi}	0.77 ^{efg}	

^a Standard deviations were under 0.05. Means ($N = 4$) with different letters in the same column are significantly different ($P < 0.05$). Abbreviations: d 1, d 14 and d 28 = days 1, 14 and 28 after fermentation. La NCFM and La L10, *L. acidophilus* strains NCFM and L10; BI HN019 and BI04, *B. animalis* subsp. *lactis* strains HN019 and BI04.

increase in the average pH of control whole yoghurts co-fermented by *L. acidophilus* NCFM and *B. lactis* strains and PFPP whole yoghurts co-fermented by *L. acidophilus* strains and *B. lactis* BI04 ($P < 0.05$). Surprisingly all the whole yoghurts with passion fruit peel powder showed higher pH than their respective controls ($P < 0.05$). However, such a scenario did not happen within the skim yoghurts group. In this case the fiber did in fact promote a significant decrease in the pH of all yoghurts, except that co-fermented by *B. lactis* BI04. A possible explanation of this dual behavior could be the simultaneous occurrence of fatty acid consumption as carbon source after sugar depletion and fiber pectin degradation to uronic acids. Prevalence of the former activity in whole yoghurts was likely responsible for alkalization, whereas its absence in skim yoghurts led to acidification.

After 14 days of shelf-life all whole yoghurts exhibited a significant increase in their titratable acidity, but they still had lower acidity level compared with the skim yoghurts ($P < 0.05$). At 14 and 28 days the highest values of average titratable acidity were

observed in skim yoghurts with passion fruit peel powder ($P < 0.05$).

Considering the whole period of shelf-life, it was observed that the average titratable acidity in yoghurts containing passion fruit peel powder was significantly higher than in their respective controls, and that in skim yoghurts higher than in the whole ones ($P < 0.05$). As far as the probiotic cultures is concerned, in general, the yoghurts co-fermented by *L. acidophilus* strains exhibited lower titratable acidity than those co-fermented by *B. lactis* strains ($P < 0.05$). Such a behavior should be indeed expected by the fact that the homolactic metabolism of the former leads to two lactic acid moles per mole of glucose consumed, while that of bifidobacteria to 1 mol of lactic acid and 1.5 mol of acetic acid.

3.3. Microorganisms viability

During the whole shelf-life, *S. thermophilus* counts were stable and ranged, as an average, from 8.6 to 10.9 Log CFU mL⁻¹ (Fig. 1). In

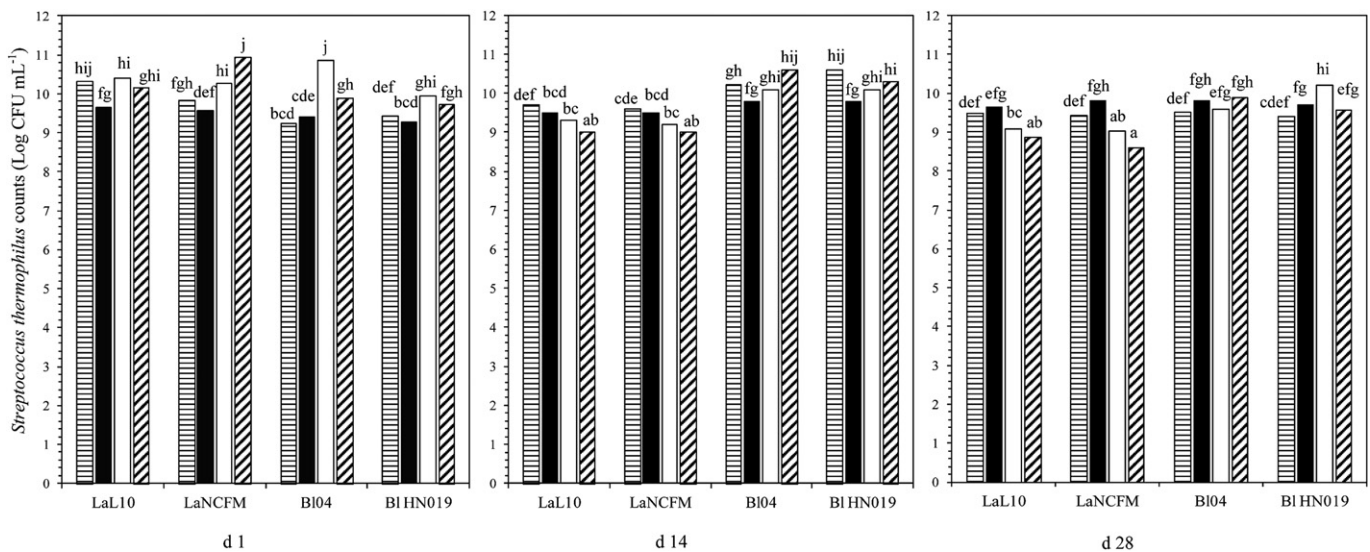


Fig. 1. *Streptococcus thermophilus* counts in control and fiber yoghurts co-fermented by different probiotic strains. Means with different letters are significantly different ($P < 0.05$). $N = 64$. Abbreviations: BI04, HN019, B94 = yoghurts co-fermented by *B. animalis* subsp. *lactis* BI04, HN019 and B94, respectively; L10 = yoghurts co-fermented by *L. acidophilus* L10. d 1, d 14 and d 28 = days 1, 14 and 28 after fermentation. □ Control skim yoghurt; ■ passion fruit peel powder skim yoghurt; ▨ control whole yoghurt; ▩ passion fruit peel powder whole yoghurt.

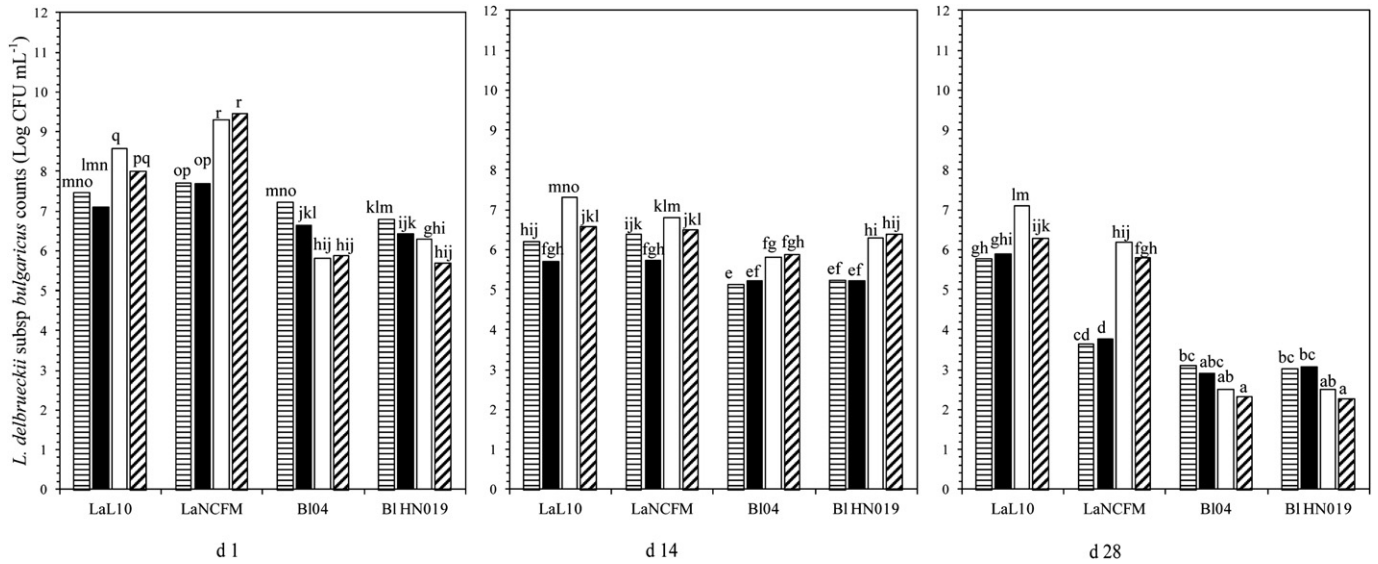


Fig. 2. *Lactobacillus delbrueckii* subsp. *bulgaricus* counts in control and fiber yoghurts co-fermented by different probiotic strains. Means with different letters are significantly different ($P < 0.05$). $N = 64$. Abbreviations: BI04, HN019, B94 = yoghurts co-fermented by *B. animalis* subsp. *lactis* BI04, HN019 and B94, respectively; L10 = yoghurts co-fermented by *L. acidophilus* L10. d 1, d 14 and d 28 = days 1, 14 and 28 after fermentation. □ Control skim yoghurt; ■ passion fruit peel powder skim yoghurt; □ control whole yoghurt; ▨ passion fruit peel powder whole yoghurt.

the period between 1 and 14 days, a mild but significant decrease in St counts occurred in all yoghurts co-fermented by *L. acidophilus* strains, but an increase in skim yoghurts co-fermented by *B. lactis* strains ($P < 0.05$).

In contrast with St counts invariability during shelf-life, *L. delbrueckii* subsp. *bulgaricus* suffered a large decrease in its counts, which ranged from 6.2 to 9.5 and from 2.9 to 7.1 Log CFU mL⁻¹ after 1 and 28 days, respectively (Fig. 2). At the end of the whole shelf-life, the highest counts of Lb were observed in yoghurts co-fermented by *L. acidophilus* strains, particularly the L10 one ($P < 0.05$). Such a symbiotic effect of *L. acidophilus* L10 on Lb was previously noticed by Espirito-Santo et al. (2010).

At the 1st day of cold storage, the probiotic counts varied from 8.5 to 10.8 Log CFU mL⁻¹ in yoghurts co-fermented by *L. acidophilus*

strains and from 7.9 to 9.9 Log CFU mL⁻¹ by *B. lactis* strains (Fig. 3). Amongst the skim yoghurts, the counts of *L. acidophilus* were about 1 Log higher than those of *B. lactis* ($P < 0.05$) in spite of the same counts of both probiotic species in the inocula. Regarding the control, a beneficial effect of passion fruit peel powder was observed only in *B. lactis* BI04 counts in skim yoghurt, but the contrary took place in whole yoghurt ($P < 0.05$).

A dramatic change in the probiotic counts profile in skim yoghurts occurred after 14 days of shelf-life. The counts of *B. lactis* raised by 1.5 Log as an average and were significantly higher than the ones of *L. acidophilus* that decreased by about 2 Log ($P < 0.05$). Furthermore, the passion fruit peel powder had a beneficial effect on the counts of *B. lactis* strains in skim yoghurts and those of *B. lactis* HN019 in whole yoghurt ($P < 0.05$), the only negative effect

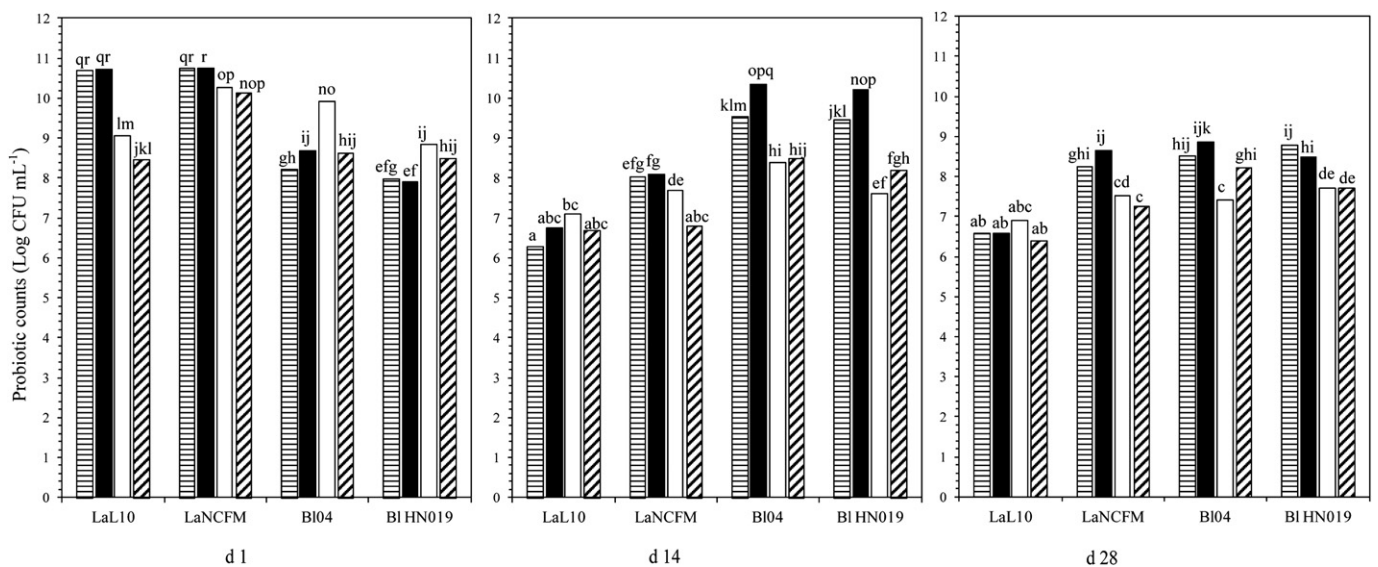


Fig. 3. Probiotic counts in control and fiber yoghurts co-fermented by different probiotic strains. Means with different letters are significantly different ($P < 0.05$). $N = 16$. Abbreviations: BI04, HN019, B94 = yoghurts co-fermented by *B. animalis* subsp. *lactis* BI04, HN019 and B94, respectively; L10 = yoghurts co-fermented by *L. acidophilus* L10. d 1, d 14 and d 28 = days 1, 14 and 28 after fermentation. □ Control skim yoghurt; ■ passion fruit peel powder skim yoghurt; □ control whole yoghurt; ▨ passion fruit peel powder whole yoghurt.

of the fiber being detected in the counts of *L. acidophilus* NCFM in whole yoghurts ($P < 0.05$) (Fig. 3). Some studies of supplementation of fermented milks with fruit or fruit fibers presented different results in the counts of *L. acidophilus* (Espírito Santo, Perego, Converti, & Oliveira, 2011). In the present study, the counts of *L. acidophilus* L10 were not affected by the addition of PFPP in the yoghurts made with the two types of milk, in spite of Kailasapathy, Harmstorf, and Phillips (2008) had reported the decrease in the counts of the same probiotic strain in fermented milk supplemented with passion fruit juice.

At the end of shelf-life, the counts of the probiotic strains ranged, as a whole, from 6.4 to 8.9 Log CFU mL⁻¹, being higher in skim yoghurts except for *L. acidophilus* L10 on which no effect due to milk type was observed. The passion fruit peel powder did not promote any significant variation in the probiotic counts, except in that of *B. lactis* BI04 in whole yoghurt that was 0.8 Log higher than its control.

Talcott, Percival, Pittet-Moore, and Celoria (2003) and Narain, Almeida, Galvão, Madruga, and Brito (2004) reported that some compounds of passion fruit, such as phenolic compounds, fatty acid esters, thiols, terpenes and alcohols can inhibit the growth of *L. acidophilus*.

According to a study of Vinderola, Costa, Regenhardt, and Reinheimer (2002), the strawberry, pineapple and kiwi juices did not influence the growth of *L. acidophilus* when the juices were previously neutralized. Likewise, the initial pH of the milk containing passion fruit peel powder – which was near the neutrality (pH 6.42) – may have attenuated the possible negative effect of the acidity from the fruit on the viability of *L. acidophilus* and *B. lactis* strains tested. Besides, the concentration of passion fruit peel powder may not have been enough to exert an inhibitory effect on the probiotics, with exception of the NCFM strain on the 14th day.

3.4. Texture profile

The texture profiles of the different yoghurts evaluated after 1, 14 and 28 days of cold storage are shown in Table 3.

Regarding only the influence of the milk type, during the cold storage the whole control yoghurts co-fermented by lactobacilli showed higher firmness, consistency and cohesiveness than the respective skim ones ($P < 0.05$). This observation is supported by some studies that pointed out that a reduction in fat content can cause a fragile texture due to weaker network of the protein gel

in yoghurts (Guven, Yasar, Karaca, & Hayaloglu, 2005; Ramchandran & Shah, 2009). As far as the influence of passion fruit peel powder is concerned, it promoted, as an average, higher values of all texture parameters in skim yoghurts co-fermented by *B. lactis* strains compared to their respective controls or to whole yoghurts with PFPP ($P < 0.05$). Meanwhile, it reduced significantly the firmness and consistency, but not the cohesiveness, of whole yoghurts co-fermented by *L. acidophilus* L10.

As expected, in general, all texture parameters significantly increased during cold storage, being the most marked increase observed after 1 and 14 days. Garcia-Perez et al. (2006) found that the addition of orange fiber below 1% concentration reduce the firmness of skim yoghurt. However, the present study shows that at the end of storage, firmness and consistency in all passion fruit peel powder skim yoghurts were higher than in their respective controls, except when using *L. acidophilus* NCFM as probiotic, while their cohesiveness was increased by the addition of the PFPP in all cases. As regards the whole yoghurts, firmness was higher in controls co-fermented by *L. acidophilus* NCFM and *B. lactis* strains ($P < 0.05$), while consistency and cohesiveness were significantly higher in the same yoghurts but that co-fermented by *B. lactis* BI04.

According to Damin et al. (2008), the firmness is higher in yoghurts lasting longer fermentation time. However, in the present study skim yoghurts co-fermented by lactobacilli – in spite of the longer fermentation time – did not show any firmness increase after 1 day of cold storage compared to the other treatments.

Cultures of lactic acid bacteria producer of exopolysaccharides (EPS) have been used to improve the texture of yoghurts (Sodini et al., 2004; Welman & Maddox, 2003). However, the high counts of EPS-producing *L. acidophilus* and *S. thermophilus* in skim yoghurts did not correspond to any increase in their textural parameters. This observation can be explained with the formation of a few weak polysaccharide–protein interactions instead of more stable protein–protein ones (Folkenberg, Dejmek, Skriver, Guldager, & Ipsen, 2006; Ramchandran & Shah, 2009), which may have contributed to lowering the firmness of yoghurts.

The results of the present study taken together suggest that the textural parameters were influenced by a combination of factors such as culture composition, milk type and passion fruit peel powder addition, which justifies further efforts in this field.

Table 3
Texture parameters of plain and skim yogurts co-fermented by different probiotic strains and with or without passion fruit fiber.

Yoghurts			Firmness (N)			Consistency (N s)			Cohesiveness (N)				
Milk type	Treatment	Probiotic	d 1	d 14	d 28	d 1	d 14	d 28	d 1	d 14	d 28		
Skim	Control	La L10	0.25 ^a	0.28 ^{ab}	0.37 ^{cd}	2.06 ^{ab}	2.19 ^{bc}	2.60 ^{cd}	0.16 ^a	0.22 ^{ab}	0.23 ^{ab}		
		La NCFM	0.25 ^a	0.31 ^{bc}	0.35 ^{cd}	1.95 ^a	2.56 ^c	2.69 ^{cd}	0.19 ^a	0.25 ^{abc}	0.24 ^c		
		BI04	0.27 ^{ab}	0.36 ^{cd}	0.39 ^{cde}	2.22 ^{abc}	2.70 ^d	2.90 ^{de}	0.22 ^{ab}	0.27 ^{bc}	0.26 ^{bc}		
		BI HN019	0.27 ^{bc}	0.41 ^{de}	0.37 ^{cd}	2.56 ^c	3.08 ^{de}	2.82 ^{cde}	0.25 ^{abcd}	0.30 ^{cd}	0.26 ^{bc}		
		La L10	0.37 ^d	0.45 ^{efg}	0.48 ^{fg}	2.95 ^{de}	3.50 ^f	3.51 ^f	0.31 ^{cde}	0.36 ^{def}	0.38 ^{efg}		
		La NCFM	0.27 ^{ab}	0.38 ^{cd}	0.38 ^{cde}	2.15 ^{ab}	2.94 ^e	2.94 ^e	0.22 ^{ab}	0.31 ^{cde}	0.30 ^{cd}		
	With passion fruit fiber	BI04	0.43 ^e	0.50 ^g	0.48 ^{fg}	3.39 ^{ef}	3.82 ^g	3.58 ^f	0.36 ^e	0.40 ^g	0.37 ^{ef}		
		BI HN019	0.37 ^d	0.48 ^{fg}	0.48 ^{fg}	3.49 ^f	3.97 ^{gh}	3.50 ^f	0.32 ^{de}	0.38 ^{efg}	0.36 ^{def}		
		Whole	Control	La L10	0.34 ^{cd}	0.45 ^{ef}	0.44 ^{ef}	2.86 ^{cde}	3.55 ^f	3.53 ^f	0.28 ^{bc}	0.31 ^{cde}	0.32 ^{de}
				La NCFM	0.31 ^{bc}	0.49 ^{fg}	0.52 ^g	2.63 ^{cd}	4.06 ^{gh}	4.18 ^h	0.26 ^{bc}	0.35 ^{def}	0.37 ^{ef}
				BI04	0.28 ^{abc}	0.31 ^{bc}	0.49 ^{fg}	2.23 ^{bc}	2.39 ^{bcd}	3.05 ^{de}	0.23 ^{ab}	0.21 ^{ab}	0.29 ^{cd}
				BI HN019	0.26 ^{ab}	0.32 ^{bc}	0.51 ^g	2.11 ^{ab}	2.35 ^{bcd}	3.28 ^{ef}	0.21 ^{ab}	0.20 ^a	0.36 ^{def}
La L10	0.24 ^a			0.39 ^{de}	0.51 ^g	1.59 ^a	2.58 ^{cd}	3.28 ^{ef}	0.22 ^{ab}	0.24 ^{abc}	0.36 ^{def}		
La NCFM	0.28 ^{abc}			0.40 ^{de}	0.47 ^{efg}	1.50 ^a	2.86 ^{cde}	3.05 ^{de}	0.26 ^{bc}	0.27 ^{bc}	0.29 ^{cd}		
With passion fruit fiber	BI04	0.28 ^{ab}	0.38 ^{de}	0.37 ^{cd}	2.11 ^{ab}	2.82 ^{cde}	2.72 ^d	0.23 ^{ab}	0.28 ^{bc}	0.29 ^{bcd}			
	BI HN019	0.28 ^{abc}	0.38 ^{de}	0.38 ^{cde}	2.15 ^{ab}	2.61 ^{cd}	2.79 ^{de}	0.23 ^{ab}	0.26 ^{bc}	0.27 ^{bc}			

Means with different letters in the same column are significantly different ($P < 0.05$). $N = 12$. All standard deviations were under 5% of the average and are not shown.

Abbreviations: d 1, d 14 and d 28 = days 1, 14 and 28 after fermentation. La L10 and La NCFM, *L. acidophilus* strains L10 and NCFM; BI04 and BI HN019, *B. animalis* subsp. *lactis* strains BI04 and HN019.

4. Conclusions

Results demonstrated that PFPP reduced significantly the maximum acidification rate in both skim and whole milks and reduced the fermentation time in all skim yoghurts, except the one fermented with *B. lactis* B104. Total titratable acidity was higher in skim yoghurts, especially in those with PFPP, indicating a lower buffering capacity of the skim milk regarding the whole one. In general, skim yoghurts presented higher counts of probiotic bacteria than the whole ones. The yoghurts with passion fruit peel powder had variable counts of probiotics but similar to those of control yoghurts in most of the cases. Passion fruit peel powder increased cohesiveness of all probiotic skim yoghurts. However, in the case of some whole yoghurts, the PFPP decreased firmness and consistency regarding the respective controls without the powder. The data show that the addition of PFPP into the yoghurt effects differently the parameters studied depending on the combination of bacteria and mainly on the milk type, being in general more favorable in the case of skim yoghurts.

Acknowledgments

The authors wish to thank Danisco Brasil Ltda (Cotia, São Paulo, Brazil) and Globalfood (São Paulo, Brazil) for providing the cultures, De Marchi for donation of passion fruit by-product and FAPESP, CNPq and CAPES for financial support.

References

- Almeida, K. E., Tamime, A. Y., & Oliveira, M. N. (2009). Influence of total solids contents of milk whey on the acidifying profile and viability of various lactic acid bacteria. *LWT – Food Science and Technology*, 42, 672–678.
- AOAC. (1995). *Fat, lactose, protein and solids in milk*. Official methods of analysis. AOAC official method 972.16. Washington, DC, USA: Association of Official Analytical Chemists.
- Chau, C. F., & Huang, Y. L. (2005). Effects of the insoluble fiber derived from *Passiflora edulis* seed on plasma and hepatic lipids and fecal output. *Molecular Nutrition and Food Research*, 49, 786–790.
- *Cordova, K. R. V., Gama, T. M. M. T. B., Winter, C. M. G., Kaskantzis Neto, G., & Freitas, R. J. S. (2005). Physico-chemical characteristics of yellow passion fruit (*Passiflora edulis* flavicarpa Degener) peel after a drying process. *Boletim do CEPPA*, 23, 221–230;
- These authors report the major nutritional components present in the by-products of the passion fruit juice production and offer a base to know the elements that could interfere in the growth and viability of the yoghurt bacteria.
- Damin, M. R., Minowa, E., Alcantara, M. R., & Oliveira, M. N. (2008). Effect of cold storage on culture viability and some rheological properties of fermented milk prepared with yoghurt and probiotic bacteria. *Journal of Texture Studies*, 39, 40–55.
- Dave, R. L., & Shah, N. P. (1996). Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* and bifidobacteria. *Journal of Dairy Science*, 79, 1529–1536.
- Demam, J. M. (1983). Consistency of fats: a review. *Journal of the American Oil Chemists' Society*, 60, 6–11.
- EMBRAPA. *Empresa Brasileira de Agricultura e Pecuária*. Centro Nacional de Pesquisa de Mandioca e Fruticultura. Retrieved in November 2010, from http://www.cnpmf.embrapa.br/index.php?p=pesquisa-culturas_pesquisadas-maracuja.php&menu.
- Espírito Santo, A. P., Perego, P., Converti, A., & Oliveira, M. N. (2011). Influence of food matrices on probiotic viability – a review focusing on the fruit bases. *Trends in Food Science and Technology*, 22, 377–385.
- *Espírito-Santo, A. P., Silva, R. C., Soares, F. A. S. M., Anjos, D., Gioielli, L. A., & Oliveira, M. N. (2010). Açai pulp addition improves fatty acid profile and probiotic viability in yoghurt. *International Dairy Journal*, 20, 415–422;
- The symbiotic effect between *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* was observed by the authors and confirmed in the present study.
- Farnworth, E. R. (2008). The evidence to support health claims for probiotics. *Journal of Nutrition*, 138, 1250–1254.
- Folkenberg, D. M., Dejmeck, P., Skriver, A., Guldager, H. S., & Ipsen, R. (2006). Sensory and rheological screening of exopolysaccharide producing strains of bacterial yogurt cultures. *International Dairy Journal*, 16, 111–118.
- García-Pérez, F. J., Sendra, E., Lario, Y., Fernández-López, J., Sayas-Barbera, E., & Pérez-Alvarez, J. A. (2006). Rheology of orange fiber enriched yogurt. *Milch-wissenschaft – Milk Science International*, 61, 55–59.
- Güven, M., Yasar, K., Karaca, O. B., & Hayaloglu, A. A. (2005). The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture. *International Journal of Dairy Technology*, 58, 180–184.
- Janebro, D. I., Queiroz, M. S. R., Ramos, A., Sabaa-Srur, A. U. O., Cunha, M. A. L., & Diniz, M. F. (2008). Effect of the flour of the yellow passion fruit peel (*Passiflora edulis* f. *flavicarpa* Deg.) in the glycemic and lipid levels of type 2 diabetes patients. *Brazilian Journal of Pharmacognosy*, 18, 724–732.
- Jordano, R., Serrano, E. C., Torres, M., & Salmeron, J. (1992). Comparison of three M17 media for the enumeration of *Streptococcus thermophilus* in fermented dairy products. *Journal of Food Protection*, 55, 999–1000.
- Kailaspathy, K., Harmstorf, I., & Phillips, M. (2008). Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* in stirred fruit yogurts. *LWT – Food Science and Technology*, 41(7), 1317–1322.
- Kaur, N., & Gupta, A. K. (2002). Applications of inulin and oligofructose in health and nutrition. *Journal of Bioscience*, 27, 703–714.
- Kodaka, H., Mizuochi, S., Teramura, H., & Nirazuka, T. (2005). Comparison of the compact dry TC method with the standard pour plate method (AOAC official method 966.23) for determining aerobic colony counts in food samples. *Journal of AOAC International*, 88, 1702–1713.
- Kourkoutas, Y., Bosnea, L., Taboukos, S., Baras, C., Lambrou, D., & Kanellaki, M. (2006). Probiotic cheese production using *Lactobacillus casei* cells immobilized on fruit pieces. *Journal of Dairy Science*, 89, 1439–1451.
- Kumar, P., & Mishra, H. N. (2003). Effect of mango pulp and soy milk fortification on the texture profile of set yoghurt made from buffalo milk. *Journal of Texture Studies*, 34, 249–269.
- Lankaputhra, W. E. V., & Shah, N. P. (1996). A simple method for selective enumeration of *Lactobacillus acidophilus* in yoghurt supplemented with *L. acidophilus* and *Bifidobacterium* spp. *Milchwissenschaft*, 51, 446–451.
- McCann, T. H., Fabre, F., & Day, L. (2011). Microstructure, rheology and storage stability of low-fat yoghurt structured by carrot cell wall particles. *Food Research International*, 44(4), 884–892.
- Narain, N., Almeida, J. N., Galvão, M. S., Madruga, M. S., & Brito, E. S. (2004). Volatile compounds in passion fruit (*Passiflora edulis* f. *flavicarpa*) and yellow mombin (*Spondias mombin* L) fruits obtained by dynamic headspace technique. *Ciência e Tecnologia de Alimentos*, 24, 212–216.
- *Ramchandran, L., & Shah, N. P. (2009). Effect of exopolysaccharides on the proteolytic and angiotensin-I converting enzyme-inhibitory activities and textural and rheological properties of low-fat yogurt during refrigerated storage. *Journal of Dairy Science*, 92, 895–906;
- The observations of these authors support some of the findings of the present study regarding the relationship between the fat content and the texture of the yoghurt.
- Rawson, H. L., & Marshall, V. M. (1997). Effect of 'ropy' strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* on rheology of stirred yoghurt. *International Journal of Food Science and Technology*, 32, 213–220.
- Salgado, J. M., Bombarde, T. A. D., Mansi, D. N., Piedade, S. M. S., & Meletti, L. M. M. (2010). Effects of different concentrations of passion fruit peel (*Passiflora edulis*) on the glycemic control in diabetic mice. *Ciência e Tecnologia de Alimentos*, 30, 784–789.
- Sendra, E., Fayos, P., Lario, Y., Fernández-López, J., Sayas-Barbera, E., & Pérez-Alvarez, J. A. (2008). Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiology*, 25, 13–21.
- *Sodini, I., Remeuf, F., Haddad, S., & Corrieu, G. (2004). The relative effect of milk base, starter, and process on yogurt texture: a review. *Critical Reviews in Food Science and Nutrition*, 44, 113–137;
- This review offers a wide vision of the main factors that can influence the texture of the yoghurt and gave some important elements to interpret and discuss our results. The authors highlight the importance of the fat content as a texture enhancer factor, which was contradicted in our study.
- Spinnler, H. E., & Corrieu, G. (1989). Automatic method to quantify starter activity based on pH measurement. *Journal of Dairy Research*, 56, 755–764.
- Staffolo, M. D., Bertola, N., Martino, M., & Bevilacqua, A. (2004). Influence of dietary fiber addition on sensory and rheological properties of yoghurt. *International Dairy Journal*, 14, 263–268.
- Talcott, S. T., Percival, S. S., Pittet-Moore, J., & Celoria, C. (2003). Phytochemical composition and antioxidant stability of fortified yellow passion fruit (*Passiflora edulis*). *Journal of Agricultural and Food Chemistry*, 51, 935–941.
- Varghese, K. S., & Mishra, H. N. (2008). Modelling of acidification kinetics and textural properties in *dahi* (Indian yogurt) made from buffalo milk using response surface methodology. *International Journal of Dairy Technology*, 61, 284–289.
- Vinderola, C. G., Costa, G. A., Regenhardt, S., & Reinheimer, J. A. (2002). Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. *International Dairy Journal*, 12, 579–589.
- Welman, A. D., & Maddox, I. S. (2003). Exopolysaccharides from lactic acid bacteria: perspectives and challenges. *Trends in Biotechnology*, 21, 269–274.
- *Yapo, B. M., & Koffi, K. L. (2008). Dietary fiber components in yellow passion fruit rinds – a potential fiber source. *Journal of Agricultural and Food Chemistry*, 56, 5880–5883;
- This study reports the nutritional components (as in Cordova et al., 2005), and emphasizes the amount and the type of fiber found in the passion fruit by-product.
- Zibadi, S., & Watson, R. R. (2004). Passion fruit (*Passiflora edulis*): composition, efficacy and safety. *Evidence Based Integrative Medicine*, 1, 183–187.
- Zibadi, S., Fari, R., Moriguchi, S., Lu, Y., Foo, L. Y., Tehrani, P. M., et al. (2007). Oral administration of purple passion fruit peel extract attenuates blood pressure in female spontaneously hypertensive rats and humans. *Nutrition Research*, 27, 408–416.