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# A potential technological application of probiotic microcapsules in lactose-free Greek-style yoghurt

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ABSTRACT

The potential of lactose-free Greek-style yoghurt as a new matrix for incorporation of spray-dried microcapsules containing the probiotic *Bifidobacterium lactis* BB-12 was evaluated. Three different microcapsule formulations were produced using gum arabic, inulin and maltodextrin as wall materials. All formulations showed encapsulation yield above 96% and good probiotic viability (>8 log cfu $g^{-1}$) throughout 30 days of storage (4 °C). Microcapsules produced with gum arabic and inulin showed the best physical properties (lowest moisture, solubility and hygroscopicity values, and highest bulk and tapped densities), and thus were selected to be added to the yoghurt. Addition of the microcapsules increased the product's pH, firmness and adhesiveness; however, it did not affect the viability of the starter cultures. After 30 days of storage, probiotic viability was above 6.5 log cfu $g^{-1}$, indicating that lactose-free Greek-style yoghurt may be an appropriate matrix for B. *lactis* BB-12.



## 1. Introduction

Functional food products play a major role in the food industry because of their health benefiting properties (Silva, Cezarino, Michelon, & Sato, 2018). However, industrial production of functional foods requires the addition of stable ingredients (Eratte, Dowling, Barrow, & Adhikari, 2018).

Within this context, probiotics are viable microorganisms that are beneficial to the host when administered in appropriate quantities (FAO/WHO, 2001). When probiotics are consumed, they colonise the intestinal tract and confer several benefits, such as stimulation of the immune system, control of serum cholesterol levels, maintenance of mucosal integrity, and production of important digestive enzymes, among others (Zoumpopoulou, Pot, Tsakalidou, & Papadimitriou, 2017). *Lactobacillus* and *Bifidobacterium* are the genera that are most commonly used as probiotics in food products (Zoumpopoulou et al., 2017).

A variety of dairy products have been formulated with the addition of different probiotic bacteria. In particular, yoghurt and other fermented milk products are the most popular food carriers for probiotic bacteria (Cruz et al., 2012). Also, Greek-style yoghurt has become popular in the past few years. Greek-style yoghurt is a concentrated semi-solid dairy product, traditionally produced by straining fermented yoghurt in cloth bags to reach the desired amount of solids (Desai, Shepard, & Drake, 2013; Gyawali & Ibrahim, 2016). However, lactose intolerance restricts consumption of these types of probiotic food products (Mani-López, Jiménez-Hernández, Palou, & López-Malo, 2017). Lactose intolerance is a gastrointestinal disorder characterised by symptoms such as abdominal pain, bloating, flatulence, and diarrhoea (Roškar et al., 2017). Approximately 70% of the world's population has a deficiency in β-galactosidase, the enzyme that is responsible for lactose hydrolysis (Lule, Garg, Tomar, Khedkar, & Nalage, 2016). Fermentation of milk into yoghurt is a process that has been used to reduce lactose content in milk (Moreira et al., 2017). Nevertheless, Greek-style yoghurt still contains significant amounts of intact disaccharide. For a food product to be considered lactose free, the concentration of lactose must be lower than 0.1 g 100 $g^{-1}$, according to a new resolution in Brazilian legislation (Brasil, 2017) and the recommendation of the European Food Safety Agency (Trani et al., 2017).

The main challenge when incorporating probiotics into food products is maintenance of viability of the probiotics until time of consumption (Mani-López et al., 2017), where the minimum count should be $10^6$ cfu $g^{-1}$ until the end of the food product's shelf life (Lalićić-Petronijević, Popov-Raljić, Lazić, Pezo, & Nedović, 2017). In

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the case of yoghurts, the viability of probiotics may be affected by several factors, such as type of inoculated probiotic strain, interactions with starter cultures, the amount of lactic acid, oxygen, and hydrogen peroxide, and also the type of substrate (Tripathi & Giri, 2014). As a result, keeping probiotics alive during the shelf life of yoghurt is not a simple task, especially in lactose-free yoghurts, where different carbohydrate profiles could affect the metabolic activity of the cultures and consequently produce derived compounds and, to some extent, also affect the survival of probiotics in the product (Vénica, Wolf, Bergamini, & Perotti, 2016).

For these reasons, new and more efficient techniques to enhance survival of probiotics have been sought. Microencapsulation is so far one of the most efficient methods for introducing viable probiotic bacteria in food products because the encapsulation matrix can provide a physical barrier against stress conditions (Chen, Li, Liu, & Meng, 2017). Among existing microencapsulation techniques, spray drying is acknowledged as one of the most convenient in terms of energy requirements, cost, and process yield (Pinto et al., 2015). In microencapsulation of probiotic bacteria, it is possible to use different types of encapsulating materials. Among the encapsulating materials employed, gum arabic has been used for encapsulating probiotic cells because of its emulsifying ability and low viscosity, as well as its non-toxicity and low cost (Zaeim, Sarabi-Jamab, Ghorani, Kadkhodaee, & Tromp, 2018). In addition, this gum has been shown to be a prebiotic dietary fibre as it arrives undigested in the gut and selectively increases levels of lactobacilli and bifidobacteria (Calame, Weseler, Viebke, Flynn, & Siemensma, 2008; Williams, 2016). Also, it is possible to find several different research works where maltodextrin and prebiotic inulin were also employed as carrier agents for microencapsulation of probiotic bacteria (Fritzen-Freire et al., 2013; Paim, Costa, Walter, & Tonon, 2016; Pinto et al., 2015).

Considering that different combinations of encapsulating materials can result in microcapsules with different structures and physical characteristics, it is important to evaluate the impact of application of microcapsules on the technological characteristics of the product as well as the viability of the probiotics. In this context, the aim of this study was to investigate the potential of spray drying microencapsulation of *Bifidobacterium animalis* ssp. *lactis* BB-12 with gum arabic, inulin and maltodextrin for production of a lactose-free Greek-style yoghurt. The microbiological, physicochemical, and texture properties of the dairy product were evaluated throughout 30 days of storage at 4 °C.

## 2. Material and methods

### 2.1. Material

The probiotic B. *animalis* ssp. *lactis* BB-12 (Nu-trish® BB-12®) was obtained from Chr. Hansen (Hørsholm, Denmark). Gum Arabic (Instantgum™ BA, Nexira, France), high-performance inulin (average DP ≥ 23, Orafti® HPX, Beneo, Tienen, Belgium) and maltodextrin DE 10 (MOR-REX 1910®, Ingredion, São Paulo, Brazil) were used as wall materials. MRS Agar, MRS Broth (Difco, Sparks, MD, USA), lithium chloride (Vetec, Rio de Janeiro, Brazil), sodium propionate (Sigma–Aldrich Co., St. Louis, MO, USA), AnaeroGen® (Oxoid, Hampshire, UK) and M17 Agar (Sigma–Aldrich Co.) were used for the microbiological assays. Ingredients used in the production of the lactose-free Greek-style yoghurt were: commercial pasteurised bovine milk containing 3.0% fat, 3.5% protein and 5.0% carbohydrate (Tirol, Treze Tílias, SC, Brazil), enzyme β-galactosidase from *Kluyveromyces lactis* (EC 3.2.1.23) (Prozyn, SP, Brazil), sucrose (Caravelas, Ariranha, SP, Brazil) and yoghurt starter culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (YF-L812, YoFlex®) obtained from Chr. Hansen.

### 2.2. Preparation of the probiotic suspension

Cell pellets of *Bifidobacterium lactis* BB-12 were prepared following the procedures proposed by Chaikham et al. (2013) with modifications. The freeze-dried culture (10 g) was rehydrated in 100 mL of MRS broth modified with lithium chloride (0.2%, m/v) and sodium propionate (0.3%, m/v) (MRS-LP broth, used to selectively promote the growth of bifidobacteria) and incubated at 37 °C for 24 h under anaerobic conditions using AnaeroGen®. The stock solution was prepared by mixing 1 mL of the incubated culture with 0.25 mL of sterile glycerol in sterile vials and kept at −80 °C until use. For the recovery of the cells the stock culture was thawed at room temperature. The culture was propagated by inoculation in MRS-LP broth at 10% (v/v), and then incubated anaerobically at 37 °C for 24 h. The resulting culture was harvested by centrifugation at 1000×*g* for 10 min at room temperature and washed twice with sterile 0.85% (m/v) NaCl solution. The pellet was re-suspended in its original volume of 0.85% (m/v) NaCl solution and subsequently used either directly as free cells (yoghurt with free cells - GYF) or subjected to microencapsulation by spray drying.

### 2.3. Microencapsulation procedure

Three different feed solutions were prepared for the production of the microcapsules containing B. *lactis* BB-12, are described in Table 1. Wall materials were homogenised into sterile distilled water and heat treated at 80 °C for 30 min. Before adding the probiotic cell suspension, the feed solutions were cooled to room temperature (±25 °C). The microencapsulation process was performed in a laboratory scale spray dryer (B-290 mini spray dryer, Buchi, Flawil, Switzerland) using the operating parameters described by Fritzen-Freire et al. (2012). Drying was performed at a constant air inlet temperature of 150 °C and outlet temperature of 50 ± 4 °C. Each drying assay was carried out in triplicate. Microcapsules were collected from the base of the cyclone, placed in sterile vials and stored without relative humidity control at 4 ± 1 °C for 30 days in a refrigerator.

### 2.4. Characterisation of the microcapsules

Characterisation of the microcapsules was performed on the first day of their storage at 4 °C. All the experiments were carried out in triplicate, except those for morphology and particle size. The morphology of the microcapsules was evaluated using a scanning electron microscope (SEM) (JEOL JSM 6390 LV, Tokyo, Japan) at an accelerating voltage of 5 kV. Samples were placed on metal stubs using double-sided adhesive tape and coated with gold using a vacuum sputtering coater (Leica, model EM SCD 500, Wetzlar, Germany), as described by Fritzen-Freire et al. (2012).

Particle size was measured from the SEM micrographs in their original magnification using the ImageJ software (version 1.51j8; https://imagej.nih.gov/ij/). Average diameter was determined by measuring 500 particles. Moisture content was determined by oven

**Table 1**
Composition of the feed solutions for each formulation used in the spray drying process for encapsulation B. *lactis* BB-12.

| Formulation | Wall material (%, m/v) | | | Concentration of culture (%, v/v) |
|---|---|---|---|---|
| | Gum arabic (GA) | Inulin (IN) | Maltodextrin (MD) | |
| GA | 20 | – | – | 10 |
| GA:IN | 10 | 10 | – | 10 |
| GA:MD | 10 | – | 10 | 10 |

drying at 102 ± 2 °C until reaching constant weight (IDF, 1993). Water activity ($a_w$) was measured at 25 °C using an Aqualab 4 TE analyser (Decagon Devices, Pullman, WA, USA).

Hygroscopicity was determined according to the methodology described by Cai and Corke (2000), with modifications. The microcapsules (1 g) were placed in an airtight desiccator containing a saturated solution of NaCl (RH 75.3%) at 25 °C. The hygroscopicity was expressed as grams of water absorbed per 100 g of dry solids after 7 days of storage.

Solubility was evaluated according to the methodology proposed by Fernandes, Borges, and Botrel (2014). One gram of microcapsules was added to 25 mL of distilled water and the mixture was agitated for 5 min using a magnetic stirrer. The mixture was then centrifuged at 760×*g* for 10 min. An aliquot of 20 mL of each supernatant was transferred to pre-weighed Petri dishes and oven dried at 105 °C overnight. The solubility was expressed as the percentage of dried supernatant in relation to the amount of the initial sample.

A Minolta Chroma Meter CR-400 (Konica Minolta, Osaka, Japan) colorimeter with illuminant D65 and 2° observation angle was used to measure the colour of the microcapsules. Results were expressed in CIELab colour space. The L* component is the lightness and ranges from 0 (black) to 100 (white); the a* parameter shows the variation from red (+) to green (−); and the b* parameter shows the variation from yellow (+) to blue (−).

Bulk and tapped density (g $mL^{-1}$) were determined according to Shishir, Taip, Aziz, and Talib (2014). From the values of bulk and tapped densities, the Hausner ratio (HR) (Hausner, 1967) and the Carr's index (CI) (Carr, 1965) were calculated to determine the flowability of the powders by means of Equations (1) and (2).

$$HR = \text{Tapped density}/\text{Bulk density} \quad (1)$$

$$CI = (\text{Tapped density} - \text{Bulk density}/\text{Tapped density}) \times 100 \quad (2)$$

### 2.5. Enumeration of *B.* lactis *BB-12 and encapsulating yield*

Viable cell counts of B. *lactis* BB-12 in the microcapsules were carried out on days 1, 15 and 30 of storage at 4 °C. The probiotic cells were released from the microcapsules according to the methodology proposed by Sheu, Marshall, and Heymann (1993). One gram of microcapsules was resuspended in 9 mL of phosphate buffer (0.1 mol $L^{-1}$, pH 7.0) followed by homogenisation for 10 min using a magnetic stirrer. Enumeration of B. *lactis* BB-12 was performed according to the method proposed by Vinderola and Reinheimer (1999), i.e., by the pour plate method using MRS agar modified with lithium chloride (0.2%, m/v) and sodium propionate (0.3%, m/v) (MRS-LP agar). The plates were incubated at 37 °C for 72 h under anaerobic conditions using AnaeroGen®. Results were expressed as log cfu $g^{-1}$.

The encapsulating yield (EY) was calculated according to Picot and Lacroix (2004) using Equation (3).

$$EY = (N/N_0) \times 100 \quad (3)$$

where N is the number of viable cells (log cfu) per gram of dry matter in the microcapsules, and $N_0$ is the number of viable cells (log cfu) per gram of dry matter in the feed solutions.

### 2.6. Manufacture of the lactose-free Greek-style yoghurt

Pasteurised milk was hydrolysed with β-galactosidase (0.15%, v/v) at 38 °C for 2 h (Moreira et al., 2017) before fermentation. The milk was added with sucrose (5.0% m/v), heated at 90 °C for 15 min and subsequently cooled to 42 °C. The mixture was inoculated with yoghurt starter culture and incubated at 42 °C until pH 4.7 was reached. After fermentation, the yoghurt was cooled to 4 °C overnight. Greek-style yoghurt was prepared by draining the yoghurt in cloth bags for 18–20 h at 4 °C, as suggested by Şanlidere Aloğlu and Öner (2013). It was then stirred with added 0.5% (v/v) of probiotic cell suspension, prepared as previously described, for the preparation of the yoghurt samples containing free cells (GYF); and with added 2.5% (m/v) of microcapsules produced with gum arabic and inulin (GA:IN), for preparation of the yoghurt samples containing microcapsules (GYM). The GA:IN microcapsules were chosen because they showed more suitable characteristics after the analyses of the microcapsules (see Results and discussion). The products obtained were packaged in 100 mL plastic cups, sealed with aluminium foil lids, and stored at 4 ± 1 °C for 30 days.

### 2.7. Technological characteristics of the lactose-free Greek-style yoghurt

#### 2.7.1. Physicochemical composition

Yoghurt samples were analysed (Day 1) for their content of total solids, protein, fat and ash according to the methods proposed by AOAC (2005). Levels of lactose were determined using an ion chromatography (IC) system (Metrohm, Compact IC Pro, model 881, Herisau, Switzerland), equipped with a pulsed amperometric detector and Metrosep Carb 2 column. Chromatographic separations were performed with a mobile phase of aqueous sodium hydroxide (5 mmol $L^{-1}$), a flow-rate of 0.3 mL $min^{-1}$ and an injection volume of 20 μL. Yoghurt samples were prepared by dilution in water (1:100) and submitted to dialysis (Metrohm Inline Dialysis, Herisau, Switzerland).

#### 2.7.2. pH, acidity and texture analysis

pH, acidity and texture analyses were carried out in triplicate on days 1, 15 and 30 of storage at 4 °C. The pH of the samples was measured using a pH-meter (Quimis model Q-400A, Diadema, SP, Brazil). Titratable acidity (% lactic acid) was determined by titrating up to pH 8.2 with 0.1 mol $L^{-1}$ NaOH.

A texture analyser model TA-XT plus (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 50 kg load cell was used to determine the texture parameters of the yoghurts. The texture profile analysis (TPA) was performed on the yoghurt samples (50 mm diameter and 20 mm height) contained in individual plastic cups using an aluminium cylinder probe of 25 mm in diameter. Yoghurts (4 °C) were compressed up to 10 mm depth with a test speed of 1.0 mm $s^{-1}$. The TPA parameters were obtained with the Texture Exponent software version 4.0.13.0 (Stable Micro Systems, Surrey, UK) and results were expressed as the average of six measurements.

#### 2.7.3. Viability of the probiotic and starter cultures

Viability of probiotic and starter cultures in the lactose-free Greek-style yoghurt samples were determined on days 1, 15 and 30 of storage at temperature of 4 °C. Twenty-five grams of samples were added to 250 mL of phosphate buffer solution (0.1 mol $L^{-1}$, pH 7.0), which was then homogenised for 10 min to release the probiotic cells from the microcapsules. Appropriate serial dilutions were prepared using peptone water (0.1%, m/v) and bacteria were enumerated by the pour plate technique. B. *lactis* BB-12 was enumerated as described in Section 2.5. Enumeration of L. *delbrueckii* subsp. *bulgaricus* was carried out using MRS agar under aerobic conditions at 37 °C for 72 h (Dave & Shah, 1996). S. *thermophilus* was enumerated using M17 agar supplemented with

lactose solution (10%, m/v) under aerobic conditions at 37 °C for 48 h (IDF, 1997).

### 2.8. Statistical analysis

Comparisons between the samples were performed by analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$) and the results were expressed as mean ± standard deviation. The data analysis was performed using the STATISTICA 7.0 software (StatSoft Inc., Tulsa, OK, USA).

## 3. Results and discussion

### 3.1. Characterisation of the microcapsules

SEM images of spray-dried microcapsules prepared with different wall material formulations are shown in Fig. 1. All formulations produced relatively spherical microcapsules with concavities and similar average particle sizes, around 10 μm (Table 2). There were no visible fractures or cracks on the surface of the microcapsules, which is important to ensure good structural integrity and protection of probiotic cells (Fritzen-Freire et al., 2012). In addition, no probiotic cells were visible either outside or on the surface of the particles, indicating that B. *lactis* BB-12 was completely trapped within the wall materials.

Different combinations of wall materials affected the shape of the particles. The GA and the GA:MD microcapsules (Fig. 1a,c) exhibited a smoother surface with more pronounced irregularities, such as concavities or dents, than the GA:IN microcapsules. However, the addition of maltodextrin (GA:MD) did not show any perceptible changes in morphology in comparison with GA microcapsules. Similar morphological shapes of the GA and the GA:MD microcapsules were obtained in other studies conducted by Di Battista, Constenla, Ramírez-Rigo, and Piña (2015) and Uekane, Costa, Pierucci, da Rocha-Leão, and Rezende (2016), respectively. In contrast, incorporation of inulin (GA:IN) (Fig. 1b) in the microcapsules resulted in a corrugated and wrinkled particle surface.

The results for particle characterisation are shown in Table 2. Moisture content of the GA microcapsules was slightly higher ($P < 0.05$) than those of GA:IN and GA:MD microcapsules. These differences can be associated with the higher amount of gum arabic used in the GA microcapsules, since the carbohydrate components of gum arabic and its highly branched structure provides a favourable environment for binding water (Phillips, Takigami, & Takigami, 1996). In the literature, different critical moisture contents have been reported for storage stability of spray-dried powders. Ghandi, Powell, Chen, and Adhikari (2012) reported a moisture content below 5% (w/w) as a good parameter for ensuring good storage stability and survival of the bacteria cells. Based on this criterion, the formulation for GA:IN microcapsules showed more suitable values for moisture. With respect to water activity, all the microcapsule formulations had values below 0.2, which is very positive to ensure microbiological stability (Huang et al., 2017; Vesterlund, Salminen, & Salminen, 2012).

**Table 2**
Characteristics of the microcapsules containing B. *lactis* BB-12 produced by spray drying.[a]

| Parameter | Microcapsules | | |
|---|---|---|---|
| | GA | GA:IN | GA:MD |
| Particle size (μm) | 9.84 ± 4.10$^{a}$ | 10.28 ± 4.09$^{a}$ | 9.93 ± 3.83$^{a}$ |
| Moisture content (%) | 5.10 ± 0.01$^{a}$ | 4.75 ± 0.01$^{b}$ | 4.76 ± 0.02$^{b}$ |
| Water activity ($a_w$) | 0.115 ± 0.003$^{b}$ | 0.123 ± 0.002$^{a}$ | 0.119 ± 0.003$^{ab}$ |
| Hygroscopicity (%) | 18.03 ± 1.42$^{a}$ | 13.28 ± 0.48$^{b}$ | 14.06 ± 0.19$^{b}$ |
| Solubility (%) | 73.65 ± 0.20$^{a}$ | 67.84 ± 0.87$^{b}$ | 73.83 ± 0.99$^{a}$ |
| Density parameters | | | |
| Bulk density (g mL$^{-1}$) | 0.38 ± 0.02$^{b}$ | 0.47 ± 0.01$^{a}$ | 0.33 ± 0.01$^{c}$ |
| Tapped density (g mL$^{-1}$) | 0.54 ± 0.01$^{a}$ | 0.57 ± 0.02$^{a}$ | 0.50 ± 0.01$^{b}$ |
| Hausner ratio | 1.41 ± 0.02$^{b}$ | 1.23 ± 0.03$^{c}$ | 1.51 ± 0.01$^{a}$ |
| Carr's index (%) | 29.27 ± 1.14$^{b}$ | 18.59 ± 2.16$^{c}$ | 33.89 ± 0.55$^{a}$ |
| Colour parameters | | | |
| L* | 97.68 ± 0.33$^{a}$ | 98.08 ± 0.03$^{a}$ | 96.25 ± 0.12$^{b}$ |
| a* | −0.67 ± 0.06$^{b}$ | −0.72 ± 0.02$^{b}$ | −0.39 ± 0.05$^{a}$ |
| b* | 5.60 ± 0.05$^{b}$ | 5.26 ± 0.06$^{c}$ | 7.47 ± 0.14$^{a}$ |

[a] Abbreviations are: GA, microcapsules produced with gum arabic; GA:IN, microcapsules produced with gum arabic and inulin; GA:MD, microcapsules produced with gum arabic and maltodextrin. Values are means ± standard deviation; means with different superscript letters in the same row are significantly different ($P < 0.05$).

Hygroscopicity is an important physical parameter because an increase in the water content through vapor diffusion at the solid–air interface may result in plasticisation and in a glassy-to-rubbery state transition, leading to changes in physical properties (stickiness, flowability, agglomeration) of the microcapsules (Zhang et al., 2018). Values for hygroscopicity of the GA microcapsules were higher ($P < 0.05$) than those of the microcapsules containing inulin (GA:IN) or maltodextrin (GA:MD). This variation in water absorption may be attributed to the higher hygroscopic nature of gum arabic as well as to the higher residual moisture content of the GA microcapsules (5.10%). A similar behaviour was reported by Premi and Sharma (2017), who noted a co-relation between hygroscopicity values and moisture content of drumstick oil encapsulated with gum arabic, maltodextrin and whey protein concentrate. Moreover, Fernandes et al. (2014) noted that use of either inulin or maltodextrin together with gum arabic caused a decrease in the hygroscopicity of rosemary essential oil, microencapsulated by spray drying.

All the microcapsules were fairly soluble, showing values that ranged from 73.7 to 67.8%. The GA and the GA:MD microcapsules showed similar values for solubility ($P > 0.05$). Uekane et al. (2016)

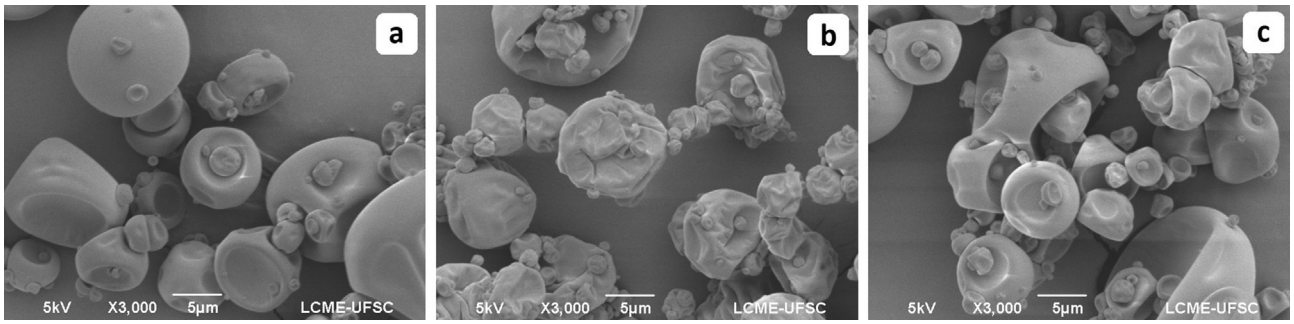


**Fig. 1.** SEM images of the spray-dried microcapsules containing B. *lactis* BB-12 produced with (a) gum arabic (GA), (b) gum arabic and inulin (GA:IN) and (c) gum arabic and maltodextrin (GA:MD).

reported similar results (76%) for microparticles containing sulphur aroma compounds produced by spray drying with gum arabic/maltodextrin. However, the presence of inulin in the GA:IN microcapsules reduced ($P < 0.05$) solubility. The long-chain structure of the inulin used in the GA:IN microcapsules, with average DP $\geq$ 23, may have contributed to the decrease in solubility. Similarly, Nale, Tontul, Aşçi Arslan, Sahin Nadeem, and Kucukcetin (2017) noted a decrease in solubility of kefir microcapsules produced with gum arabic/maltodextrin with added inulin.

Bulk and tapped densities are important physical properties that affect handling, packing, and transportation of spray-dried powders (Fernandes et al., 2014). Thus, production of high density powders is desirable since they can be stored in smaller containers in comparison with a product of low bulk density (Rodríguez-Restrepo, Giraldo, & Rodríguez-Barona, 2017). The highest bulk and tapped densities were noted with the microcapsules containing inulin (GA:IN), which differed ($P < 0.05$) from the other microcapsules, except for the tapped density of the GA microcapsules. The same behaviour was noted by Fernandes et al. (2014), who studied the effects of partial replacement of gum arabic with modified starch, maltodextrin or inulin on the properties of rosemary essential oil microencapsulated by spray drying. On the other hand, the microcapsules containing maltodextrin (GA:MD) showed the lowest bulk and tapped density values ($P < 0.05$). As reported by Goula and Adamopoulos (2008), maltodextrin is considered a skin-forming material. Thus, using maltodextrin as wall material can induce accumulation and trapping of air inside the particles leading them to become less dense. A decrease in bulk density due to addition of maltodextrin was also noted by Caparino et al. (2012) in spray-dried mango powder. Also, the flowability properties of the microcapsules were significantly ($P < 0.05$) affected by the different formulations of wall materials studied. The GA:IN microcapsules exhibited a Hausner ratio of 1.23 and a Carr's index of 18.59%, which indicates 'fair' flow characteristics according to the scale of flowability described by Lebrun et al. (2012). However, the GA and the GA:MD microcapsules corresponded to 'poor' and 'very poor' flowability powders, respectively, showing a tendency to agglomerate.

The colour of the microcapsules is an important quality parameter since it may affect the colour of the final product. All samples showed quite high L* values (luminosity), indicating that wall materials conferred a light colour to the microcapsules. Moreover, values for a* and b* of all the samples were located in the second quadrant (-a*, +b*), showing a tendency to the colours green and yellow, respectively. These results are in agreement with those reported by Gul (2017), who evaluated the stability of microencapsulated *Lactobacillus casei* Shirota by spray drying using blends of gum arabic, maltodextrin and reconstituted skim milk as wall materials. Microcapsules produced with maltodextrin (GA:MD) showed the lowest L* values and the most intense yellow colour ($P < 0.05$). On the other hand, the microcapsules containing inulin showed the least intense yellow colour ($P < 0.05$), which might allow their application in dairy products without affecting the products' appearance.

In conclusion, the results of the characterisation of the microcapsules showed that the GA:IN formulation resulted in more suitable characteristics, such as lower values for moisture, hygroscopicity and solubility, as well as higher bulk and tapped density values and 'fair' flowability characteristics.

Thus, considering the physical characteristics of the GA:IN microcapsules and also the prebiotic properties of the wall materials used, this formulation was chosen to be applied in the lactose-free Greek-style yoghurt.

### 3.2. Encapsulation yield and viability of microencapsulated *B.* lactis BB-12

The effect of microencapsulation on the viability of B. *lactis* BB-12, expressed in terms of encapsulation yield (EY), and the viable cell counts of the microcapsules during storage at 4 °C are shown in Table 3. All formulations exhibited a high encapsulation yield (above 96%), which suggests that B. *lactis* BB-12 has adapted well to the spray drying conditions used. In spite of the GA formulation showing slightly higher encapsulation yield than the GA:IN and the GA:MD formulations, no significant difference ($P > 0.05$) was verified among them. In comparison with other similar studies, the encapsulation yield found in this study can be considered satisfactory. Nunes et al. (2018) reported encapsulation yields of 94.26 and 93.12% for *Lactobacillus acidophilus* La-5 spray dried with himaize and inulin, respectively. Also, an encapsulation yield of 97.43% was obtained by Verruck et al. (2017) for *Bifidobacterium* BB-12 microencapsulated by spray drying using full-fat goats' milk as wall material.

As can be seen in Table 3, the viable cell counts for the microcapsule formulations were very similar and remained stable ($P > 0.05$) during storage at 4 °C, with no significant ($P > 0.05$) differences among them. Results for encapsulation yield and viability during refrigerated storage noted in this study indicated that all combinations of wall materials were compatible with the probiotic strain used. Moreover, the high cell survival noted may be associated with the relatively low outlet temperatures (50 ± 4 °C) during the spray drying process and also with the amount of wall materials used (20%, m/v). According to Huang et al. (2017), a total solids content between 20 and 30% was considered as optimal to ensure high residual viability of different lactic acid strains. In addition, Perdana, Zubia, Kutahya, Schutyser, and Fox (2015) confirmed that an increase in outlet temperatures used in spray drying negatively affected survival of *Lactobacillus plantarum* WCFS1, especially at temperatures higher than 70 °C.

### 3.3. Technological characteristics of the lactose-free Greek-style yoghurt

#### 3.3.1. Physicochemical composition

On the first day post-manufacture, the yoghurt with free cells (GYF) contained (%) 20.68 ± 0.05 total solids, 6.67 ± 0.01 protein, 6.12 ± 0.02 fat, and 0.74 ± 0.02 ash while the yoghurt with the microcapsules (GYM) contained (%) 22.68 ± 0.03 total solids, 6.68 ± 0.03 protein, 5.95 ± 0.03 fat and 0.78 ± 0.01 ash. An expected consequence of addition of the microcapsules was an increase ($P < 0.05$) in the total solids content. A similar behaviour was also noted by Pinto et al. (2017) in samples of Greek-style yoghurt.

The yoghurt samples prepared with hydrolysed milk showed lactose values < 0.1 g 100 $g^{-1}$, representing a great decrease in the concentration of this disaccharide (yoghurt without hydrolysed

**Table 3**
Encapsulation yield (EY) and viability of microencapsulated B. *lactis* BB-12 during storage (4 ± 1 °C).[a]

| Microcapsules | EY (%) | Viable cell count (log cfu $g^{-1}$) | | |
|---|---|---|---|---|
| | | Day 1 | Day 15 | Day 30 |
| GA | 97.62 ± 0.37 | 8.43 ± 0.07 | 8.36 ± 0.13 | 8.35 ± 0.01 |
| GA:IN | 96.35 ± 0.08 | 8.33 ± 0.04 | 8.26 ± 0.01 | 8.33 ± 0.03 |
| GA:MD | 96.50 ± 0.94 | 8.39 ± 0.15 | 8.32 ± 0.02 | 8.34 ± 0.01 |

[a] Abbreviations are: GA, microcapsules produced with gum arabic; GA:IN, microcapsules produced with gum arabic and inulin; GA:MD, microcapsules produced with gum arabic and maltodextrin. Values are means ± standard deviation (n = 3).

milk, ± 3.6 g 100 $g^{-1}$ of lactose). These results are in agreement with values established by Brazilian legislation (Brasil, 2017) and can therefore be considered a lactose-free product.

### 3.3.2. pH, acidity and texture analyses

Values for pH and titratable acidity of the lactose-free Greek-style yoghurts during storage at 4 °C are shown in Table 4. The pH values decreased ($P < 0.05$) and the titratable acidity increased ($P < 0.05$) in similar manner for both yoghurt formulations during the storage period. This behaviour probably resulted from the residual activity of the starter cultures, especially *Lactobacillus bulgaricus*, which showed an increase in its count throughout storage (Table 5). The GYM yoghurt showed slightly higher pH values ($P < 0.05$) than the GYF yoghurt during the entire storage period. However, the addition of the microcapsules had no significant effect ($P > 0.05$) on the titratable acidity values. Other authors have reported higher pH values in yoghurts containing microencapsulated probiotics in comparison with their free form (Pinto et al., 2017; Shoji et al., 2013). Conversely, results obtained by Dimitrellou et al. (2016) indicated that incorporation of microencapsulated L. *casei* cells had no effect on the pH and titratable acidity values of fermented milks.

Fig. 2 shows the evolution of texture parameters (firmness, adhesiveness and cohesiveness) of the lactose-free Greek-style yoghurts during refrigerated storage. Firmness and adhesiveness of both yoghurts increased gradually ($P < 0.05$), whereas cohesiveness decreased ($P < 0.05$). This behaviour could be related to the decrease in pH during storage, causing the gel to contract and consequently increasing the firmness of the yoghurts (McCann, Fabre, & Day, 2011). Other studies have also shown similar changes in the texture parameters during refrigerated storage of probiotic yoghurts (Bedani, Campos, Castro, Rossi, & Saad, 2014; do Espírito Santo, Perego, Converti, & Oliveira, 2012). Addition of microcapsules contributed to an increase ($P < 0.05$) in the firmness and the adhesiveness of the GYM yoghurt, and had no influence ($P > 0.05$) on the cohesiveness values. The higher firmness values of the GYM yoghurt may be because of its higher total solids content, which increased from 20.68 to 22.68 as a result of the incorporation of the microcapsules. Besides the total solids, the presence of gum arabic and inulin in the formulation of the microcapsules might also have contributed to greater firmness and adhesiveness of the GYM yoghurt. According to Gyawali and Ibrahim (2016) gum arabic is a good stabiliser because it has sufficient hydrophobic groups to act as bonding points as well as hydrophilic groups that reduce surface tension in a liquid–liquid interface. These authors also state that inulin is able to act as a stabiliser, immobilising the water molecules in the food matrix and improving the overall texture of yoghurts.

### 3.3.3. Viability of the probiotic and starter cultures

Table 5 shows the viable cell count of the starter cultures (L. *bulgaricus* and S. *thermophilus*) and B. *lactis BB-12* in the lactose-free Greek-style yoghurts during the storage period of 30 days at 4 °C. Incorporation of the microcapsules in to the lactose-free yoghurts did not influence ($P > 0.05$) viability of S. *thermophilus* and L. *bulgaricus* throughout the entire storage period. Similar results were reported by Dimitrellou et al. (2016) for fermented milk with added L. *casei* ATCC 393 cells microencapsulated by spray drying using skim milk as the carrier. Moreover, the results obtained in this study showed that the starter cultures produced good cell viability values in both lactose-free yoghurt samples, since the S. *thermophilus* counts remained stable ($P > 0.05$) and the L. *bulgaricus* counts showed a slight increase ($P < 0.05$) during storage. Moreover, viable cell counts for S. *thermophilus* and L. *bulgaricus* were greater than 7 log cfu $g^{-1}$, and thus are in accordance with the quality parameters established by Brazilian legislation (Brasil, 2007) for fermented milks.

Regarding the B. *lactis* BB-12 counts, no difference ($P > 0.05$) was noted between the yoghurts with either free cells or microencapsulated cells added at the end of the storage period. This could indicate that the lactose-free Greek-style yoghurt provided an appropriate environment for B. *lactis* BB-12 despite the low pH of the samples (Table 4). This behaviour may be attributed to the high intrinsic resistance to the acidic pH of the B. *animalis* species. It has been reported that the B. *animalis* subsp. *lactis* is able to withstand acidic environments because of its $F_1F_0$-ATPase activity (Jayamanne & Adams, 2009; Matsumoto, Ohishi, & Benno, 2004). $F_1F_0$-ATPase is an enzyme responsible for the maintenance of intracellular pH, which takes place by pumping protons from the cytoplasm to the extracellular environment (Sánchez, Ruiz, Gueimonde, Ruas-Madiedo, & Margolles, 2013).

**Table 5**
Viable cell counts of B. *lactis* BB-12 and starter cultures in the lactose-free Greek-style yoghurts during storage at 4 °C.[a]

| Microorganism | Storage (days) | Yoghurt samples | |
|---|---|---|---|
| | | GYF | GYM |
| *Streptococcus thermophilus* | 1 | 9.48 ± 0.10 | 9.36 ± 0.07 |
| | 15 | 9.39 ± 0.02 | 9.33 ± 0.02 |
| | 30 | 9.36 ± 0.06 | 9.38 ± 0.06 |
| *Lactobacillus bulgaricus* | 1 | $8.80 \pm 0.10^{aB}$ | $8.64 \pm 0.11^{aB}$ |
| | 15 | $8.87 \pm 0.08^{aAB}$ | $8.74 \pm 0.09^{aB}$ |
| | 30 | $9.03 \pm 0.09^{aA}$ | $9.03 \pm 0.14^{aA}$ |
| *B. lactis* BB-12 | 1 | $6.85 \pm 0.01^{aA}$ | $6.65 \pm 0.04^{bAB}$ |
| | 15 | $6.44 \pm 0.10^{bB}$ | $6.72 \pm 0.04^{aA}$ |
| | 30 | $6.72 \pm 0.09^{aA}$ | $6.52 \pm 0.04^{aB}$ |

[a] Abbreviations are: GYF, lactose-free Greek-style yoghurt with free cells; GYM, lactose-free Greek-style yoghurt with microcapsules produced with gum arabic and inulin. Values, in log cfu $g^{-1}$, are expressed as mean ± standard deviation; different superscript lowercase letters within a row denote significant differences between samples ($P < 0.05$); different superscript uppercase letters within a column denote significant differences between storage days ($P < 0.05$).

**Table 4**
pH and titratable acidity of the lactose-free Greek-style yoghurts containing free or microencapsulated B. *lactis* BB-12 during storage.[a]

| Analysis | Yoghurt samples | Storage days at 4 °C | | |
|---|---|---|---|---|
| | | 1 | 15 | 30 |
| pH | GYF | $4.61 \pm 0.02^{bA}$ | $4.38 \pm 0.03^{bB}$ | $4.23 \pm 0.02^{bC}$ |
| | GYM | $4.68 \pm 0.03^{aA}$ | $4.46 \pm 0.04^{aB}$ | $4.33 \pm 0.02^{aC}$ |
| Titratable acidity (% lactic acid) | GYF | $0.84 \pm 0.01^{aB}$ | $0.93 \pm 0.01^{aA}$ | $0.94 \pm 0.01^{aA}$ |
| | GYM | $0.85 \pm 0.01^{aB}$ | $0.93 \pm 0.01^{aA}$ | $0.95 \pm 0.01^{aA}$ |

[a] Abbreviations are: GYF, lactose-free Greek-style yoghurt with free cells; GYM, lactose-free Greek-style yoghurt with microcapsules produced with gum arabic and inulin. Values are expressed as mean ± standard deviation (n = 3); different superscript lowercase letters within a column denote significant differences between samples for each analysis ($P < 0.05$); different superscript uppercase letters within a row denote significant differences between storage days ($P < 0.05$).

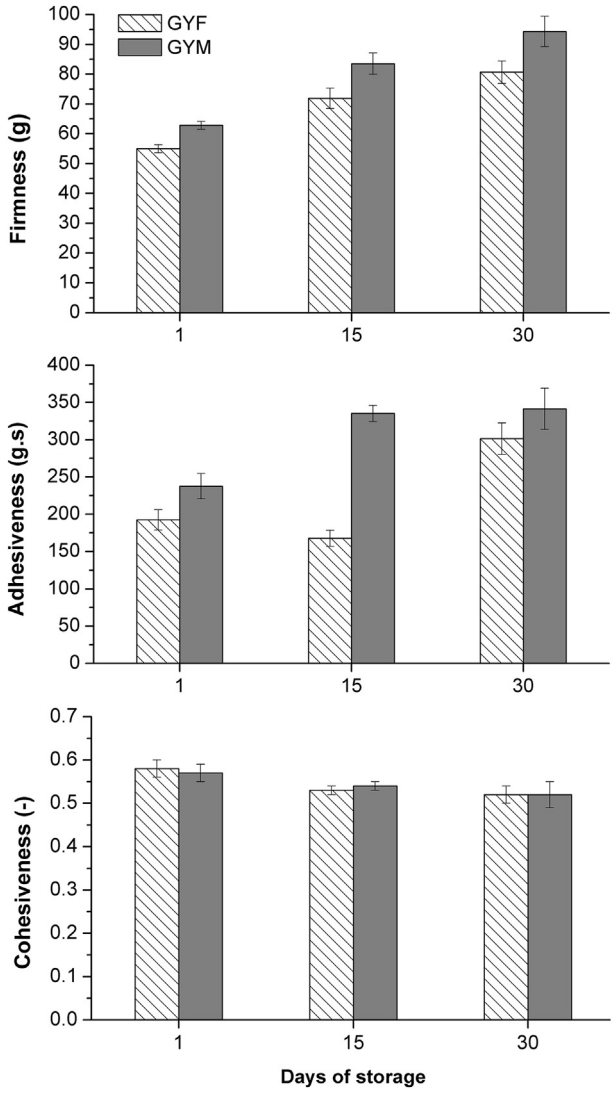


**Fig. 2.** Textural properties of the lactose-free Greek-style yoghurts with free (GYF, ▨: lactose-free Greek-style yoghurt with free cells) or microencapsulated (GYM, ■: lactose-free Greek-style yoghurt with microcapsules produced with gum arabic and inulin) B. *lactis* BB-12 over 30 days of storage at 4 °C.

## 4. Conclusion

The results for encapsulation yield and viability of B. *lactis* BB-12 showed that all combinations of wall materials were able to protect the probiotic cells during spray dying process and storage of the microcapsules at 4 °C for 30 days. Also, the SEM images showed no visible fractures or cracks in all the microcapsules evaluated, which is an important aspect to guarantee good protection of probiotic cells. However, the use of gum arabic and either inulin or maltodextrin as wall materials conferred different morphological and physical characteristics to the microcapsules. Microcapsules produced with gum arabic and inulin were selected as the best to be used in the lactose-free Greek-style yoghurt since they showed more appropriate characteristics (lower values for moisture, solubility and hygroscopicity; higher bulk and tapped densities and 'fair' flowability; and less intense yellow colour). Also, the prebiotic potential of these wall materials was considered.

Results of this work showed that it is possible to produce a lactose-free Greek-style yoghurt with addition of microencapsulated B. *lactis* BB-12 since the viability of S. *thermophilus* and L. *bulgaricus* and the pH and titratable acidity values were not critically affected during storage. Moreover, it was possible to note an improvement in firmness and adhesiveness as a consequence of the wall materials employed (gum arabic and inulin). However, the microencapsulation did not provide any additional protection for B. *lactis* BB-12. Even so, the results suggest that lactose-free Greek-style yoghurt may be considered a potential matrix for delivering of this probiotic strain, following the increasing trend in the development of new lactose-free dairy products.

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