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Microcapsules loaded with the probiotic *Lactobacillus paracasei* BGP-1 produced by co-extrusion technology using alginate/shellac as wall material: Characterization and evaluation of drying processes

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ABSTRACT

Microcapsules containing *Lactobacillus paracasei* BGP-1 were produced by co-extrusion technology using alginate and alginate-shellac blend as wall materials. Sunflower oil and coconut fat were used as vehicles to incorporate BGP-1 into the microcapsules. The microcapsules were evaluated with regard the particle size, morphology, water activity and survival of probiotics after 60 days of storage at room temperature. Fluidized bed and lyophilization were used to dry the microcapsules and the effect of these processes on probiotic viability was also evaluated. Next, dried microcapsules were exposed to simulated gastrointestinal fluids to verify the survival of BGP-1. Microcapsules dried by fluidized bed had spherical shape and robust structures, whereas lyophilized microcapsules had porous and fragile structures. Dried microcapsules presented a medium size of 0.71–0.86 mm and a_w ranging from 0.14 to 0.36, depending on the drying process. When comparing the effects of drying processes on BGP-1 viability, the fluidized bed was less aggressive than lyophilization. The alginate-shellac blend combined with coconut fat as core effectively protected the encapsulated probiotic under simulated gastrointestinal conditions. Thus, the production of microcapsules by co-extrusion followed by drying using the fluidized bed is a promising strategy for protection of probiotic cells.

1. Introduction

Probiotics are microorganisms that may improve health when administered in adequate amounts (FAO/WHO, 2002). Several studies revealed that probiotics may also reduce the incidence of cancer, allergies, inflammatory diseases, lactose intolerance, diarrhea, as well as improve the defenses of the immune system, leading to an additional protection against pathogens (Pandey, Naik, & Vakil, 2015; Parvez, Malik, Kang, & Kim, 2006).

Probiotic products have to contain 10^6–10^9 CFU/g to be effective and to confer health benefits to the consumers (Champagne, Ross, Saarela, Hansen, & Charalampopoulos, 2011). However, many factors may affect the viability of probiotics in food, such as the pH, water activity, storage conditions and processing, thereby challenging researchers during the development of new probiotic products for food industry. To overcome these problems, encapsulation technologies can be used to protect probiotics and to control their release into the intestine (Fávaro-Trindade, Heinemann, & Pedroso, 2011). One of these technologies is the microencapsulation by vibration, which consists in extruding a solution through a nozzle and applying simultaneously a vibrational frequency to produce a laminar jet (Whelehan & Marison, 2011). Depending on the device, it is used a monocentric or concentric nozzle system, which produces different types of microcapsules, respectively, by extrusion and co-extrusion. The concentric system presents internal and external nozzles that allow the production of reservoir type microcapsules. Shinde, Sun-Waterhouse, and Brooks (2014) evaluated the co-extrusion using alginate and apple skin polyphenols to protect *Lactobacillus acidophilus* in a milk beverage at 4 °C. After 50 days of storage, the decrease on cell viability was very low, indicating that the co-extrusion technology was efficient to protect probiotics.

The wall of microcapsules may be composed of several materials. In this context, alginate is one of the most used polymers for encapsulation of cells because it has a food-grade status (non-toxic) and it is cheap

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(Kailasapathy, 2002; Vos, Faas, Spasojevic, & Sikkema, 2010). However, microcapsules produced with alginate may present a wall with high porosity, leading to loss of core material throughout the storage. To overcome this problem, blends of alginate and other polymers such as shellac may be used to reduce wall porosity (Burgain, Gaiani, Linder, & Scher, 2011; Chew, Tan, Long, & Nyam, 2015). Shellac is a natural polymer from the insect *Kerria lacca* and it is considered a food additive by FDA (The United States Food and Drug Administration). In addition, it has a protective effect in the gastric fluid, which is an advantage when used in microcapsules to improve probiotic resistance (Schell & Beermann, 2014; Strummer et al., 2010).

Many materials may be used to form the core of microcapsules produced by co-extrusion. One example are the lipids, which are protective matrices for probiotics, as demonstrated by some studies using the spray chilling technology to produce solid lipid microparticles (Okuro, Thomazini, Balieiro, Liberal, & Fávaro-Trindade, 2013; Pedroso, Thomazini, Heinemann, & Fávaro-Trindade, 2012). However, solid lipid microparticles produced by spray chilling are matrix-type particles, so the probiotic are dispersed in the whole structure, including at the surface where they are unprotected. On the other hand, the co-extrusion of a lipid matrix containing probiotics (core) with alginate (wall material) creates a reservoir-type microcapsule, which may efficiently protect the microorganisms. In this context, it is important to select an adequate vehicle to carry and protect the probiotics. The coconut oil has a high content of saturated fatty acids, which may contribute to reduce oxidative processes and improve stability at higher temperatures when used in microcapsules. This material has some characteristics that are important to application in foods, such as a melting point between 25 and 28 °C, pleasant flavor and good aroma (Che Man & Marina, 2006). Another lipid material that may be used as carrier is the sunflower oil, which is rich in polyunsaturated fatty acids, besides presenting important antioxidant activity due to the high alpha-tocopherol content (Grompone, 2005).

For industrial applications, the microcapsules have to be dried to avoid deterioration, fermentation and loss of viable cells during storage. According to Morgan, Herman, White, and Vesey (2006), low water activity may improve the survival rates of probiotics in the microcapsules. One of the most used methods to dry microcapsules is the lyophilization, which consists on removing the water by sublimation. However, lyophilization is an expensive process and requires time to complete the drying (up to 48 h), and the dried microcapsules are usually very porous and fragile (Albadran, Chatzifragkou, Khutoryanskiy, & Charalampopoulos, 2015). Another drying process is the fluidized bed, which consists on suspending particles in a rising air upstream. This process is fast (approximately 30 min) and can be conducted using air in mild temperatures, which is important when working with probiotics (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012).

Considering the potential of lipids to protect probiotics and the advantages of the co-extrusion process, this study aimed to produce and characterize microcapsules containing the probiotic *Lactobacillus paracasei* BGP-1 dispersed into sunflower oil or coconut fat, and co-extruded with alginate or with the alginate-shellac blend. In addition, the effect of drying processes on different parameters was also evaluated.

2. Material and methods

2.1. Materials

The core of microcapsules was composed of lyophilized *L. paracasei* BGP-1, which was kindly donated by Sacco Brasil (Campinas, Brazil), sunflower oil (Scamark, France) or coconut fat (Nadya, France). Alginate Algogel 3001 with mannuronic/guluronic acid ratio of 0.64 (Cargill, France) and aqueous shellac solution (Norelac B20, Norevo, Germany) were used as wall materials. Calcium chloride (Sigma, France) was used for gelation of alginate. All the chemicals used in this work were of analytical grade.

2.2. Production of microcapsules loaded with probiotics

The microcapsules loaded with probiotics were produced using the Encapsulator B390 (Buchi, Switzerland). Firstly, the lyophilized probiotics were added at 1% (w/w) into sunflower oil (25 °C) or molten coconut fat (40 °C), and the mixture was homogenized at 4000 rpm using an Ultra-Turrax (Ika T-25, Staufen, Germany) for 1 min. The mixture was loaded into a syringe and pumped into the encapsulator at 2 mL/min, while the alginate solution (18 g/L) or the blend of alginate (18 g/L) and shellac (10 g/L) were pumped at 16 mL/min using compressed air. The encapsulator contained an internal nozzle of 450 μm for the core material (lipid matrix with probiotics), and an external nozzle of 700 μm that was responsible for the co-extrusion process with the wall materials (alginate or alginate-shellac blend). A vibrational frequency of 100 Hz was applied while pumping of the core and wall materials to produce the microcapsules by breaking the jet into droplets of equal sizes. The droplets were immediately collected in a calcium chloride solution (32 g/L) and kept under magnetic stirring for 30 min. After production, the wet microcapsules were dried as described in Section 2.2.1.

The composition of each formulation used to produce microcapsules loaded with *L. paracasei* BGP-1were designated as A – alginate/sunflower oil, B – alginate/coconut fat, C – alginate-shellac/sunflower oil, and D – alginate- shellac/coconut fat. After fluidized bed drying, the formulations were designated as A-FB, B-FB, C-FB and D-FB, while lyophilized microcapsules were designated as A-L, B-L, C-L and D-L.

2.2.1. Drying of microcapsules

In order to improve the viability of probiotics and to compare the effects of different drying methods, the microcapsules were dried by different methods. For this, the microcapsules were divided in two groups, in which one of them was dried by fluidized bed and the other was lyophilized. The drying by fluidized bed (Glatt, Binzen, Germany) was conducted as described by Albadran et al. (2015), with some modifications, using an inlet air temperature of 27 °C for 45 min and air volumetric flow rate of 183 m^3/h. The lyophilization was performed using a Christ Alpha 1-2 lyophilizer (Osterode, Germany) for 48 h. Next, all formulations were stored in lidded polypropylene flasks at room temperature for up to 60 days for stability studies (Section 2.4.2).

2.3. Physical characterization of microcapsules

The morphology of wet and dried microcapsules was evaluated using an optical microscope (Leica Wild M3C, France) and the captured images were analyzed using the software ImageJ 1.47v (USA). The average size of microcapsules, core and membrane thickness were evaluated by measuring one hundred microcapsules. Firstly, the external diameter (d_{ext}, corresponding to the size of the microcapsule) was measured, followed by the measurement of the internal diameter (d_{int}, corresponding to the size of the core). Thus, the membrane thickness was calculated using the following equation:

$$m_t = \frac{(d_{ext} - d_{int})}{2}$$

In this equation, m_t is the membrane thickness of microcapsules, d_{ext} is the external diameter of microcapsules, and d_{int} is the internal diameter of microcapsules.

The water activity was measured using a Pre Water Activity Analyzer Aqualab (Decagon Devices Inc., USA) after the drying processes and during storage.

2.4. Microbiological analysis

2.4.1. Resistance of L. paracasei BGP-1 to encapsulation and drying processes

Since the encapsulation process may affect the viability of probiotics, in the present study, the viable bacterial cells were enumerated before, during (probiotics incorporated into the lipid matrix) and after encapsulation. Lyophilized probiotics and probiotics incorporated into the lipid matrix (core material) were tenfold diluted in 2% (w/w) sodium citrate solution for enumeration. The bacterial suspensions were serially diluted, inoculated on MRS (De Man, Rogosa and Sharpe) agar and incubated under anaerobiosis (Anaerocult, Merck, Germany) at 37 ± 1 °C for 48 h. Similarly, the enumeration of encapsulated probiotics was performed by dispersing 15 g of wet microcapsules in 135 mL of 2% (w/w) sodium citrate solution previously heated at 40 ± 1 °C, followed by homogenization using a stomacher (Grosseron, Coueron, France) for 15 min to release the probiotics. The bacterial suspensions were serially diluted, inoculated on MRS (De Man, Rogosa and Sharpe) agar and incubated under anaerobiosis (Anaerocult, Merck, Germany) at 37 ± 1 °C for 48 h. After the drying step, probiotics encapsulated into dried microcapsules were enumerated as previously described, however, considering the dry matter contained in 15 g of wet microcapsules.

2.4.2. Probiotic viability during storage

The microcapsules were stored in lidded polypropylene flasks without humidity control at room temperature, and the encapsulated probiotics were enumerated after 0, 15, 30 and 60 days of storage as described in Section 2.4.1. For comparison purposes, the viability of probiotics in wet microcapsules was also evaluated.

2.4.3. Survival of free and encapsulated L. paracasei BGP-1 under in vitro gastrointestinal conditions

Probiotics loaded into dried microcapsules and lyophilized probiotics (free cells) were evaluated with regard to the survival under *in vitro* simulated gastrointestinal conditions, as described by Gbassi, Vandamme, Ennahar, and Marchioni (2009), with modifications. For this, 1 g of dried microcapsules was added to 9 mL of simulated gastrointestinal fluid (SGF: 9 g/L of NaCl and 3 g/L of pepsin, pH 1.8) and incubated at 37 °C under constant stirring (100 rpm). After 120 min, 10 mL of simulated intestinal fluid (SIF: 9 g/L of NaCl, 10 g/L of pancreatin, 10 g/L of trypsin and 3 g/L of bile salts, pH 6.5) were added to the previous mixture and incubated at 37 °C for further 180 min. Thus, the analysis was conducted over 300 min, and aliquots were removed for bacterial enumeration (Section 2.4.1) after 0, 60, 120, 210 and 300 min of incubation.

2.5. Statistical analysis

All experiments were performed as independent triplicates and the results were evaluated by analysis of variance (ANOVA) followed by Tukey's post-test (95% confidence interval), using the software SAS v9.1.3 (Statistic Analysis Software, SAS Institute Inc., USA).

3. Results and discussion

3.1. Physical characterization of microcapsules

The micrographs of wet microcapsules revealed that all formulations presented spherical shapes, as displayed in Fig. 1. The core of formulations B and D were larger than the cores of formulations A and C, since coconut fat presents a melting temperature between 25 and 28 °C, as previously mentioned, resulting in a solid core at room temperature. Furthermore, the size of microcapsules was very homogeneous, as presented in Fig. 1. The main factor that affects the size of the droplets is the nozzle diameter, while the frequency and flow rate are set-up conjointly to reach an optimal breakage of the jet containing the core and wall materials. Preliminary tests were performed to select optimal parameters for encapsulation of probiotic cells. Smaller microcapsules were not produced due to the granulometry of lyophilized probiotics dispersed into the lipid matrix. To overcome this problem, the size of the internal nozzle could not be smaller than 450 μm. Furthermore, it was also observed that an increase on frequency resulted in microcapsules with irregular shapes. Similar results have been previously reported in the literature. Wang, Waterhouse, and Sun-Waterhouse (2013) evaluated different

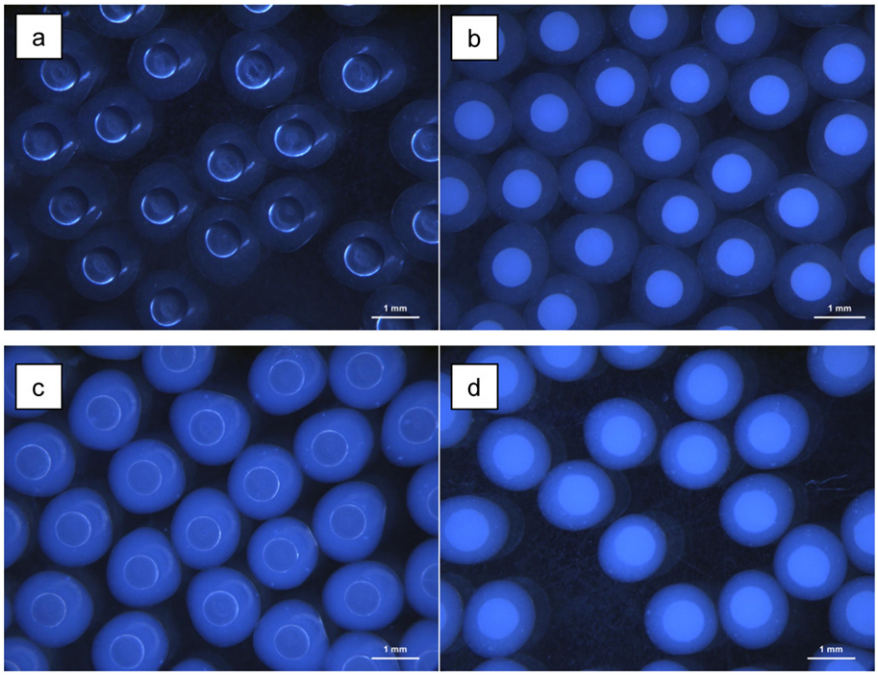

Fig. 1. Photomicrographs of wet microcapsules obtained by co-extrusion. The images were acquired using an optical microscope at 16×. In this figure: a) formulation A (alginate/sunflower oil), b) formulation B (alginate/coconut fat), c) formulation C (alginate-shellac/sunflower oil) and d) formulation D (alginate-shellac/coconut fat).

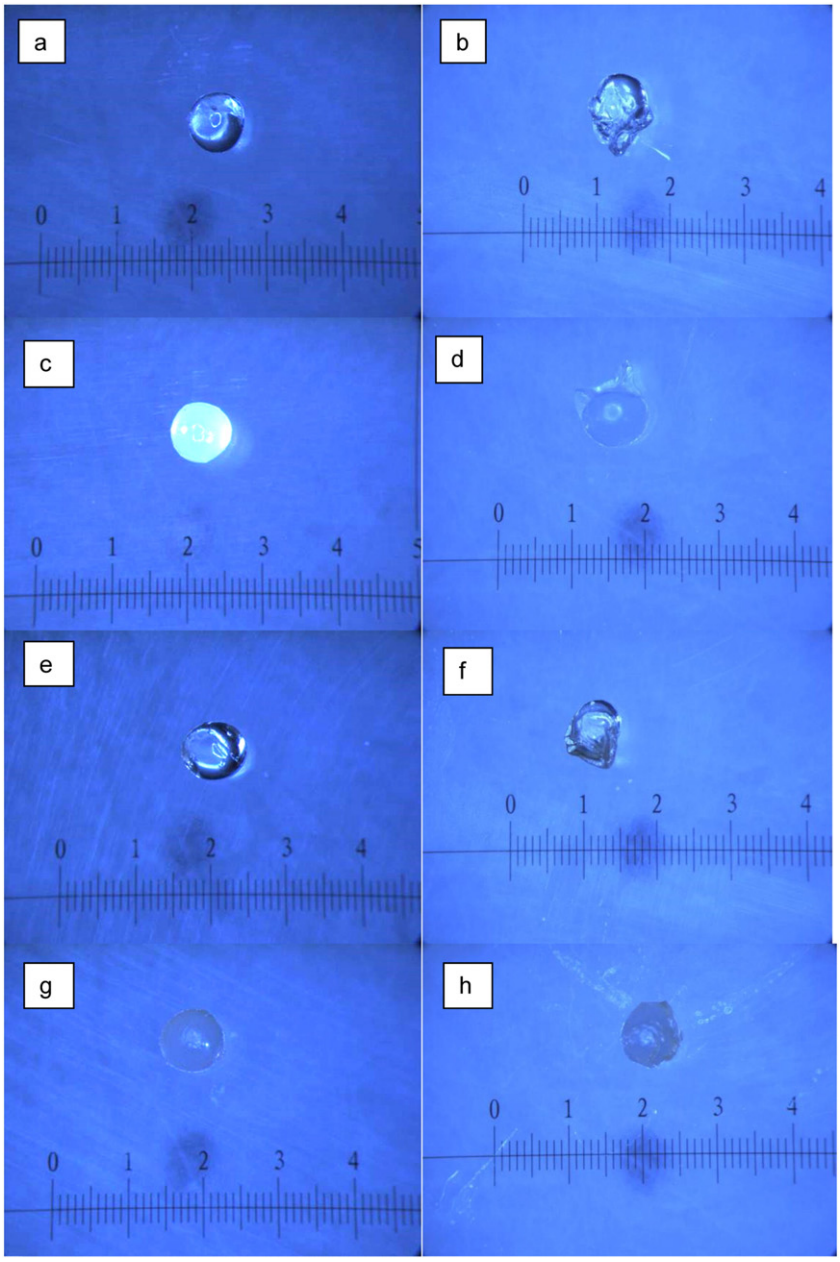

Fig. 2. Photomicrographs of microcapsules obtained by co-extrusion and dried by fluidized bed (FB) or lyophilization (L). The images were acquired using an optical microscope at 16× magnification. In this figure: a) A-FB (alginate/sunflower oil dried by fluidized bed); b) A-L (alginate/sunflower oil dried by lyophilization); c) B-FB (alginate/coconut fat dried by fluidized bed); d) B-L (alginate/coconut fat dried by lyophilization); e) C-FB (alginate-shellac/sunflower oil dried by fluidized bed); f) C-L (alginate-shellac/sunflower oil dried by lyophilization); g) D-FB (alginate-shellac/coconut fat dried by fluidized bed); h) D-L (alginate-shellac/coconut fat dried by lyophilization).

parameters during co-extrusion of canola oil fortified with antioxidants, resulting in microcapsules with spherical shape, similar to those reported in the present study.

The microcapsules dried by lyophilization or fluidized bed were analyzed using an optical microscope, and the micrographs are presented in Fig. 2. Microcapsules dried by fluidized bed presented spherical shape

Table 1
Diameter (mm) of wet and dried microcapsules produced by co-extrusion and loaded with *Lactobacillus paracasei* BGP-1.

	Wet microcapsules (diameter, mm)			Dried microcapsules (diameter, mm)	
Formulations	Shell	Core	Membrane	Fluidized bed	Freeze-dried
A	1.37 ± 0.10^{bA}	0.65 ± 0.09^{bB}	0.36 ± 0.05^{bC}	0.76 ± 0.05^{aA}	0.76 ± 0.10^{bA}
B	1.42 ± 0.11^{abA}	0.70 ± 0.07^{abB}	0.36 ± 0.06^{bC}	0.71 ± 0.03^{aB}	0.86 ± 0.09^{aA}
C	1.48 ± 0.08^{aA}	0.68 ± 0.06^{bB}	0.40 ± 0.04^{aC}	0.75 ± 0.04^{aB}	0.82 ± 0.11^{aA}
D	1.48 ± 0.08^{aA}	0.74 ± 0.06^{aB}	0.37 ± 0.05^{abC}	0.74 ± 0.05^{aA}	0.78 ± 0.10^{bA}

Values with the same upper case letter in a row and values with the same lower case letter in a column are not statistically different ($p > 0,05$). In this table, the formulations are composed of: A) alginate/sunflower oil; B) alginate/coconut fat; C) alginate-shellac/sunflower oil; D) alginate-shellac/coconut fat.

Table 2
Water activity (a_w) values of microcapsules dried by fluidized bed and lyophilization and stored at room temperature for up to 60 days.

	Microcapsules dried by fluidized bed			Microcapsules dried by lyophilization				
Formulations	Initial	15 days	30 days	60 days	Initial	15 days	30 days	60 days
A	0.312 ± 0.002^{aC}	0.313 ± 0.002^{abC}	0.341 ± 0.004^{aB}	0.357 ± 0.003^{aA}	0.137 ± 0.002^{aD}	0.147 ± 0.002^{bC}	0.184 ± 0.004^{cB}	0.209 ± 0.006^{cA}
B	0.304 ± 0.003^{bC}	0.308 ± 0.003^{bBC}	0.316 ± 0.006^{cB}	0.330 ± 0.002^{cA}	0.136 ± 0.003^{aC}	0.147 ± 0.002^{bC}	0.228 ± 0.002^{aB}	0.333 ± 0.012^{aA}
C	0.311 ± 0.003^{aB}	0.316 ± 0.003^{aB}	0.332 ± 0.005^{abA}	0.342 ± 0.005^{bA}	0.139 ± 0.003^{aD}	0.151 ± 0.004^{abC}	0.198 ± 0.003^{bB}	0.244 ± 0.004^{bA}
D	0.311 ± 0.002^{aC}	0.313 ± 0.002^{abC}	0.327 ± 0.003^{bcB}	0.351 ± 0.004^{abA}	0.136 ± 0.002^{aD}	0.154 ± 0.002^{aC}	0.188 ± 0.002^{cB}	0.225 ± 0.003^{cA}

Values with the same upper case letter in a row and values with the same lower case letter in a column are not statistically different ($p > 0,05$). In this table, the formulations are composed of: A) alginate/sunflower oil; B) alginate/coconut fat; C) alginate-shellac/sunflower oil; D) alginate-shellac/coconut fat.

and rigid structure (Fig. 2 a, c, e and g). However, microcapsules dried by lyophilization had a nearly round shape with very fragile structures (Fig. 2 b, d, f and h). During the lyophilization process, the microcapsules lost part of the core material, which negatively affected the results. On the other hand, the drying by fluidized presented better results since the oil was kept inside of the microcapsules due to a less porous structure. Smrdel, Bogataj, and Mrhar (2008) investigated the influence of lyophilization, fluidized bed and air-drying conditions on the size and morphology of microcapsules. Microcapsules dried by lyophilization presented higher porosity and fragility on the touch, which may be attributed to the fast sublimation of water from alginate, leading to pore formation in these areas. In this context, the alginate-shellac has been evaluated to improve oil retention into the microcapsules (formulation C).

The diameters of wet and dried microcapsules were measured and the values are presented in Table 1. According to the results, wet microcapsules showed an average diameter between 1.37 and 1.48 mm, and an average core size between 0.65 and 0.74 mm. Similar results were reported by Gandomi, Abbaszadeh, Misaghi, Bokaie, and Noori (2016) for probiotic microcapsules produced by extrusion using alginate and chitosan, which presented an average diameter between 1.33 and 1.48 mm. As expected, the size of microcapsules decreased after the drying process, resulting in a thin layer of membrane, which may facilitate the application of the microparticles. Dried microcapsules presented diameters ranging from 0.71 to 0.86 mm (Table 1). Despite the large size, these microcapsules may be used in solid foods, in which the texture is not affected by the particle size, such as chocolate and cereal bars. Wang, Yu, Xu, Aguilar, and Wei (2016) encapsulated a *Lactobacillus plantarum* strain using sodium alginate with or without inulin as inner layer and skim milk as outer layer, followed by lyophilization. Those authors also reported large particles, with an average size of 1.5 ± 0.1 mm, similar to the results of the present study. The diameters of all formulations dried by fluidized bed were not significantly different ($p > 0.05$), indicating that the microcapsules dried by this method showed homogeneous sizes. On the other hand, the diameter of microcapsules dried by lyophilization were significantly different ($p < 0.05$), depending on the composition of each formulation.

Water activity is another important parameter to be evaluated because it may affects the viability of encapsulated probiotic, since at high a_w values the microorganisms remain metabolically active and at low a_w values they remain in latent state (Vesterlund, Salminen, & Salminen, 2012. In the present study, the water activity of microcapsules dried by lyophilization and fluidized bed were evaluated throughout storage period and the values are presented in Table 2. As the microcapsules were not stored under vacuum or controlled humidity to simulate real situations, they absorbed moisture from the atmosphere since all samples showed a slight increase on a_w values during storage. The microcapsules dried by lyophilization had lower a_w values than those dried by fluidized bed, considering the microcapsules at the beginning of storage. However, microcapsules dried by lyophilization presented a more pronounced increased on a_w throughout the time than those dried by fluidized bed. Despite of that, both methods were efficient to produce microcapsules with a_w values below 0.35, which is important to guarantee that the microorganisms are not metabolically active in the microcapsules. Other studies also evaluated the effects of drying methods on encapsulated probiotic and, at low a_w, the probiotics kept viable but at a low metabolic state during the storage period (Albadran et al., 2015; Poddar et al., 2014).

In the present study, microcapsules loaded with *L. paracasei* BGP-1 were produced by co-extrusion, representing an alternative to vehiculate probiotics in food, especially in solid food due to the size of the microcapsules. In this context, some studies have shown the potential applications of similar microparticles in different food products. Alvim, Stein, Koury, Dantas, and Cruz (2016) encapsulated ascorbic acid by spray chilling using stearic acid and hydrogenated vegetable fat, and these microparticles were effectively applied in biscuits. Another application was investigated by Bampi et al. (2016) for probiotics encapsulated by the same technology above mentioned, in which the microparticles were satisfactorily incorporated in cereal bars. Thus, the microcapsules produced in the present study have potential for application in solid and non-dairy foods, such as dark chocolate and cereals bars, offering an alternative for those consumers that do not ingest dairy products. This type of products would also contribute to keep the low water activity of the microcapsules, thereby playing a role on maintaining the viability of encapsulated probiotics, as previously discussed.

Table 3
Enumeration of viable *L. paracasei* BGP-1 during the encapsulation process, expressed as log CFU/g.

Formulations	Free cells	Suspension*	Wet microcapsules
A	9.92 ± 0.04^{a}	8.35 ± 0.17^{a}	8.17 ± 0.12^{a}
B	9.82 ± 0.12^{a}	8.39 ± 0.30^{a}	7.93 ± 0.04^{a}
C	9.86 ± 0.06^{a}	8.60 ± 0.43^{a}	8.29 ± 0.16^{a}
D	9.91 ± 0.06^{a}	8.41 ± 0.19^{a}	8.12 ± 0.17^{a}

Values with the same lower case letter in a column are not statistically different ($p > 0,05$). In this table, the formulations are composed of: A) alginate/sunflower oil; B) alginate/coconut fat; C) alginate-shellac/sunflower oil; D) alginate-shellac/coconut fat.

* The suspension refers to the mixture of lipid matrix and probiotic.

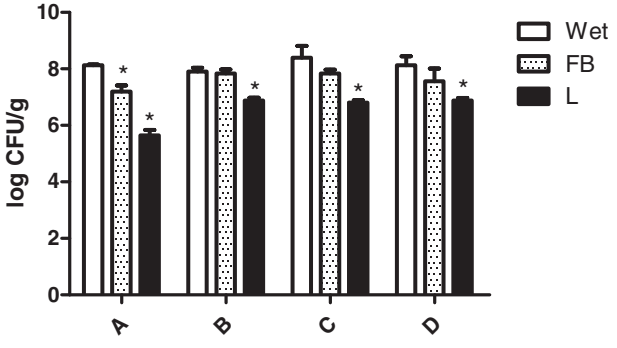

Fig. 3. Effect of drying processes (FB: fluidized bed; L: lyophilization) on the survival of *Lactobacillus paracasei* BGP-1 encapsulated by co-extrusion. In this figure, the formulations are composed of: a) alginate/sunflower oil; b) alginate/coconut fat; c) alginate-shellac/sunflower oil; d) alginate-shellac/coconut fat. Significant reductions ($p < 0.05$) on probiotic populations compared to the wet microcapsules were highlighted with an asterisk.

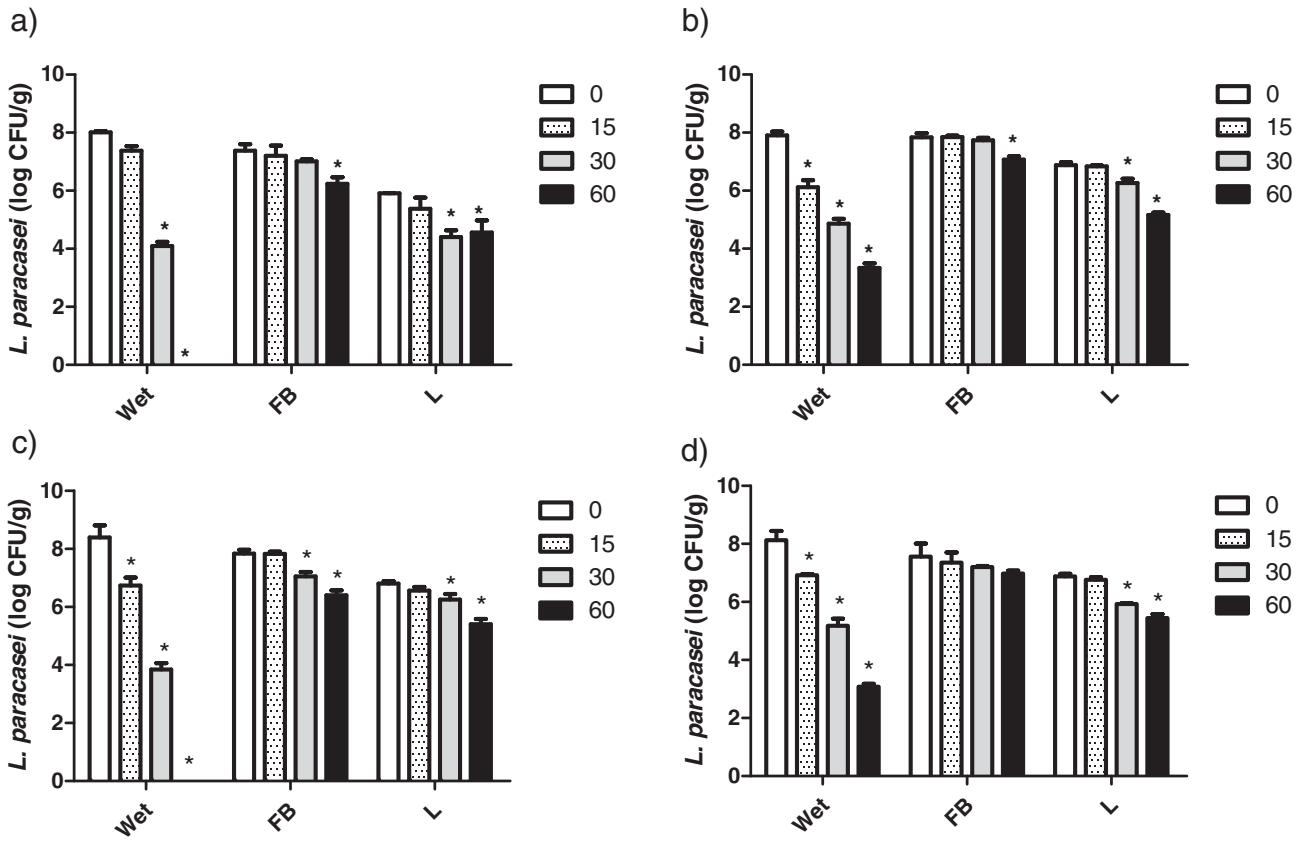

Fig. 4. Viability of encapsulated *Lactobacillus paracasei* BGP-1 loaded into microcapsules obtained by co-extrusion and stored at room temperature for up to 60 days. The microcapsules were analyzed wet or after drying by fluidized bed (FB) or lyophilization (L). White bar - initial time, white bars with points - 15 days of storage, grey bar - 30 days of storage and black bar - 60 days of storage. In this figure, the formulations are composed of: a) alginate/sunflower oil; b) alginate/coconut fat; c) alginate-shellac/sunflower oil; d) alginate-shellac/coconut fat. Significant reductions ($p < 0.05$) on probiotic populations compared to the initial population (100%) were highlighted with an asterisk.

3.2. Resistance of *L. paracasei* BGP-1 to encapsulation and drying processes

The resistance of *L. paracasei* BGP-1 to the encapsulation process was evaluated and the results are presented in Table 3. Initially, lyophilized cells presented *ca.* 9.9 log CFU/g and the core material (lipid matrix) *ca.* 8.4 log CFU/g. This reduction on bacterial population was expected due to the dilution of cells in the lipid matrix (1% w/w). However, these data revealed that the homogenization and heating used to melt the coconut fat did not affect the viability of probiotics. Next, the suspension of lipid matrix and probiotic cells was used to produce the microcapsules, leading to a decrease on the probiotic viability of approximately 0.3 log CFU/g, which indicates that co-extrusion is a process that do not significantly affect the viability of encapsulated probiotics.

The effect of drying processes on the survival of encapsulated probiotic was also evaluated and the results are presented in Fig. 3. According to the results, microcapsules produced with alginate and sunflower oil (formulation A) presented significant reductions on probiotic population after drying by fluidized bed (0.9 log CFU/g) or lyophilization (2.5 log CFU/g), when compared to wet microcapsules. However, probiotic populations in formulations B, C and D were affected only by the lyophilization process, since there were significant reductions when compared to probiotic population in wet microcapsules. Based on these results, the drying by fluidized bed was less aggressive for the encapsulated probiotic when compared to lyophilization, for all formulations.

The lyophilization process depends on a preliminary step, the freeze of the material, which may contribute with an initial reduction on probiotic viability. This negative effect of lyophilization was demonstrated by Wang et al. (2016) while evaluating the viability of *L. plantarum* loaded into lyophilized alginate beads with or without inulin. Those authors reported that when the beads were added of inulin the probiotic population reduced 0.4 log CFU/g and, when the inulin was absent, the probiotic population reduced 0.9 log CFU/g. In addition, the water sublimation during lyophilization may increase the microcapsule porosity, leading to a reduced protection from external conditions and allowing the release of the core, especially when using the sunflower oil. This effect, however, was reduced in formulation C due to the alginate-shellac blend, since the latter improves the wall structure. On the other hand, the fluidized bed drying is a fast process, with no pre-operational steps and with mild temperatures, which reduces the negative effects on probiotics loaded into microcapsules. Albadran et al. (2015) evaluated the viability of *L. plantarum* in alginate microcapsules coated with chitosan after drying processes. Those authors indicated that the fluidized bed drying is performed in relatively low temperatures and in a short period of time, which may contribute to probiotic survival, when compared to lyophilization.

3.3. Probiotic viability during storage

The probiotic was incorporated into lipid matrices and co-extruded with alginate or an alginate-shellac blend to produce microcapsules, and microcapsules were further dried by lyophilization or fluidized bed in order to improve the viability of the probiotics during storage for 60 days at room temperature, as demonstrated at Fig. 4.

The probiotics were incorporated into sunflower oil and encapsulated using alginate or alginate-shellac blend, respectively, in formulations A and C (Fig. 4a and c), which presented a significant reduction on viable

cells ($<5 \times 10^2$ CFU/g) after 60 days of storage when the microcapsules were not dried. Otherwise, probiotic population showed a reduction only *ca.* 1 log CFU/g when the microcapsules (formulation A) were dried by fluidized bed or lyophilization. Nevertheless, microcapsules produced with the alginate-shellac blend, and dried by fluidized bed, presented the best results. Thus, the porosity of alginate microcapsules may have contributed to the reduction on viable probiotics due to the exposure to the environment conditions and due to the leakage of sunflower oil throughout the storage, especially in lyophilized microcapsules.

Coconut fat was used to load *L. paracasei* BGP-1 into formulations B and D (Fig. 4b and d), resulting in a significant reduction of 5 log CFU/g on probiotic population after 60 days of storage in wet microcapsules. When the microcapsules were dried by lyophilization, the probiotic population in formulations B and D reduced approximately 1.5 log CFU/g. On the other hand, when the microcapsules were dried by fluidized bed, only formulation B presented a significant reduction on viable probiotic after 60 days of storage, and no significant reduction on probiotic population was observed ($p < 0.05$) in formulation D, reinforcing the advantage of using the alginate-shellac blend.

Poddar et al. (2014) evaluated the effects of lyophilization, fluidized bed and spray drying on *L. paracasei* viability in whole milk, and observed better results with drying by fluidized bed. Those authors suggested that some aspects as low porosity, larger size and rigid structure may avoid the absorption of water, thereby keeping the viability of the probiotic population. Similarly, in the present study, the drying by fluidized bed was important to keep the viability of encapsulated probiotics mainly due to the less porous structure of the microcapsule, which avoided the leakage of the core. Another factor to be considered is the drying temperature in fluidized bed, which is relatively low (27 °C) and contributes to obtain good survival rates for probiotics after the process. With regard to the lipid matrices, the coconut fat kept the probiotic cells into microcapsules because the storage temperature was lower than its melting point, thereby preventing the probiotic release and improving probiotic counts in these formulations (B and D).

All formulations dried by fluidized bed presented probiotic populations up to 6 log CFU/g after 60 days of storage, which is the minimum amount suggested to confer health benefits to consumers. Similar results were also reported by Albadran et al. (2015) for alginate beads coated with chitosan and kept at 30 °C for 45 days. Those authors reported that probiotics loaded into beads dried by fluidized bed presented better viability when compared with probiotics loaded into lyophilized beads. However, Holkem et al. (2016) evaluated the viability of *Bifidobacterium* BB-12 loaded into alginate beads during storage. Those authors reported that lyophilized beads presented counts of approximately 6 log CFU/g for up to 60 days, despite that the initial population was higher than the initial population in lyophilized microcapsules obtained in this work.

3.4. Survival of free and encapsulated L. paracasei BGP-1 under in vitro gastrointestinal conditions

The survival of free and encapsulated probiotic in simulated gastrointestinal fluids was evaluated and the results are presented in Fig. 5. Free cells reduced 3.7 log CFU/g after 120 min under simulated gastric fluid (SGF) and, at the end of the analysis, the probiotic population was 3.3 log CFU/g. These values demonstrate that the microorganism was fragile under these conditions, which justify the encapsulation to improve probiotic survival. The formulations A and B (produced only with alginate) and dried by fluidized bed showed, respectively, 5.4 and 6.2 log CFU/g of viable probiotics after exposure to simulated gastrointestinal fluids. Interestingly, the probiotic populations in microcapsules produced with the alginate-shellac blend and sunflower oil (formulation C) or coconut fat (formulation D) were *ca.* 6.7 and 7.6 log CFU/g, respectively, after 300 min. Similar results were also

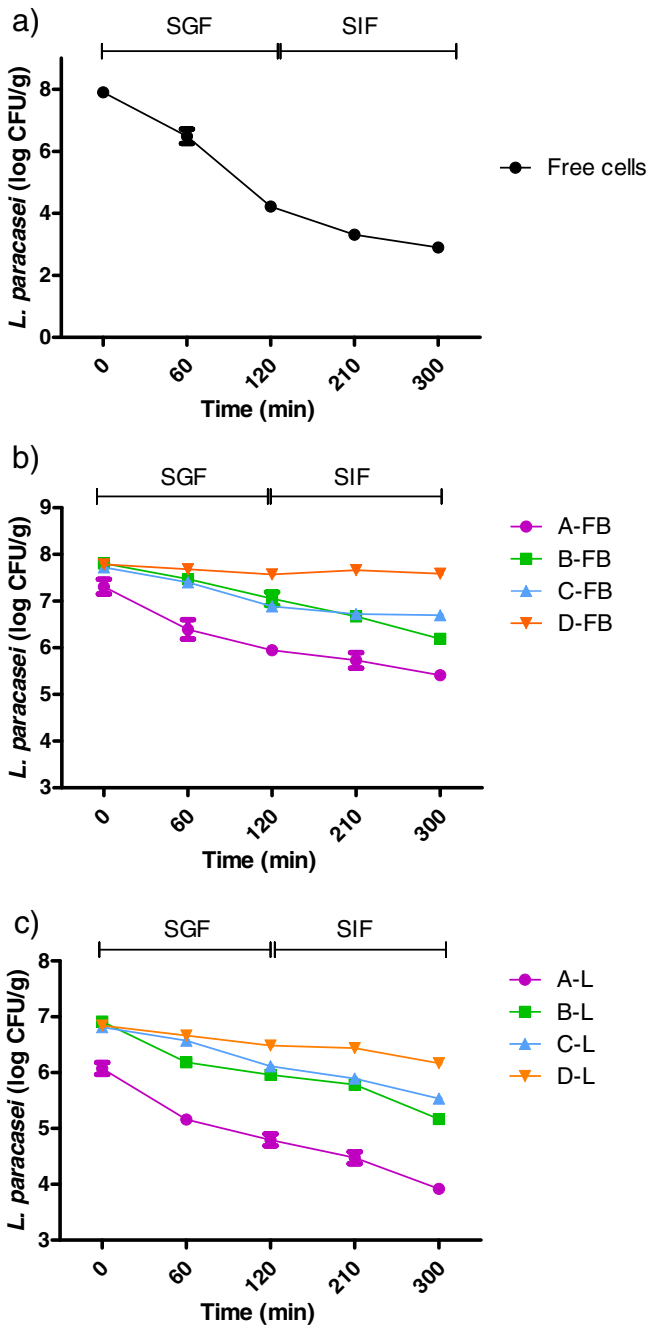

Fig. 5. Survival of free (a) and encapsulated *Lactobacillus paracasei* BGP-1 (b, c) under *in vitro* simulated gastrointestinal conditions. Free cells and microcapsules were exposed to simulated gastric fluid (SGF) followed by simulated intestinal fluid (SIF) for up to 300 min. The formulations were dried by fluidized bed (FB, Fig. 5b) or by lyophilization (L, Fig. 5c). In this figure, the formulations are composed of: A) alginate/sunflower oil; B) alginate/coconut fat; C) alginate-shellac/sunflower oil; D) alginate-shellac/coconut fat.

observed for the microcapsules dried by lyophilization, since formulations A, B, C and D presented, respectively, 3.9, 5.2, 5.5 and 6.2 log CFU/g of viable cells. Thus, these results indicate that the alginate-shellac blend and the core of coconut fat may confer an additional protection to the encapsulated probiotics. According to the literature, the consumers should ingest 10^6–10^9 CFU of viable probiotics per day to obtain health benefits (FAO/WHO, 2002). Thus, the formulations B, C and D dried by fluidized bed and the formulation D dried by lyophilization would meet this criterion. These results also revealed that the

immobilization of *L. paracasei* BGP-1 in coconut fat, followed by co-extrusion covering with alginate or alginate-shellac blend is a very effective technique to protect this microorganism and improve the possibilities of its application and consumption.

In the recent years, different authors have studied the protection of probiotics by encapsulation technologies. Etchepare et al. (2016) encapsulated *L. acidophilus* in alginate beads with resistant starch (Hi-maize) and investigated the probiotic survival under simulated gastrointestinal conditions. Those authors reported that probiotic populations reduced to approximately 5.4–5.8 log CFU/g after exposure to simulated gastrointestinal fluids, which is similar to the values reported in the present study. Okuro et al. (2013) evaluated different lipids to co-encapsulate L. *acidophilus* and prebiotics by spray chilling aiming to protect probiotics under *in vitro* gastrointestinal conditions. Those authors reported that the probiotic population reduced from 8 log CFU/g at the beginning of the assay to approximately 5 log CFU/g at the end of the experiment. Similarly, Pedroso, Dogenski, Thomazini, Heinemann, and Favaro-Trindade (2013) encapsulated *L. acidophilus* in cocoa butter using the spray chilling technology and revealed that the viability of encapsulated cells was enhanced by 67% in simulated gastrointestinal fluids. In the present study, the lipids matrices, especially the coconut fat, were effective on protecting the probiotic under gastrointestinal simulated fluids. Moreover, the highest viability of probiotic during storage was observed when the probiotics were dispersed in this matrix, indicating the potential of lipids to carry and protect probiotics.

4. Conclusions

Fluidized bed drying improved the viability of encapsulated probiotics, mainly due to the robust structure of microcapsules obtained with this method, while microcapsules dried by lyophilization presented a fragile structure. In this sense, the blend of alginate-shellac also improved the microcapsule structure by reducing the porosity, and the coconut fat was more effective on keeping the cells into the microcapsules, since the temperature of storage was lower than the melting point. Microcapsules presented diameters between 0.71 and 0.86 mm, which encourage their application in solid foods, such as cereal bars, dark chocolate and mixed nuts. Thus, this approach may represent an alternative to vehiculate probiotics in non-dairy products. After 60 days of storage at 25 °C, the viability of probiotic loaded into microcapsules dried by fluidized bed was up to 6 log CFU/g, corresponding to 90% of the initial probiotic population. In addition, the formulation produced with alginate-shellac and coconut fat was the most effective on improving probiotic survival in simulated gastrointestinal fluids, mainly by reducing the porosity of microcapsules, in which 7.5 log CFU/g of probiotics (95%) survived at the end of the assay. Thus, the immobilization of probiotics in coconut fat co-extruded with alginate-shellac blend followed by fluidized bed drying is a promising technology to protect and extended the viability of probiotics in functional foods.

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