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Influence of cofermentation by amylolytic *Lactobacillus* strains and probiotic bacteria on the fermentation process, viscosity and microstructure of gruels made of rice, soy milk and passion fruit fiber

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ABSTRACT

Gruels tailored to school-age children and made of soy milk and rice flour with or without total dietary fiber from passion fruit by-product were fermented by amylolytic lactic acid bacteria strains (*Lactobacillus fermentum* Ogi E1 and *Lactobacillus plantarum* A6), by commercial probiotic bacteria strains (*Lactobacillus acidophilus* L10, *Lactobacillus casei* L26 and *Bifidobacterium animalis* subsp. *lactis* B94) and by co-cultures made of one amylolytic and one probiotic strain. The influence of ingredient composition and bacterial cultures on kinetics of acidification, α-amylase activity of the bacteria, apparent viscosity and microstructure of the fermented products was investigated. During fermentation of the gruels, α-amylase activity was determined through the Ceralpha method and apparent viscosity, flux behavior and thixotropy were determined in a rotational viscometer. Rheological data were fitted to Power Law model. The combination of amylolytic and probiotic bacteria strains reduced the fermentation time of the gruels as well as increased the α-amylase activity. The addition of passion fruit fiber exerted less influence on the apparent viscosity of the fermented products than the composition of the bacterial cultures. Scanning electron microscopy provided evidence of exopolysaccharide production by amylolytic bacteria strains in the food matrices tested. The co-cultures made of amylolytic and probiotic bacteria strains are suitable to reduce the fermentation time of a soy milk/rice matrix and to obtain a final product with pH and viscosity similar to yoghurt.

1. Introduction

According to Food Processing (2009) and Granato, Branco, Cruz, Faria, and Shah (2010), the market of food products containing functional ingredients such as probiotics, prebiotics, soy derivatives and dietary fiber, grows approximately 5% per year worldwide and the selling of these products is expected to be over US$19.6 billion in 2013. About 65% of the sales of functional foods correspond to probiotic products (Granato et al., 2010; Stanton, Ross, Fitzgerald, & Van Sinderen, 2005). FAO/WHO (2001) defines probiotics as live microorganisms that when administered in adequate amounts, are able to colonize the gastrointestinal tract conferring health benefits to the host. Prevention of diarrhea caused by rotavirus (common in schoolchildren), lowering of serum cholesterol, modulation of the immune system response and prevention of colon cancer are amongst the benefits commonly attributed to probiotics consumption (Farnworth, 2008). Fermented dairy products have been the most utilized food matrix for probiotic intake, but the development of probiotic vegetable beverages has been increasing as an alternative to attend to the needs of individuals with lactose intolerance or milk allergy (Espírito-Santo et al., 2012). Besides, the great majority of disadvantaged populations cannot afford a dairy-based functional food. In Brazil, the National Program for Nutrition in the School (PNAE) stipulates that 30% of the recommended daily nutritional requirements for school-age children should be provided by the school meal service, challenging the formulation of a diversified menu (Brasil, 2009).

The use of a vegetal food matrix with high protein quality such as hydrosoluble extract of soybeans (soy milk) instead of a milk base is a cheaper way to develop probiotic beverages, which can be more affordable to poor communities in soybean producer countries, such as those of South America and South Asia (Tou et al., 2007). Beyond the health benefits of probiotics themselves, fermentation by probiotic bacteria has been noticed as beneficial to increase functional aspects of soy milk by generating antihypertensive peptides (Donkor, Henriksson, Vasiljevic, & Shah, 2005), promoting normobiosis in the intestinal tract (Cheng et al., 2005) and reducing the content of nondigestible

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oligosaccharides (LeBlanc, Garro, & de Giori, 2004). Fermentation of soy milk can also reduce the beany off-flavor of the soybean (LeBlanc et al., 2004; Wang, Zhou, & Chen, 2008).

On the other hand, promoting a balanced daily energy intake for children, mainly those exposed to precarious economic situation, is a must according to the World Health Organization (Mouquet & Trèche, 2001; WHO, 1998). A cheap way to increase the energy value of soy milk is the addition of an amylaceous ingredient such as rice flour, which also acts as food texturing agent (Nguyen et al., 2007; Sabanis & Tzia, 2009). The nutritional quality of a rice based food can be improved through fermentation by amylolytic lactic acid bacteria (ALAB) which can increase the availability of lysine and improve the digestibility of starch in young children (Gobbetti, De Angelis, Corsettic, & Di Cagno, 2005; Lee, Gilliland, & Carter, 2001; Lee, Lee, Park, Hwang, & Ji, 1999). The fermentation of amylaceous matrix by ALAB offers a technological benefit by eliminating the need to add exogenous α-amylases for starch liquefaction (Haydersah et al., 2012; Mouquet & Trèche, 2001; Nguyen et al., 2007). Amylolytic activity was also observed in some probiotic strains of *Lactobacillus* and *Bifidobacterium* (Knudsen et al., 2013; Lee et al., 2001; Ryan, Fitzgerald, & van Sinderen, 2006). However, besides starch as carbon source, probiotic bacteria can be particularly demanding in amino acids, vitamins, minerals or other growth stimulant factors such as non-digestible carbohydrates, known as prebiotic fibers (Espírito-Santo et al., 2012). Many by-products from fruit processing industry, such as the yellow passion fruit rinds, are rich source of fibers and minerals which can be used as ingredient to support the bacteria growth during fermentation and to increase the nutritional and functional aspects of the food product (Cordova, Gama, Winter, Kaskantzis Neto, & Freitas, 2005; Espírito-Santo et al., 2013; Yapo & Koffi, 2008).

Considering the mentioned elements and the protein/energy daily needs of 2–6 years old children living in the poorest communities of Brazil (IBGE, 2006; Silva, Martins, Oliveira, & Miyasaka, 2010), our group formulated fermented products containing probiotic lactic acid bacteria as a base for a dessert that could be an alternative to probiotic yoghurt and integrate a school meal. To reach this purpose, gruels made of soy milk and rice flour with or without total dietary fiber from passion fruit rinds were fermented by amylolytic (*Lactobacillus fermentum* Ogi E1 and *Lactobacillus plantarum* A6) and commercial probiotic bacteria strains (*Lactobacillus acidophilus* Lafti L10, *Lactobacillus casei* Lafti L26 and *Bifidobacterium animalis* subsp. *lactis* Lafti B94) in single culture or in co-culture. The commercial probiotic bacteria selected have the status of GRAS (Generally Recognized as Safe) and QPS (Qualified Presumption of Safety), as reported by Jankovic, Sybesma, Phothirath, Ananta, and Mercenier (2010), and have been applied to the fermentation of soy milk (Donkor, Tsangalis, & Shah, 2007). The selected ALAB have been used in the fermentation of rice-based foods (Nguyen et al., 2007). The structural characteristics and rheological parameters of a fermented food are profoundly affected by the composition of the ingredients and the selection of bacteria strains (Donkor et al., 2007; Haydersah et al., 2012; Mouquet & Trèche, 2001; Nguyen et al., 2007; Wang et al., 2008). Thus, the co-cultures made of one amylolytic and one probiotic strain were employed in the fermentation of gruels made of rice flour and soy milk, in order to verify if they are more able than the single cultures in promoting an improvement of some biotechnological and physical aspects of the fermented product. The aim of this study was to evaluate the influence of the composition of ingredients of the food matrix and of bacterial culture on the apparent viscosity and flux behavior during fermentation of rice/soy milk gruels. In order to explain the findings on rheology, supplementary experiments were done on kinetics of acidification and α-amylolytic activity of the bacteria as well as the analysis of microstructure of the fermented products through scanning electron microscopy. To the best of our knowledge, it is the first time that amylolytic lactic acid bacteria isolated from vegetable matrices are used in co-culture with commercial and dairy-adapted probiotic bacteria strains in the fermentation of a soy milk/rice food matrix for the production of a yoghurt-like food.

2. Material and methods

2.1. Preparation of the raw material

The fruits of yellow passion fruit (*Passiflora edulis* var. *flavicarpa* Deg., Passifloraceae) were acquired in a market of organic products in the city of Curitiba, Parana State, Brazil. The fruits were decontaminated by immersion into a solution of 5 ppm of chlorine active hypochlorite for 30 min and then thoroughly washed under running tap water. Afterwards, the peel and pulp were separated and the peels – which represented around 60% of the weight of the fruit – were dried in oven under air flow at 50 °C until constant weight, milled to fine powder (passion fruit fiber, PF) and the particle size was standardized to less than 42 μm.

Hydrosoluble soybean extract (soy milk) was prepared as described by Mandarino and Carrao-Panizzi (1999), with some modifications. Briefly, 600 g of soybeans were cooked in 1.5 L of boiling water for 10 min to inactivate the lipoxygenase, responsible for the color degradation and beany off-flavor of the soybean. Then, the cooking water was drained and the grains were washed, decorticated by rubbing them between the palms of the hands and cooked in 3 L of water for 5 min. After cooling, the soybeans were milled in a knives blender for 15 min. The dough obtained was cooked for 10 min under constant stirring, cooled and passed through sieves to obtain a final product with particle size smaller than 50 μm. The resulting soy milk was freeze-dried in a lyophilizer (Christ model Alpha 1–2 LD plus, Passau, Germany) and stored at 4 °C until use.

2.2. Preparation of the gruels

The dry matter content of the ingredients and of the different food matrices before and after fermentation was determined in an infrared moisture analyser (Sartorius, MA30, Gottingen, Germany). The dry matter contents of the rice flour, freeze-dried soy milk and total dietetic fiber from passion fruit rinds were of 88.4 ± 0.3, 95.1 ± 0.5 and 90.9 ± 0.2%, respectively. To obtain 100 g of base gruel (RS) with 20 g of dry matter (DM), white rice flour (Moulin des Moines, France) and lyophilized soy milk were mixed at 10 g of dry matter each in deionized water. Passion fruit fiber was added at 1 g of dry matter to the base formulation (RS) in order to obtain a fiber-enriched gruel (RSPF) with 21 g of dry matter in 100 g of product. A gruel made of rice flour at 10% of DM was also prepared. The starchy formulations were cooked at 90 °C under constant mixing for 15 min to obtain the total gelatinization of the starch and ensure a heat treatment to the food matrix. The gruels were then distributed in portions of 10 g into sterile falcon tubes, cooled and stored at 4 °C until inoculation.

2.3. Microorganisms and fermentation process

In this study, three freeze-dried commercial cultures of probiotic bacteria were used, specifically *L. acidophilus* LAFTI L10 (DSM, Moorebank, NSW, Australia), *L. casei* LAFTI L26 (DSM) and *B. animalis* subsp. *lactis* LAFTI B94 (DSM) and two amylolytic lactic acid bacteria (ALAB) strains from the collection of IRD, *L. fermentum* OgiE1 and *L. plantarum* A6 (LMG 18053, BCCM, Gent, Belgium), both isolated from fermented cereal-based foods. The bacterial strains were cultivated and activated in MRS broth (Difco™, Becton, Dickinson and Co, Le Point de Croix, France) as described by Nguyen et al. (2007).

The bacteria count in each inoculum was 8 log of CFU·mL^{-1} and the inoculation rate was 1 ml per 100 g of food matrices. Rice gruel and soy milk were fermented by single cultures (one of the 5 bacteria strains). RS and RSPF gruels were fermented by single bacteria cultures or by co-culture made of one amylolytic strain and one probiotic strain, performing 6 different bacteria co-cultures (PA, PB and PC = *L. plantarum* A6 in co-culture with *L. acidophilus* L10, *B. lactis* B94 and *L. casei* L26, respectively; FA, FB and FC = *L. fermentum* OgiE1 in co-culture with *L. acidophilus*

L10, *B. lactis* B94 and *L. casei* L26, respectively). The control of each food matrix received sterile solution of NaCl 0.9 g. 100 mL^{-1} instead of the bacteria inoculum. The fermentation of rice gruel and soy milk aimed at demonstrating the influence of each bacterial strain on the kinetics of acidification and rheology of the basic components of the gruels RS and RSPF.

After inoculation, the tubes were capped and placed in water bath at 40 °C until the pH 4.5 was reached or until 24 h of fermentation. One tube of each treatment was capped with an electrode and connected to a pH meter (WTW, pH 3310, Weilheim, Germany) which recorded the temperature and the pH decrease at every 10 min. From the pH decreasing curve, it was possible to calculate the kinetic parameters of fermentation as the maximum acidification rate (V_{max}), calculated as the −dpH/dt, expressed as pH units·h^{-1}, and identified in the acidification curve, time to reach V_{max} (T_{Vmax}) in hours, final pH in pH units and fermentation time in hours. The fermentation was interrupted by cooling the tubes to 5 °C in ice bath.

2.4. Determination of α-amylolytic activity

A preliminary screening to determine the amylolytic activity of the bacteria strains was performed through pour plate technique. Each bacteria strain was inoculated separately at the concentration of 6 log CFU·mL^{-1} in MRS agar (de Man–Rogosa–Sharpe) containing 20 g of soluble starch. L^{-1} (Sigma, ACS reagent 9005-84-9) as the carbon source. The petri dishes were maintained at 37 °C during 24 h in aerobiosis for *L. plantarum* A6 and *L. fermentum* Ogi E1 and during 72 h in anaerobiosis provided by Anaerocult® A (Merck, Darmstadt, Germany) for the probiotic strains. Afterwards, the colonies were enumerated and then, the culture plates were covered with some drops of Lugol iodine and the colonies with halo of starch hydrolysis were counted (Sanni, Morlon-Guyot, & Guyot, 2002). The results were expressed as % of colonies presenting amylolytic activity.

Alpha-amylase activity was determined in triplicate every 2 h during fermentation of the gruels using the kit Megazyme International Ireland Ltd. (Ceralpha method, 2004; ICC Standard No. 303), following the instructions of the manufacturer and expressed as Ceralpha Units (CU) per gram of product in dry matter, as described by McCleary, McNally, Monaghan, and Mugford (2002). One Ceralpha unit is the amount of α-amylase required to release 1 μM of *p*-nitrophenol from the substrate per 1 min, under the assay conditions.

2.5. Viscosity measurements and flow behavior of the fermented products

Apparent viscosity was determined in triplicate at every 2 h during the fermentation of gruels. Measurements were performed at 40 °C in a rotational viscometer (VT550, Haake, Champlan, France) with concentric cylinders using sensor SV-DIN, controlled by a PC with RheoWin software 2.67 (Haake Laboratories, Karlsruhe, Germany). About 7 g of sample was placed in the stationary cup. Temperature was controlled by a circulating water bath through the jacket surrounding the cup assembly. One cycle of shear rate (γ') ranging from 20 to 100 s^{-1} of upward and downward curves in 60 s was performed, and the corresponding shear stress (τ) data were computed by the software. Apparent viscosity (η_{app}) was obtained at $\gamma' = 20\ s^{-1}$ in the downward flow curve, and expressed in Pa·s. Data were fitted with the Power Law model (Heldman & Singh, 1981):

$$\tau = K \cdot (\gamma')^n$$

where τ is the shear stress, K is the consistency index, expressed in Pa·s, and n is the Power Law index.

The thixotropy of each sample was given by the RheoWin software 2.67, as the area between the downward and upward curves of shear rate, and represents the ability of the gruels to recover the former structure during the decreasing of γ'; so, the higher the area of thixotropic loop, the higher the structure recovery (Joly & Mehrabian, 1976). The thixotropy loop is expressed in Pa·s^{-1}.

2.6. Microstructural analysis

One sample of each type of fermented gruel at pH 4.5 was freeze-dried. Afterwards, the samples were homogenized thoroughly and stuck on stubs with double-face tape and coated with 15 nm of a gold–palladium layer applied by a cathodic coater Polaron SC500 (Polaron, Hertfordshire, West Sussex, UK). Eight fields of each sample were observed in a field-emission scanning electron microscope (SEM S-4000, Hitachi, Japan), operating at a voltage of 10.0 kV and photomicrographs were registered under magnifications from 500 to 10000×.

2.7. Statistical analyses

Four food matrices (rice gruel, soy milk, RS and RSPF) were fermented by 5 different bacteria cultures, performing 20 different treatments. Moreover, RS and RSPF gruels were also fermented by 6 different co-cultures made of amylolytic and probiotic bacteria, adding others12 treatments. In sum, the study involves 32 treatments, being each one fermented in three independent batches (N = 96). The two-way ANOVA was applied to the experimental data and the means were compared by Fisher test at $P < 0.05$ using the software Statgraphics plus 5.1 (Statpoint, Warrenton, USA).

3. Results and discussion

In general, amylolytic and probiotic bacteria counts ranged around 7–8 log CFU·g^{-1} in fermented rice gruels, around 8–9 log CFU·g^{-1} in fermented soy milks and around 8–10 log CFU·g^{-1} in soy milk/rice gruels — with or without passion fruit fiber (data not shown).

The dry matter content of RS and RSPF gruels was reduced ($P < 0.05$) from 20 g and 21 g % to 19.0 ± 0.2 g and 20.0 ± 0.2 g %, respectively in the fermented products. No significant differences were observed between gruels fermented by different bacterial cultures ($P > 0.05$). The reduction of dry matter in fermented products can be ascribed to partial proteolysis of the matrix by the bacteria (Hou, Yu, & Chou, 2000).

Some members of our group described the odor and taste of the fermented RS and RSPF gruels as similar to yoghurt, but sweeter (personal report), which can be ascribed to the degradation of starch into monomers and dimers of carbohydrates by bacterial amylase.

3.1. Kinetics of acidification

The addition of passion fruit fiber had no significant influence ($P > 0.05$) on the initial pH of the rice/soy milk gruel, which was 6.3 ± 0.2 and 6.2 ± 0.2 in RS and RSPF gruels, respectively.

The results concerning the effects of bacteria composition as well as the addition of passion fruit fiber on the parameters of acidification of different cereal and soy milk-based matrices are summarized in Table 1. As far as the fermentation of the basic constituents are concerned, it was observed that the bacteria strains were not able to ferment the rice gruel until pH 4.5 within 24 h, and only *B. lactis* and *L. acidophilus* fermented soy milk until the desired pH in about 15 and 19 h, respectively ($P < 0.05$). This result is somehow expected as the rice gruel is poor in nitrogen source, which limits the metabolism of the selected strains (Hofvendahl & Hahn-Hägerdal, 2000). Lee et al. (1999) supplemented rice medium with L-cysteine and yeast extract to guarantee the growth of *Bifidobacterium* strains and Yun, Wee, Kim, and Ryu (2004) observed that the addition of starch to the rice bran is needed to increase the production of lactic acid by *Lactobacillus* strains. On the other hand, as sucrose, raffinose and stachyose are the main carbon source in soy milk, the extension of fermentation of this food matrix relies on the ability of the bacteria to ferment these sugars (Hati et al.,

Table 1
Parameters of the kinetics of acidification of the different matrices fermented by amylolytic and probiotic bacteria alone or in co-culture.

Matrice	Microorganism	V_{max} (pH units.h^{-1})	$T_{v\ max}$ (h)	Final pH	Time of final pH (h)
Gruel made of rice at 10 g. 100 g^{-1}	*L. fermentum*OgiE1	0.4 ± 0.1bcd	1.3 ± 0.1ab	5.3 ± 0.1 h	24 m
	L. plantarum A6	0.4 ± 0.1e–h	0.4 ± 0.1a	4.6 ± 0.0abc	24 m
	L. acidophilus L10	0.5 ± 0.1ij	3.9 ± 0.6 h–k	4.6 ± 0.0abc	24 m
	B. lactis B94	0.7 ± 0.1 k	0.3 ± 0.2a	4.6 ± 0.0bcd	24 m
	L. casei L26	0.5 ± 0.1j	6.9 ± 0.2 l	4.9 ± 0.1 g	24 m
Soy milk at 10 g. 100 g^{-1}	*L. fermentum* OgiE1	0.4 ± 0.0c–e	3.4 ± 0.5 g–j	4.7 ± 0.0f	24 m
	L. plantarum A6	1.0 ± 0.2 m	6.8 ± 0.9 l	4.6 ± 0.0cde	24 m
	L. acidophilus L10	0.5 ± 0.0hi	2.6 ± 0.3c–g	4.5 ab	19.3 ± 0.5jk
	B. lactis B94	0.4 ± 0.0d–h	2.8 ± 0.7d–h	4.5 ab	15.5 ± 0.3i
	L. casei L26	0.8 ± 0.1 l	4.8 ± 0.1 k	4.6 ± 0.0ef	24 m
Gruel made of rice and soy milk at 10 g each. 100 g^{-1} (RS)	*L. fermentum* OgiE1	0.5 ± 0.0ij	1.5 ± 0.0abc	4.5 ab	13.8 ± 0.3 h
	L. plantarum A6	0.3 ± 0.0ab	3.2 ± 2.1e–j	4.5 a	13.0 ± 0.9gh
	L. acidophilus L10	0.4 ± 0.0b–f	3.3 ± 1.6f–j	4.5 a	11.6 ± 0.1f
	B. lactis B94	0.2 ± 0.0a	3.4 ± 1.1f–j	4.5 a	12.7 ± 0.7gh
	L. casei L26	0.3 ± 0.1ab	4.5 ± 0.2jk	4.5 a	13.7 ± 0.6 h
	PA	0.4 ± 0.0d–h	1.8 ± 0.0b–e	4.5 a	6.7 ± 0.1ab
	PB	0.4 ± 0.0c–g	1.7 ± 0.1bcd	4.5 a	5.9 ± 0.1a
	PC	0.3 ± 0.0bcd	2.1 ± 0.1b–f	4.5 a	7.7 ± 0.1c
	FA	0.5 ± 0.1ij	2.7 ± 0.6c–h	4.5 a	6.9 ± 0.4ab
	FB	0.4 ± 0.0e–h	2.5 ± 0.0b–g	4.5 a	6.7 ± 0.4ab
	FC	0.5 ± 0.0hi	2.4 ± 0.1b–g	4.5 a	9.1 ± 0.1 cd
Gruel made of rice and soy milk at 10 g each. 100 g^{-1} and passion fruit fiber at 1 g. 100 g^{-1} (RSPF)	*L. fermentum* OgiE1	0.5 ± 0.0hi	3.3 ±0.0f-j	4.5 a	16.5 ± 0.2i
	L. plantarum A6	0.2 ± 0.0a	4.8 ± 2.8 k	4.5 a	11.0 ± 1.4ef
	L. acidophilus L10	0.3 ± 0.0abc	4.2 ±0.0ijk	4.5 a	10.0 ± 0.2de
	B. lactis B94	0.3 ± 0.0abc	4.0 ± 0.2 h–k	4.5 a	9.7 ± 0.2de
	L. casei L26	0.3 ± 0.0ab	4.3 ± 0.5jk	4.5 a	8.7 ± 0.1 cd
	PA	0.3 ± 0.0bcd	3.4 ± 0.6f–j	4.5 a	6.8 ± 0.5ab
	PB	0.3 ± 0.0bcd	4.0 ± 0.0 h–k	4.5 a	7.1 ± 0.4b
	PC	0.3 ± 0.0abc	4.2 ± 0.2ijk	4.5 a	8.7 ± 0.1d
	FA	0.4 ± 0.0f–i	3.3 ± 0.0f–j	4.5 a	6.5 ± 0.0ab
	FB	0.5 ± 0.0ghi	3.3 ± 0.0f–j	4.5 a	6.2 ± 0.0ab
	FC	0.4 ± 0.0e–h	2.8 ± 0.1c–i	4.5 a	9.4 ± 0.2de

Means (n = 3) ± standard deviation with different letters in the same column are significantly different ($P < 0.05$).
Abbreviations: V_{max} = maximum rate of acidification; $T_{v\ max}$ = time to reach V_{max};; PA, PB and PC = *L. plantarum* A6 in co-culture with *L. acidophilus* L10, *B. lactis* B94 and *L. casei* L26, respectively; FA, FB and FC = *L. fermentum* OgiE1 in co-culture with *L. acidophilus* L10, *B. lactis* B94 and *L. casei* L26, respectively.

2013; Wang, Yu, Yang, & Chou, 2003). The averages of kinetic parameters, especially V_{max} and T_{vmax}, of the fermentation of soy milk by *B. lactis* and *L. fermentum* are in accordance to the observations of Garro, Valdez, and Giori (2004), which carried out the soy milk fermentation at 42 °C.

The combination of rice and soy milk (RS gruel) was sufficient to reach pH 4.5 and to decrease the fermentation time in all combinations of bacteria cultures, probably because these ingredients together offer the basic nutrients for growth and metabolism of the bacteria tested. Considering the RS fermented by single cultures, *L. fermentum* developed the highest V_{max} in the shortest time (T_{vmax}), $P < 0.05$. However, *L. acidophilus* was able to ferment RS until the desired pH in the shortest time (Table 1). The kinetic parameters of fermentation of the RS gruel by amylolytic bacteria strains A6 and Ogi E1 are near the findings reported by Nguyen et al. (2007) for a similar product made of rice and soy flours.

All RS and RSPF gruels fermented by co-cultures had shorter fermentation time than the same gruels fermented by single strains of ALAB ($P < 0.05$), Table 1. Still with respect to the fermentation of RS and RSPF gruels, the association ALAB-probiotic exerted only mild influence on the averages of V_{max} and T_{Vmax} which had the tendency to be near the averages of the gruels fermented by single strains of ALAB (Table 1). Notwithstanding, it was observed that the gruels fermented by the co-cultures made of one ALAB strain and *L. casei* had longer fermentation time than the gruels fermented by the other co-cultures ($P < 0.05$), which is in accordance with the poor acidifying capacity of *L. casei* observed in rice gruel and soy milk separately (Table 1). Considering the fermentation of RS and RSPF gruels, the time to reach pH 4.5 ranged from 5.9 to 9.4 h, which were near the fermentation time needed to obtain yoghurt at the same final pH through fermentation by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Espírito-Santo et al., 2012).

Compared to the RS gruel, the addition of passion fruit fiber to the RS gruel had no significant influence on averages of the maximum acidification rate (V_{max}) but increased the time to reach V_{max} in the RSPF gruels fermented by ALAB, probiotic strains (except *L. casei*) and by all co-cultures, indicating a longer time of adaptation of the bacteria to the RSPF matrix (Table 1). This finding indicates that all strains took, in general, longer time to adapt to the presence of passion fruit fiber. Notwithstanding, the fiber reduced in about 2 h the fermentation time of the RSPF gruels fermented by *L. plantarum* and *B. lactis* and in about 5 h the RSPF gruel fermented by *L. casei*, but increased in 3 h the fermentation time of the same gruel fermented by *L. fermentum* ($P < 0.05$). Although the passion fruit fiber had influenced significantly the fermentation time of the RSPF fermented by the single cultures (except *L. acidophilus*), it had no effect when the gruel was fermented by co-cultures (Table 1).

The comparison between the means revealed that the statistical differences observed in the fermentation time of RS and RSPF gruels are rather due to the composition of microorganisms ($P < 0.05$) than to the presence of passion fruit fiber ($P > 0.05$).

3.2. Amylolytic activity

The screening for the amylolytic activity of bacteria strains in MRS–starch medium evidenced that 98 ± 1% of the *L. plantarum* A6 and 96 ± 2% of the *L. fermentum* Ogi E1 colonies presented a halo of starch hydrolysis indicative of amylolytic activity, but only about one-third of the colonies of probiotic bacteria exhibited halo in the same culture medium: 31 ± 5% of *L. acidophillus* L10, 33 ± 5% of the *L. casei* and 36 ± 3% of the *B. lactis* B94 colonies formed. The large variability of amylolytic activity amongst the colonies of probiotic bacteria can be due to the heterogeneous population of bacteria in the commercial strains, which is consistent with the observations of Espírito-Santo et al. (2012) and Sybesma, Molenaar, Van Ijcken, Venema, and Kort (2013).

Determination of *in situ* α-amylase activity through the Ceralpha method in the rice gruel at 24 h of fermentation by single cultures revealed that *L. casei* L26 presented the same activity as *L. plantarum* A6 and *L. fermentum* Ogi E1 was the strain with the highest α-amylase activity, $P < 0.05$ (Fig. 1). The pH 5.3 of the rice gruel after 24 h of fermentation by *L. fermentum* was near the optimum pH for α-amylase activity, pH 5.0 (Lee et al., 1999), which can explain the highest enzymatic activity of this bacteria regarding the others at pH near 4.6 (Table 1, Fig. 1). *B. lactis* developed the lowest α-amylase activity (16.3 CU · 100 g^{-1}) followed by *L. acidophilus* (25.4 CU · 100 g^{-1}), $P < 0.05$. The low α-amylase activity of *B. lactis* could be due to the low content of cysteine, an essential amino acid for the growth and metabolism of *Bifidobacterium*, in the rice gruel (Lee et al., 1999). So, the rice gruel was supplemented with L-cysteine. HCl (Sigma, C1276, Germany) at 0.05%, and the fermentation with *B. lactis* and evaluation of its α-amylase activity was repeated in triplicate, revealing an increase in the enzymatic activity from 16.3 to 25.8 CU · 100 g^{-1}. This result corresponds to the level of α-amylase activity of the other two probiotic strains and confirms that cysteine was a limiting factor to *B. lactis* B94 in rice gruel (Fig. 1).

By the end of fermentation, the α-amylase activities in gruels fermented by single cultures were, in average, between 50.5 and 69.2 CU · 100 g^{-1} in RS and between 47.1 and 73.7CU · 100 g^{-1} in RSPF gruels (Fig. 2A and B). Even lowering the time to achieve pH 4.5, passion fruit fiber increased significantly the α-amylase activity of single cultures of *L. plantarum* and of *L. acidophillus*, but the mechanism of this positive effect remains unclear and requires further studies. The enzymatic activity was also higher in RSPF gruels fermented by *L. fermentum* but, regarding its curve of α-amylase activity (Fig. 2B), this effect can be due rather to the increase in the fermentation time promoted by passion fruit fiber (Table 1) than to a direct effect of the fiber on the activity or production of the enzyme. However, the lower final α-amylase activity in RSPF fermented by *L. casei* can be associated to the shorter fermentation time induced by the passion fruit fiber (Table 1, Fig. 2B).

Regarding the fermentation of the food matrices by the co-cultures, the α-amylase activity varied from 71.4 to 116.8 CU · 100 g^{-1} in RS gruels and from 77.9 to 117.5 CU · 100 g^{-1} in RSPF gruels (Fig. 2). Although the association of bacterial cultures decreased the fermentation time (Table 1), it increased significantly the enzyme activity ($P < 0.05$), Fig. 2C and D, probably due to the sum of amylase production by both bacteria types (Fig. 1). As far as the fermentation of RS gruel by co-cultures is concerned, in most cases the associations of a given probiotic strain with *L. fermentum* presented higher α-amylase activity than the same probiotic bacteria associated with *L. plantarum* (Fig. 2C). This finding might probably be ascribed to a positive effect of the probiotic bacteria on the expression of α-amylase by *L. fermentum* in RS gruel, however further experiments are needed to confirm this hypothesis.

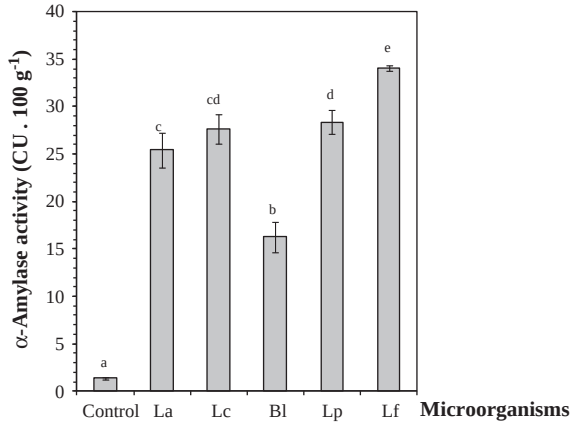

Fig. 1. α-Amylase activity during the fermentation of rice gruel by different bacteria in single cultures. Means with different letters indicate significant differences ($P < 0.05$) between products at 24 h of fermentation, n = 3. Abbreviations: La = *L. acidophilus* L10; Lc = *L. casei* L26; Bl = *B. lactis* B94; Lp = *L. plantarum* A6; Lf = *L. fermentum* OgiE1.

Considering the fermentation of the rice/soy milk gruels by the same co-cultures, the addition of passion fruit fiber increased significantly the α-amylase activity in the co-cultures of *L. plantarum* with *L. acidophilus* and *L. casei* ($P < 0.05$) and with *B. lactis* but not significantly in this case ($P > 0.05$). The addition of passion fruit fiber reduced the final enzyme activity in RSPF gruels fermented by the co-cultures made of *L. fermentum* and *L. acidophilus* or *L. casei*, however in the case of the co-culture with *L. casei*, the activity between 2 and 8 h was higher in RSPF. No effect on the enzyme activity of the amylolytic cultures associated to *B. lactis* was observed. Considering the fermentation of the gruels by the co-cultures, a positive and moderate correlation ($r = 0.46$) between fermentation time and α-amylolytic activity was observed. So, the higher amylolytic activity observed in RSPF gruels fermented by *L. plantarum* in co-culture with probiotic bacteria can be correlated to the tendency of a longer fermentation time promoted by passion fruit fiber addition.

3.3. Viscosity and flow behavior

Regarding the apparent viscosity of non-fermented control (4.4 Pa·s), the apparent viscosity of rice gruel was significantly reduced by the amylolytic strains, *L. plantarum* (3.7 Pa·s) and *L. fermentum* (3.3 Pa·s) and by the probiotic strains *L. acidophilus* and *L. casei* (both 3.8 Pa·s), $P < 0.05$ (Fig. 3A). *B. lactis* was not able to reduce the viscosity significantly, which can be explained by the poor α-amylase activity in rice gruel without supplementation with L-cysteine (Fig. 1). In all cases, the averages of apparent viscosity of the fermented rice gruels were consistent with the α-amylase activity of the bacteria (Figs. 1 and 3A). The area of thixotropic loop (Fig. 3B) is representative of the breakdown and partial recovering of the food structure during the development of up and downwards curves of shear rate and is proportional to the thixotropy of the food (Fonseca, O'Sullivan, Nagira, Yasuda, & Gourlay, 2013; Joly & Mehrabian, 1976). In this sense, the fermentation of rice gruels by *L. acidophilus*, *L. plantarum* and *L. fermentum* produced products with lower thixotropy ($P < 0.05$).

In soy milk, the apparent viscosity increased significantly as the result of fermentation (Fig. 3A). The highest η_{app} was observed in the soy milk fermented by *L. fermentum*. On the other hand, *L. plantarum*, *L. acidophilus* and *B. lactis* promoted the lowest η_{app} which were not different between them, in spite of the significant differences in the fermentation time (Fig. 3A, Table 1). The soy milk fermented by *L. casei* and *B. lactis* presented the highest ($P < 0.05$) area of thixotropic loop which points out that these bacteria either produced exopolysaccharides that stabilized the protein gel network or promoted less proteolysis.

Whereas the η_{app} of the fermented rice-based food is dependent of the amylolytic activity degree, in soy milk it is mainly dependent on the strengthening of protein gel, which is an equilibrium between denaturing effect of acidification on the proteins, activity of proteolytic enzymes (optimal pH around 4.5) of fermenting bacteria, and rearrangement of peptides through electrostatic and hydrophobic interactions, hydrogen and disulfide bonding (Ringgenberg, Alexander, & Corredig, 2013; Roesch, Juneja, Monagle, & Corredig, 2004). Various Lactobacilli species, amongst them *L. fermentum* and *L. plantarum*, are able to promote proteolysis of food matrices (Kunji, Mierau, Hagfing, Poolman, & Konings, 1996; Williams & Banks, 1997). Furthermore, Donkor et al. (2005) reported that remarkable proteolytic activity of the probiotic strains *L. acidophilus* LAFTI® L10, *B. lactis* LAFTI® B94, and *L. casei* LAFTI® L26 – the same used in this study – is one of the responsible factors for the production of antihypertensive peptides in soy yoghurts at pH 4.5. So, the lowest η_{app} of soy milks fermented by *L. acidophilus* and *B. lactis* can be attributed to a more extensive proteolytic activity by these microorganisms at pH around 4.5.

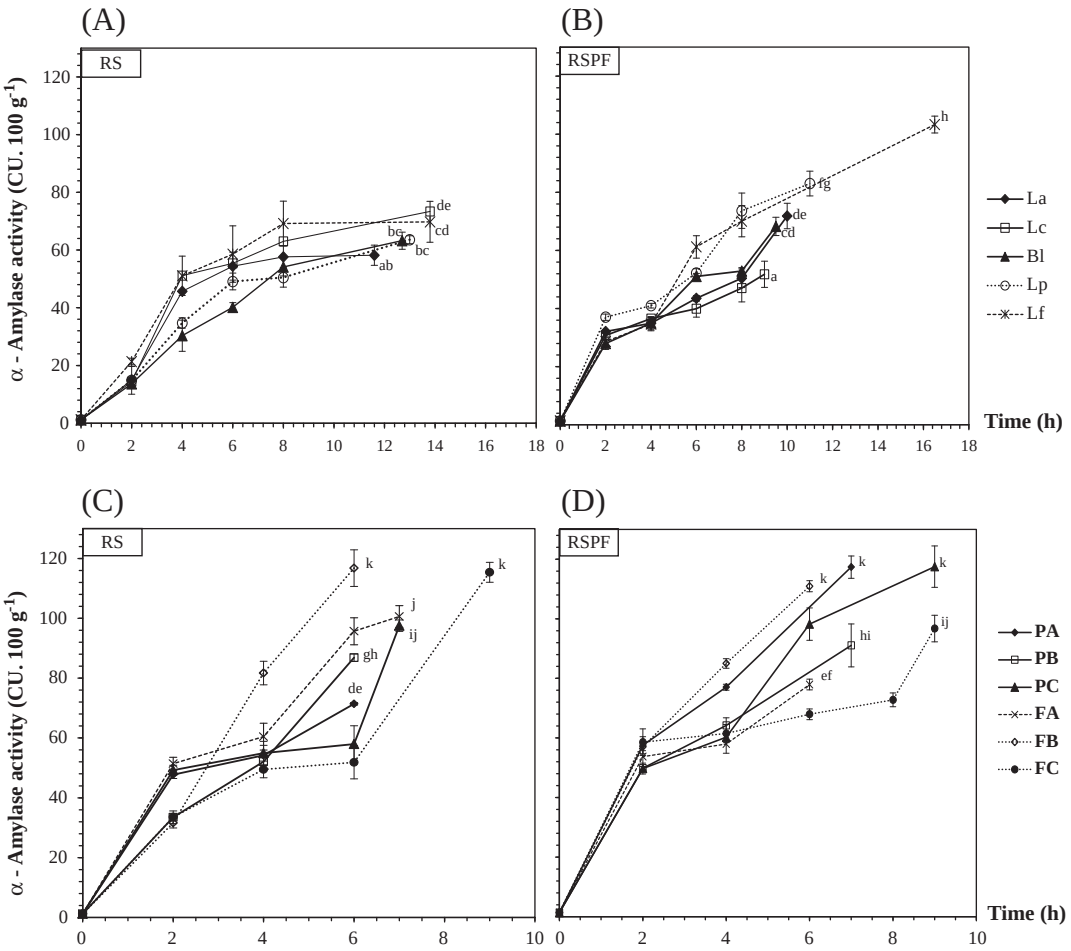

Fig. 2. α-Amylase activity during the fermentation of porridges (RS and RSPF) by different bacteria in single (A and B) or co-cultures cultures (C and D). Means with different letters indicate significant differences ($P < 0.05$) between fermented products by the end of fermentation, n = 3. Abbreviations: RS = Porridge made of rice flour and soy milk at 10 g. 100 g^{-1} of dry matter each; RSFP = RS porridge with addition of passion fruit fiber at 1 g. 100 g^{-1} of dry matter. La = *L. acidophilus* L10; Lc = *L. casei* L26; Bl = *B. lactis* B94; Lp = *L. plantarum* A6; Lf = *L. fermentum* OgiE1. PA, PB and PC = *L. plantarum* A6 in co-culture with *L. acidophilus* L10, *B. lactis* B94 and *L. casei* L26, respectively. FA, FB and FC = *L. fermentum* OgiE1 in co-culture with *L. acidophilus* L10, *B. lactis* B94 and *L. casei* L26, respectively.

Other important factor that can play a role in the apparent viscosity is the production or not of exopolysaccharides by the bacteria. Exopolysaccharides can be produced by strains of *L. acidophilus*, *L. casei* (Cerning et al., 1994; De Vuyst & Degeest, 1999; Mozzi, Giori, Oliver, & Valdez, 1996) and *B. lactis* (Kailasapathy, 2006; Kailasapathy & Masondole, 2005) and are used in food industry as thickeners, stabilizing or emulsifying agents (Savadogo et al., 2004; Sutherland, 1994). Production of exopolysaccharides by several strains of *L. plantarum* and *L. fermentum* has also been reported (Desai, Akolkar, Badhe, Tambe, & Lele, 2006; Fukuda et al., 2010; Savadogo et al., 2004; Tallon, Bressollier, & Urdaci, 2003). The possible production of exopolysaccharides in different amounts, can also explain the

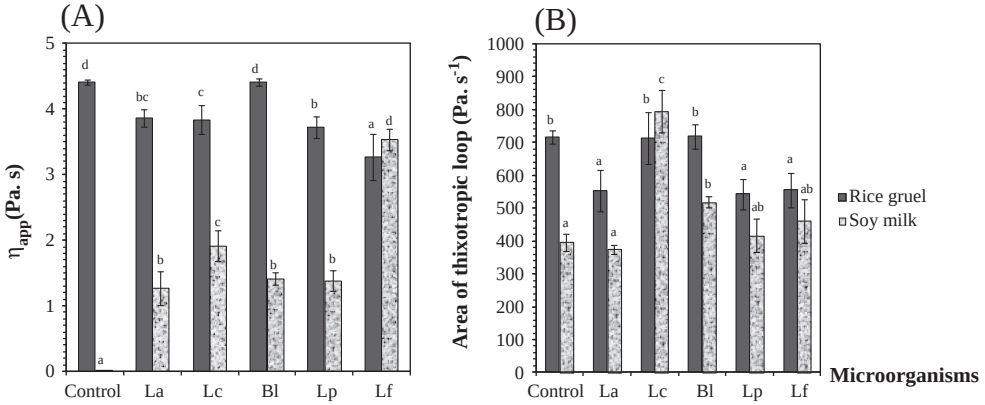

Fig. 3. Influence of the fermentation by different bacteria strains on the (A) apparent viscosity (η_{app}) at $\gamma' = 100\ s^{-1}$ and (B) area of thixotropic loop of two different food matrices: rice gruel and soy milk, both at 10 g of dry matter. 100 g^{-1} of product. Means with different letters for the same food matrix indicate significant differences ($P < 0.05$) between the final fermented products, n = 3. Abbreviations: La = *L. acidophilus* L10; Lc = *L. casei* L26; Bl = *B. lactis* B94; Lp = *L. plantarum* A6; Lf = *L. fermentum* OgiE1.

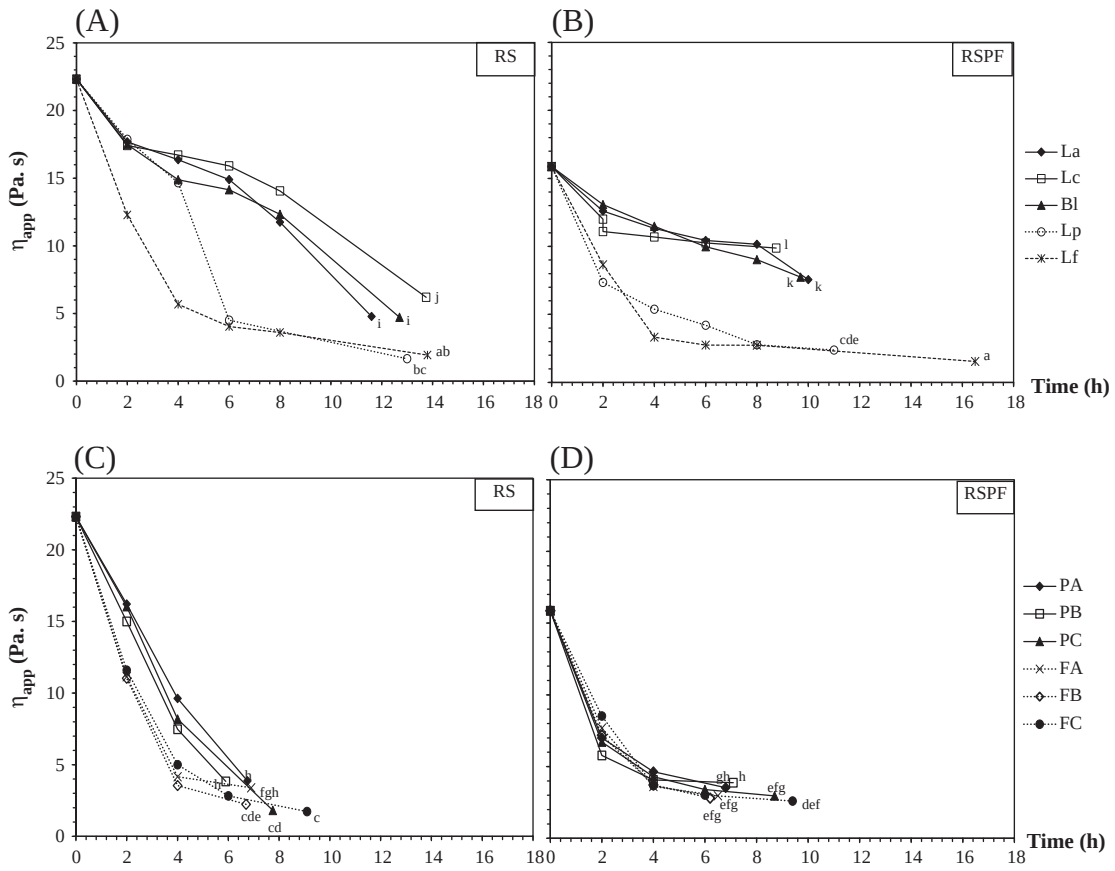

Fig. 4. Apparent viscosity (η_{app}) at $\gamma' = 20\ s^{-1}$ of gruels made of rice flour and soy milk at 10 g. 100 g^{-1} of dry matter each as basic formulation (RS) or with addition of passion fruit fiber at 1 g. 100 g^{-1} (RSPF) and fermented by single (A and B) or co-cultures (C and D). Means with different letters indicate significant differences ($P < 0.05$) between fermented products at pH 4.5, n = 3. Abbreviations: La = *L. acidophilus* L10; Lc = *L. casei* L26; Bl = *B. lactis* B94; Lp = *L. plantarum* A6; Lf = *L. fermentum* OgiE1. PA, PB and PC = *L. plantarum* A6 in co-culture with *L. acidophilus* L10, *B. lactis* B94 and *L. casei* L26, respectively. FA, FB and FC = *L. fermentum* OgiE1 in co-culture with *L. acidophilus* L10, *B. lactis* B94 and *L. casei* L26, respectively.

Table 2
Flow behavior predicted by the Power Law model of food matrices fermented by different combinations of amylolytic and probiotic bacteria.

Matrice	Microorganism	K	n	Area of thixotropic loop ($Pa \cdot s^{-1}$)
Gruel made of rice and soy milk at 10 g each. 100 g^{-1} (RS)	Control	228.9 ± 53.1i	0.2 ± 0.0a	11674.7 ± 1448.7 l
	L. fermentum OgiE1	4.6 ± 1.7a	0.5 ± 0.1fgh	274.8 ± 89.0ab
	L. plantarum A6	12.4 ± 0.5bc	0.5 ± 0.0 h	495.7 ± 52.1a–d
	L. acidophilus L10	25.6 ± 2.1cde	0.4 ± 0.0 fg	1629.0 ± 146.1hi
	B. lactis B94	21.5 ± 1.9b–e	0.4 ± 0.0 fg	1608.3 ± 623.8hi
	L. casei L26	41.9 ± 1.5ef	0.4 ± 0.0 cd	2459.7 ± 837.5jk
	PA	16.5 ± 1.2bc	0.5 ± 0.0fgh	1254.3 ± 43.2fgh
	PB	19.9 ± 1.4bcd	0.5 ± 0.0fgh	1165.3 ± 54.4e–h
	PC	13.8 ± 0.5bc	0.5 ± 0.0fgh	428.1 ± 87.9abc
	FA	15.0 ± 1.1bc	0.5 ± 0.0fgh	1333.7 ± 168.4gh
	FB	14.4 ± 0.7bc	0.5 ± 0.0fgh	854.9 ± 66.9b–g
	FC	15.5 ± 4.8bc	0.5 ± 0.0fgh	713.1 ± 61.7a–f
Gruel made of rice and soy milk at 10 g each. 100 g^{-1} and passion fruit fiber at 1 g. 100 g^{-1} (RSPF)	Control	120.1 ± 40.8 h	0.3 ± 0.1bc	3682.0 ± 89.1jk
	L. fermentum OgiE1	4.7 ± 0.8a	0.3 ± 0.1b	312.5 ± 8.4a
	L. plantarum A6	11.3 ± 0.6bc	0.5 ± 0.0gh	456.8 ± 26.5a–d
	L. acidophilus L10	46.5 ± 2.4 fg	0.4 ± 0.0de	2052.0 ± 240.8ij
	B. lactis B94	39.8 ± 3.8def	0.4 ± 0.0ef	2058.0 ± 361.7ij
	L. casei L26	64.7 ± 2.2 g	0.4 ± 0.0d	2941.3 ± 412.8 k
	PA	18.2 ± 4.0bc	0.5 ± 0.0fgh	1031.9 ± 260.3d–h
	PB	19.9 ± 1.4bcd	0.5 ± 0.0fgh	930.1 ± 176.3c–g
	PC	13.8 ± 0.5bc	0.5 ± 0.0fgh	623.0 ± 41.2a–e
	FA	15.0 ± 1.1bc	0.5 ± 0.0fgh	690.0 ± 90.5a–f
	FB	14.4 ± 0.7bc	0.5 ± 0.0fgh	699.9 ± 18.0a–f
	FC	12.3 ± 0.9bc	0.4 ± 0.0gh	645.6 ± 94.0a–e

Means ± standard deviation with different letters in the same column and for the same food matrix are significantly different ($P < 0.05$), n = 3.
Abbreviations: PA, PB and PC = *L. plantarum* A6 in co-culture with *L. acidophilus* L10. *B. lactis* B94 and *L. casei* L26, respectively; FA, FB and FC = *L. fermentum* OgiE1 in co-culture with *L. acidophilus* L10. *B. lactis* B94 and *L. casei* L26, respectively.

differences of η_{app} of a food matrix fermented by single strains in the same total fermentation time (Fig. 3).

The η_{app} of RS and RSPF gruels fermented by single probiotic strains was always higher than of the same gruels fermented by single amylolytic strains (Fig. 4A and B), independently of the differences of fermentation time between them (Table 1). Although the addition of passion fruit fiber had increased significantly the final η_{app} of gruels fermented by probiotic bacteria, had no effect on the apparent viscosity of gruels fermented by *L. fermentum* and *L. plantarum*. The higher apparent viscosity in RSPF gruels fermented by probiotic strains can be explained by the decreasing of fermentation time – remarkably of the gruels fermented by *B. lactis* and *L. casei* – promoted by passion fruit fiber addition, which reduced the extension of amylolytic (Fig. 2A and B) and probably proteolytic activities.

The association of one amylolytic to one probiotic strain had a remarkable effect on the pattern of the viscosity decreasing curves (Fig. 4C and D). In RS gruels fermented by co-cultures, the η_{app} of the final product was between 1.7 and 3.8 Pa·s, in average, being the lowest in gruels fermented by co-cultures made of one amylolytic strain and *L. casei* ($P < 0.05$), which can probably be ascribed to their longer fermentation time that allowed longer action of amylolytic enzymes (and possibly also of proteolytic enzymes). The averages of apparent viscosities of the gruels fermented by co-cultures are between the limits of a so considered spoonable food (Krokida, Maroulis, & Saravacos, 2001; Mouquet & Trèche, 2001).

Compared to the non-fermented controls, fermentation by all bacteria combinations reduced significantly the consistency index (K) and increased the power law index (n) of RS and RSPF gruels ($P < 0.05$). In average, the K of the fermented gruels approached K of yoghurts enriched or not with passion fruit fiber (Espírito-Santo et al., 2013). The n was higher in RS control gruel than in RSPF control gruel (Table 2) and in all fermented gruels it was typical of a cereal-based food (Drozdek & Faller, 2002; Vaikousia, Biliaderisa, & Izydorczyk, 2004).

Regarding the RS gruels fermented by co-cultures, the addition of passion fruit fiber increased η_{app} of gruels fermented by an amylolytic strain in co-culture with *L. casei* and had no effect on apparent viscosity of the others RSPF fermented gruels. In general, passion fruit fiber decreased the area of thixotropic loop, but significantly only in the RSPF gruel fermented by *L. fermentum* in co-culture with *L. acidophilus*

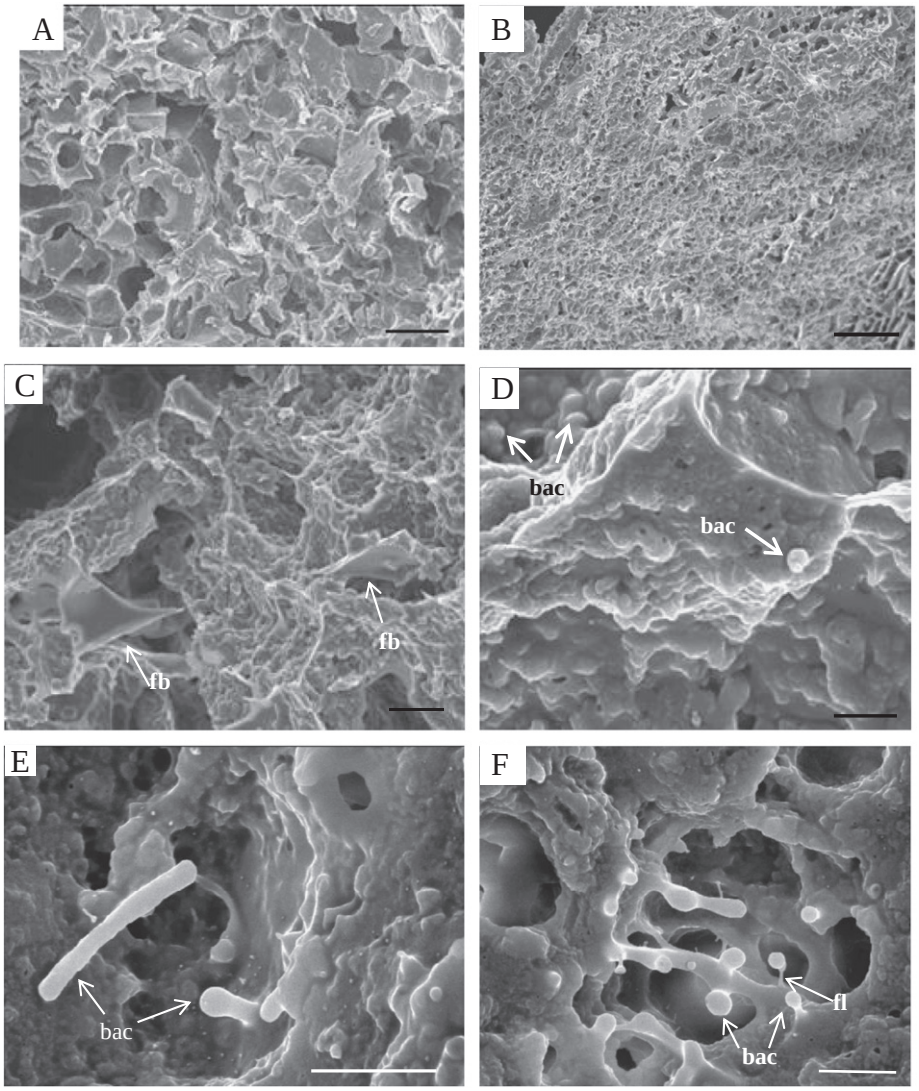

Fig. 5. Microstructure of the gruels: A — control non fermented gruel made of rice flour and soy milk at 10 g. 100 g^{-1} of dry matter each (RS), B — RS gruel fermented by *L. plantarum* A6, barrs = 30 μm. C and D — RS gruels with addition of passion fruit fiber at 1 g. 100 g^{-1} of dry matter, fermented by *L. plantarum* A6 in co-culture with *B. lactis* B94, bars = 30 and 3 μm, respectively. E and F — RS gruels fermented by *L. plantarum* A6 and *L. fermentum* OgiE1, respectively, bars = 3 μm. Abbreviations: bac = bacteria; fb = fiber; fl = filament suggesting exopolysaccharide.

(Table 2). Surprisingly, in spite of the presence of pectin in passion fruit fiber – about 10–20% (Yapo & Koffi, 2008) – , the addition of this ingredient had smaller influence on η_{app} than it could be expected for a thickener agent. Such a result can be due to the partial structure breaking promoted by the blade-shaped total dietary fiber from passion fruit rinds during the time of exposure to the shear rate (Fig. 5C).

Besides composition of the ingredients of a fermented cereal or soy-based food product, the viscosity of the final product depends on several factors such as the kinetics of acidification, the amylolytic and proteolytic activities of the fermenting bacteria and the exopolysaccharides produced (or not) by them, the degree of soy protein gelation and protein–starch interactions (Grygorczyk & Corredig, 2013; Mouquet & Trèche, 2001; Nguyen et al., 2007).

3.4. Microstructure

Remarkable differences could be seen between the microstructures of non-fermented (Fig. 5A) and fermented (Fig. 5B) gruels observed through SEM. As can be seen in Fig. 5A, the gruels were thoroughly gelatinized before the inoculation step and no grains of starch could be seen. Non-fermented gruel was characterized by a coarser and irregular structure with large and heterogeneous fragments while fermented gruels made more homogeneous and smoother structure with smaller fragments.

The only noteworthy difference observed between RS and RSPF fermented gruels was the presence of passion fruit fiber in blade-shape or forming cavities recovered by the gruel and nesting large amount of bacteria (Fig. 5C and D). This structural relationship between passion fruit fiber, food matrix and fermenting bacteria was also observed in yoghurts supplemented with the same fruit fiber (Espírito-Santo et al., 2013).

In spite of having no reports about the production of exopolysaccharides by *L. fermentum* Ogi E1 and *L. plantarum* A6, the micrographs (Fig. 5E and F) suggest EPS production and release by these amylolytic bacteria strains since the bacteria surroundings presented a smoothie and mucous aspect if compared to other parts without appearing bacteria, in which the structure seems grainy and opaque. Moreover, filaments with mucous aspect were seen always associated to probiotic and amylolytic bacteria in numerous fields of the samples (Fig. 5F).

4. Conclusions

This study demonstrated, for the first time, the amylolytic activity in commercial probiotic strains *L. acidophilus* Lafti L10, *L. casei* Lafti L26 and *B. animalis* subsp. *lactis* Lafti B94. However, the association of the probiotic strains to amylolytic bacteria *L. fermentum* Ogi E1 and *L. plantarum* A6 was necessary to reduce the pH, the fermentation time and the apparent viscosity of a rice/soy milk gruel and obtain a yoghurt-like product. The addition of passion fruit fiber exerted less influence on the apparent viscosity of the fermented products than the composition of the bacterial cultures. Photomicrographs suggest production of exopolysaccharides by amylolytic lactic acid bacteria strains.

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