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FOOD TECHNOLOGY

Encapsulation of *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12 by spray drying and evaluation of its resistance in simulated gastrointestinal conditions, thermal treatments and storage conditions

Graciele Lorenzoni Nunes[1] Mariana Heldt Motta[2] Alexandre José Cichoski[1] Roger Wagner[1] Édson Irineu Muller[3] Cristiane Franco Codevilla[1] Cristiane de Bona da Silva[2] Cristiano Ragagnin de Menezes[1*]

[1]Departamento de Ciência e Tecnologia de Alimentos, Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: cristiano.ufsm@gmail.com. *Corresponding author.
[2]Departamento de Farmácia, Centro de Ciências da Saúde (CCS), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[3]Departamento de Química, Centro de Ciências Naturais e Exatas (CCNE), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.

ABSTRACT: *Lactobacillus acidophillus La-5 (ML) and Bifidobacterium Bb-12 (MB) microparticles were produced at different temperatures by spray dryer. The influence of different temperatures on the viability, encapsulation efficiency, water activity and moisture were evaluated. Microparticles that presented more viability were submitted to thermal resistance, gastrointestinal simulation, storage stability, morphology and particle size analyses. Drying temperature of 130°C showed higher encapsulation efficiency, 84.61 and 79.73% for Lactobacillus acidophillus (ML) and Bifidobacterium Bb-12 (MB) microparticles, respectively. In the evaluation of thermal resistance and gastrointestinal simulation, the microparticles of Lactobacillus acidophillus La-5 (ML) presented higher survival than Bifidobacterium Bb-12 (MB) under these conditions. In storage viability only the Lactobacillus acidophillus La-5 (ML) microparticles remained viable at all evaluated temperatures during the 120 days. The particle sizes reported were 4.85 for Lactobacillus acidophillus La-5 (ML) and 8.75 for Bifidobacterium Bb-12 (MB), being in agreement with the desired values for products obtained by spray dryer. Finally, the Lactobacillus acidophilus La-5 (ML) microparticles were shown to be more resistant under the conditions evaluated in this study.*
Key words: *spray dryer, viability, probiotics.*

Encapsulação de *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12 por spray dryer e avaliação da sua resistência em condições gastrointestinais simuladas, tratamentos térmicos e em condições de armazenamento

RESUMO: *Micropartículas de Lactobacillus acidophillus La-5 (ML) e Bifidobacterium Bb-12 (MB) foram produzidas em diferentes temperaturas de secagem no spray dryer. A influência das diferentes temperaturas sobre a viabilidade, eficiência de encapsulação, atividade de água e umidade foram avaliadas. As micropartículas que apresentaram maior viabilidade foram submetidas a análises de resistência térmica, simulação gastrointestinal, estabilidade ao armazenamento, morfologia e tamanho de partícula. A temperatura de secagem de 130°C mostrou maior eficiência de encapsulação, 84.61 e 79.73% para micropartículas de Lactobacillus acidophillus (ML) e Bifidobacterium Bb-12 (MB), respectivamente. Na avaliação da resistência térmica e simulação gastrointestinal as micropartículas de Lactobacillus acidophillus La-5 (ML) apresentaram maior sobrevivência que Bifidobacterium Bb-12 (MB) nestas condições. Na viabilidade ao armazenamento somente as micropartículas Lactobacillus acidophillus La-5 (ML) mantiveram-se viáveis em todas as temperaturas avaliadas durante os 120 dias. Os tamanhos de partícula encontrados foram de 4.85 para Lactobacillus acidophillus La-5 (ML) e 8.75 para Bifidobacterium Bb-12 (MB), estando de acordo aos valores desejáveis para produtos obtidos por spray dryer. Por fim, as micropartículas de Lactobacillus acidophilus La-5 (ML) demostraram ser mais resistentes frente as condições avaliadas neste estudo.*
Palavras-chave: *spray dryer, viabilidade, probióticos.*

INTRODUCTION

Probiotics are live microorganisms, which when consumed in adequate amounts, conferring a health benefit for the host. In this context, in recent years there has been a great deal of interest in its use and application in food especially in fermented dairy products (yogurt, fermented milk and cheese). However, there is also increasing interest for the use of probiotics in non-dairy products such as fruit and vegetable juices, soy and some cereals (FAO/OMS, 2001; DAS & GOYAL, 2015; FIJAN, 2014;

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RANADHEERA et al., 2017). *Bifidobacterium lactis* and *Lactobacillus acidophillus* are the probiotic bacteria most widely studied and frequently used in food (FELICIO et al., 2016; HOMAYOUNI et al., 2008). RANADHEERA et al. (2015) microencapsulated *Lactobacillus acidophilus* La-5, *Bifidobacterium* Bb-12 and *Propionibacterium jansenii* 702 by spray drying in goat's milk. Recently, Kavitek et al. (2018) demonstrated recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods. According to ANAL & SINGH (2007), the ability of probiotic microorganisms to survive and develop in the host will directly influence their probiotic effects. Therefore, the microorganism that is metabolically stable in the product and survive the passage through the gastrointestinal tract reaching the intestine with high viability will be able to develop its beneficial effects. Application of microencapsulation processes has been studied as an alternative to maintain high viability of these microorganisms (FUNG et al, 2011; KIM et al., 2011). Among different microencapsulation techniques, spray drying is commonly used for its advantages such as low operating costs, high production rates, low moisture content in the final product and possibility of application on an industrial scale (CORCORAN et al., 2004). However, this process requires high temperatures, which may affect the survival of probiotic microorganisms (BOZA et al., 2004). In this sense, optimization of the spray-drying conditions as well as the composition of the encapsulation solution are parameters of great importance in order to achieve high survival of the probiotic microorganisms during this process (CORCORAN et al., 2004; FRITZEN-FREIRE et al., 2012; RAJAM et al., 2013; SIMPSON et al., 2005).

In this context, the aim of this study was to evaluate the influence of different temperatures by spray drying on the viability, encapsulation efficiency, water activity and moisture of microparticles containing *Lactobacillus acidophillus* La-5 and *Bifidobacterium* Bb-12. Subsequently, the microparticles that presented the highest viability and best physical-chemical characteristics were evaluated in relation to their thermal resistance, gastrointestinal simulation and storage stability. In addition, the morphology and particle size were also evaluated.

MATERIALS AND METHODS

To produce microparticles, the following compounds were used: Gum arabic (CNI, São Paulo, Brazil); Maltodextrin (Ingredion, São Paulo, Brazil); Tween 80 (Vetec, Rio de Janeiro, Brazil); Glycerol (Vetec, Rio de Janeiro, Brazil), and probiotic culture *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12 obtained by Chr. Hansen from Brazil (Valinhos, São Paulo).

Inoculum

The *Lactobacillus acidophilus* La-5 probiotic culture (Chr. Hansen, São Paulo, Brazil) was activated in MRS broth (Himedia Curitiba, Parana, Brazil) and incubated for 15 h at 37°C. The *Bifidobacterium* Bb-12 culture was rehydrated in reconstituted milk (Molico, Nestlé, São Paulo, Brazil) at a concentration of 12% and incubated for 5 hours at 37°C. Then, it was centrifuged at 4670 g for 15 min and washed in NaCl solution (0.85%). Cells were then suspended in saline to obtain a solution containing about 12 and 10log CFU/g^{-1}.

Production of microparticles by spray drying

Feed solutions were prepared with gum arabic (8g), maltodextrin (2g), glycerol (1.9mL), tween 80 (0.1mL) containing *Lactobacillus acidophilus* La-5 (SL) and *Bifidobacterium* Bb-12 (SB) to a final concentration of 12% m/v. Microencapsulation process was performed in a lab spray dryer (MSD 1.0 Labmaq, Sao Paulo, Brazil). Initially, the feed solutions (SL and SB) were submitted to different drying temperatures, 110, 120, 130 and 140°C. Next, the microparticles produced at the inlet temperature of 130°C were chosen to be further evaluated. Different feed solutions, kept stirring, were introduced into the drying chamber using a peristaltic pump with feed rate of 0.48L/h, drying air flow rate of 40L/min, and air pressure of 0.6 MPa. The microparticles (ML and MB) were collected at the base of the cyclone, transferred to sterile vials, and stored in a desiccator.

Viable cell count

Serial dilutions for *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12 were transferred to sterile Petri plates containing MRS agar (Himedia Curitiba, Paraná, Brazil), in triplicate. The MRS agar used for *Bifidobacterium* Bb-12 was added lithium chloride (0.1%) and L-cysteine (0.05%), according to manufacturer recommendations (Chr Hansen, 1999). Plates were incubated at 37°C for 72h in anaerobic jars with an anaerobic generator (Oxoid, São Paulo, Brazil).

Dilution of the microparticles comprised weighing 1g of microparticles followed by the addition of 9mL sterile phosphate buffer solution (pH

7.5), following the methodology described by SHEU et al. (1993). Results were shown as log colony forming units per gram (log CFU/g^{-1}).

Efficiency of encapsulation (EE)

The efficiency of encapsulation (EE) is the survival rate of the microorganisms during the microencapsulation process, calculated according to Eq. (1), as proposed by MARTIN et al. (2013):

$EE\% = (N/N_0) \times 100$ (1)

Where N is the number of viable cells (log CFU/g^{-1}) released from the microparticles and N_0 is the number of viable cells (log CFU/g^{-1}) free in the feed solution before the spray-drying process. Viable cell count was performed as described in Section "*Viable cell count"*.

Moisture and Water Activity (Aw)

Moisture content of the microparticles was determined in an oven at 105°C until constant weight, according to the methodology proposed by AOAC (2005). Water activity was measured at 25°C using Aqualab 4TE equipment (Decagon Devices, Pullman, WA, USA) after prior stabilization of the samples for 15min.

Microparticle morphology and size

Morphology of the microcaparticles was evaluated using an optical microscope (Carl Zeiss Axio Scope. A1, Oberkochen, Germany) equipped with an Axio Cam MRc digital camera (Carl Zeiss) and scanning electronic microscope (SEM; JEOL JM6360, Tokyo, Japan). Distribution of microparticle size was measured using a Mastersizer 3000 (Malvern, Germany), with water as the dispersion medium.

Resistance to heat treatment

Thermal resistance was assessed as proposed by ZHANG et al. (2015), with some adaptations. Microparticles and free culture (1g) were transferred to 9ml of peptone water in test tubes. Contents were then subjected to thermal conditions of 72°C for 15 seconds and 63°C for 30 minutes, after which tubes were immediately cooled by immersion on ice for 10min. Finally, aliquots were collected and probiotic cultures were counted according to Section "*Viable cell count"*.

Assessment of the survival of encapsulated Lactobacillus acidophilus La-05 and Bifidobacterium Bb-12 exposed to simulated gastrointestinal conditions

The method proposed by MADUREIRA et al. (2011), with some adaptations, was used to submit the microparticles to simulated gastrointestinal conditions. Viability of the bacteria was determined in media simulating the different sections of the gastrointestinal tract, such as esophagus/stomach (addition of pepsin, pH adjusted to 2.0 for 90min), duodenum (addition of pancreatin and bile salts, pH adjusted to 5.0 for 20min), and ileum (pH adjusted to 7.5 for 90min). Analysis was conducted on a TE 421 Shaker (Tecnal, Piracicaba, SP, Brazil) at a temperature of 37°C, simulating the temperature of the human body. Finally, aliquots were removed after 90min (esophagus or stomach), 110min (duodenum), or 200min (ileum) to determine the survival of free and microencapsulated *Lactobacillus acidophilus* La-5. Probiotic cultures were counted in MRS medium as described in Section "*Viable cell count"*.

Viability of microparticles during storage at different temperatures

Viability of the microencapsulated microorganisms was determined by enumeration in MRS agar, as described in Section 2.3. Microparticles were examined after storage for 0, 15, 30, 45, 60, 75, 90, 105, and 120 days at 25°C, −18°C and 7°C.

Statistical analysis

Data were submitted to analysis of variance (ANOVA) using Statistic version 7.0 software (2004; Statsoft Inc., Tulsa, OK, USA), followed by Tukey's means comparison test at a level of 5% significance of treatments showing possible significant differences. All experiments were performed in triplicate; data are expressed as means ± standard deviations.

RESULTS AND DISCUSSION

Viability, encapsulation efficiency, water activity and moisture of microparticles produced t different drying temperatures

The viability of the microparticles containing *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at the different drying temperatures can be seen in table 1. The air inlet temperature of 140°C had a significant effect ($P<0.05$) in relation to the other conditions evaluated, presenting the lowest results for viability for both microparticles studied (ML and MB). Temperature of 130°C showed the greatest viability for *Lactobacillus acidophilus* La-5 (ML) and Bifidobacterium Bb-12 (MB) microparticles, but there was no significant difference ($P<0.05$) among temperatures of 110, 120 and 130°C. Similar results were reported by BUSTAMANTE et al. (2017) when encapsulating *Lactobacillus acidophilus*

Table 1 – Viability, encapsulation efficiency, water activity and moisture of microparticles containg *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at different inlet temperatures in the spray dryer.

ML (*Lactobacillus acidophilus* La-5)	Initial viability log CFU/g	Temperatures inlet	Post-encapsulation viability log CFU/g	Encapsulation efficiency (EE%)	Water activity	Moisture (%)
		110°C	10.18 ± 0.05^{b}	83.30 ± 0.28^{a}	0.289 ± 0.06^{a}	5.71 ± 0.10^{a}
	12.22 ± 0.20^{a}	120°C	10.22 ± 0.05^{b}	83.63 ± 0.33^{a}	0.275 ± 0.02^{a}	5.41 ± 0.33^{a}
		130°C	10.34 ± 0.10^{b}	84.61 ± 0.51^{a}	0.270 ± 0.05^{a}	5.26 ± 0.20^{ab}
		140°C	9.92 ± 0.07^{c}	81.17 ± 0.39^{b}	0.237 ± 0.11^{b}	4.60 ± 0.33^{b}
----------	----------	----------	MB (*Bifidobacterium* Bb-12)	----------	----------	----------
		110°C	8.19 ± 0.07^{b}	77.92 ± 0.39^{a}	0.228 ± 0.07^{a}	5.34 ± 0.06^{a}
	10.51 ± 0.02^{a}	120°C	8.25 ± 0.11^{b}	78.49 ± 0.55^{a}	0.213 ± 0.05^{a}	4.96 ± 0.07^{b}
		130°C	8.38 ± 0.12^{b}	79.73 ± 0.60^{a}	0.208 ± 0.09^{a}	4.83 ± 0.04^{c}
		140°C	7.81 ± 0.15^{c}	74.31 ± 0.77^{a}	0.195 ± 0.08^{a}	4.61 ± 0.04^{d}

ML: microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Lactobacillus acidophilus* La-5; MB: microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Bifidobacterium* Bb-12. Means followed by the same letter, lowercase in the column, do not differ statistically from each other by the Tukeytest at 5% significance. Means found in triplicate.

with mucilage extracted from the chia seed at two different spray dryer temperatures (110°C and 140°C). These authors reported lower survival of encapsulated *Lactobacillus acidophilus* at 140°C. ARSLAN et al. (2015) reported that an increase in the air inlet temperature of the spray dryer resulted in decreased viability and lower survival rates of *Saccharomyces cerevisiae var. boulardi*. Increase in the inlet temperature of the spray dryer consequently causes an increase in the outlet temperature. PISPAN et al. (2013) explained that an increase in the outlet temperature directly increases the temperature at which the microparticles are exposed. Conversely, a reduction in the outlet temperature results in a longer drying time. Thus, viability losses during the spray drying process can arise from dehydration and high temperatures. These two mechanisms occurring at the same time cause a negative effect on the survival of probiotic microorganisms (PEIGHAMBARDOUST et al., 2011; RIVEROS et al., 2009). The encapsulation efficiency (Table 1) ranged from 81.17 to 84.61% and 74.31 to 79.73% for the microparticles containing *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB), respectively. Therefore, it is possible to observe that *Lactobacillus acidophilus* La-5 presented greater resistance on the spray-drying conditions compared to *Bifidobacterium* Bb-12. RANADHEERA et al. (2015) evaluated viability of *Bifidobacterium* Bb-12, *Lactobacillus acidophilus* La-5 and *Propionibacterium jansenii* encapsulated in spray dryer reported greater loss viability for *Bifidobacterium* Bb-12. The current study showed greater resistance to the drying conditions used for the *Lactobacillus acidophilus* (LAC4) culture compared to *Bifidobacterium lactis* (B01). The highest encapsulation efficiency for both microparticles produced, ML (84.61%) and MB (79.73%) was observed at 130°C. FAVARO-TRINDADE AND GROSSO (2002) and LIAN et al. (2002) reported that different strains of microorganisms may vary in their ability to tolerate the high temperatures imposed during spray drying.

In the evaluation of the effect of different input temperatures on the water activity (Table 1) of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles, we observed that the temperature of 140°C had a significant influence ($P<0.05$), presenting the lowest water activity content for both studied microparticles. Nonetheless, the water activity reported in the different evaluated temperatures for the *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 microparticles ranged from 0.195 to 0.289. Thus, these results are as expected for microparticles dried by spray dryer (0.150 to 0.300) to ensure their microbiological stability (CORCORAN et al., 2004; ARSLAN et al., 2015).

The microparticles moisture content ranged from 4.60% to 5.71% (Table 1) and the lowest moisture contents were observed as the inlet temperature of the spray dryer was raised. FERRARI et al. (2012) reported that higher temperatures imply a higher rate of heat transfer to the microparticles, resulting in a higher water evaporation and consequently, low

moisture contents are obtained. Results obtained in the present research are in accordance with those reported by other authors who recommend that the moisture content should be around 4-5% to guarantee better storage stability (CHAVEZ & LEDEBOER, 2007). In this sense, studies have shown that a lower inlet temperature and, consequently output, results in increased post-encapsulation viability; however, this condition may imply greater moisture and water activity, which adversely affects the prolonged storage of powders (PEIGHAMBARDOUST et al., 2011; VESTERLUND et al., 2012). Thus, the relevance of the study of different drying temperatures, not only on the viability of the microorganisms, but also their influence on the physical characteristics of the microparticles is emphasized. MORGAN et al. (2006) reported that spray-drying temperatures are of great importance for the viability of bacteria and need to be optimized individually for every new application. In this context, *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles produced at 130°C were chosen to be evaluated in this study.

Morphology and size of microparticles

Scanning electron microscopy of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles can be observed in figure 1. The microparticles produced presented a rounded shape containing concavities. The same was observed by FAVARO-TRINDADE & GROSSO (2002) and FRITZEN-FREIRE et al. (2012). These authors reported that these concavities are typical of spray-dried products. Moreover, it is possible to observe that microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) presented high porosity (Figure 1a) and ruptures in their structure (Figure 1b). This fact may be related to the low solids concentration (12% m/v) used in the formulations. Similar results were shown by PINTO et al. (2015) in the production of microparticles containing *Bifidobacterium* Bb-12 and using a concentration of 10% w/v in combinations with liquid whey, whey retentate, inulin and polydextrose.

The particle size observed for the microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) was 4.85 and 8.75, respectively. RAJAM & ANANDHARAMAKRISHNAN (2015) reported particle sizes from 6.68 to 23.89*µm for* microparticles containing *Lactobacillus plantarum* (MTCC 5422) using oligofructose, whey protein isolate and denatured whey protein isolate at a concentration of 20% (w/v). ARSLAN et al. (2015) verified particle sizes that ranged between 8.56 and 21.38*µm by encapsulating Saccharomyces cerevisiae var. boulardii* using gelatin, gum Arabic, maltodextrin, modified starch,

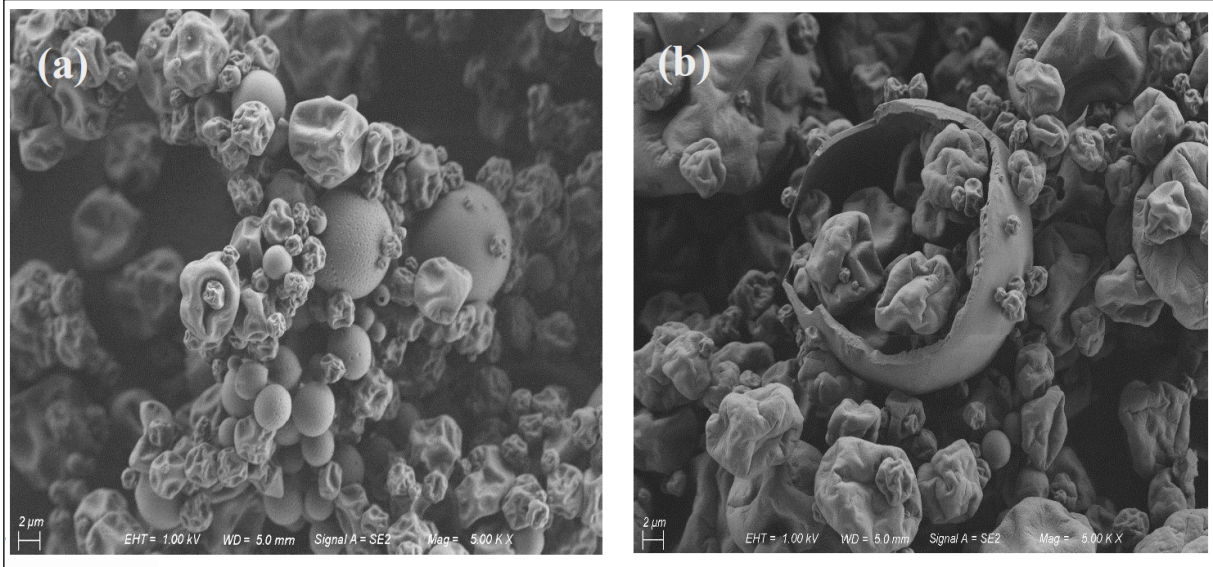

Figure 1 - Micrographs of the microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Lactobacillus acidophilus* La-5 (ML) e microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Bifidobacterium* Bb-12 (MB).

whey protein concentrate and pea protein isolate as encapsulating agents. Smaller particle sizes shown in the present research may be related to the different film-forming and gelling properties of the materials used in the microencapsulation process. In addition, according to KUROZAWA et al. (2009), higher concentrations of encapsulating agents in the feed solution promote an increase in particle size. However, it is worth mentioning that microparticles obtained by spray dryer presented a desirable size, once that smaller particles are preferred to ensure homogeneity and quality when applied to food (BURGAIN et al., 2011).

Resistance of microparticles to heat treatment

The microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) were evaluated to the heat treatments of 63°C/30min and 72°C/15s (Table 2). The microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) presented reductions of 1.91 and 1.93, at 63°C/30min and 1.36 and 1.42 at 72°C/15s, respectively. Thus, *Lactobacillus acidophilus* La-5 (ML) microparticles presented higher resistance to the thermal treatments studied. FAVARO-TRINDADE & GROSSO (2002) and LIAN et al. (2002) have shown in previous studies that different strains of microorganisms can vary their ability to resist high temperatures. Bifidobacteria are known to be more susceptible to high temperatures than lactobacilus (DOLEYRES & LACROIX, 2005). However, it is noteworthy that for both thermal treatments studied, all viable cell counts of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles were superior than 6log CFU/g^{-1}.

Regarding the different applied thermal treatments, the higher temperature and the shorter time (72°C/15s) resulted in higher survival for the *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles. These results are in accordance with those reported by ZHANG, et al. (2015) and NUNES et al. (2017), who reported better survival of *Lactobacillus salivarius* NRR B-30514 and *Lactobacillus acidophilus* La-5 encapsulated by the emulsion and spray drying methods, respectively, when subjected to heat treatment at 72°C/15s in relative to 63°C/30min.

Exposure of microparticles to simulated gastrointestinal conditions

Table 3 shows viable cell counts of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles exposed to simulated gastrointestinal conditions. After 90 min incubation in the presence of a pepsin solution and pH adjusted to 2.0 (simulated esophagus/stomach), there was significantly decreased ($P<0.05$) of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles compared to the initial count of 3.58 and 2.85log cycles, respectively. HOLKEN et al. (2016) and NUNES et al. (2017) verified a similar behaviour when encapsulating *Bifidobacterium* Bb-12 by emulsification/internal gelation and *Lactobacillus acidophillus* by spray drying, respectively. These authors explained that this reduction in log cycles does not necessarily imply a loss of viability, since the microparticles should not have ruptured at a pH value 2.0. Subsequently, when the microparticles are in contact with bile salts and pH 5.0 (section of the gastrointestinal tract comprising the duodenum), it was observed an increased number of viable cells (Table 4). Similar results were reported by HOLKEM et al. (2016), NUNES et al. (2017) and RAJAM et al. (2013). The increase in viable cell count under these conditions probably resulted from a recovery of the sub-injured cells (PICOT & LACROIX, 2004). In the

Table 2 – Effect of heat treatments on the viability of microparticles containg *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at inlet temperature of 130°C in the spray dryer.

Heat treatments	ML (*Lactobacillus acidophilus* La-5)	MB (*Bifidobacterium* Bb-12)
Initial count log CFU/g	10.34 ± 0.10^{aA}	8.38 ± 0.12^{aB}
63°C/30min	8.43 ± 0.03^{cA}	6.45 ± 0.10^{cB}
72°C/15s	8.98 ± 0.15^{bA}	6.96 ± 0.17^{bB}

ML: microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Lactobacillus acidophilus* La-5; MB: microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Bifidobacterium* Bb-12. Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by the Tukey test at 5% significance. Means found in triplicate.

Table 3 – Viability of microparticles containing *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at inlet temperature of 130°C in the spray dryer against simulated gastrointestinal.

	ML (*Lactobacillus acidophilus* La-5)	MB (*Bifidobacterium* Bb-12)
Initial count log CFU/g	10.34 ± 0.10^{aA}	8.38 ± 0.12^{aB}
Esophagus/stomach 90min/pH 2,0	6.76 ± 0.07^{dA}	5.53 ± 0.06^{cB}
Duodenum 20min/pH 5,0	7.06 ± 0.09^{cA}	5.69 ± 0.05^{cB}
Ileum 90min/pH 7,5	8.62 ± 0.08^{bA}	6.34 ± 0.06^{bB}

ML: microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Lactobacillus acidophilus* La-5; MB: microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Bifidobacterium* Bb-12. Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by the Tukey test at 5% significance. Means found in triplicate.

last section of the simulated gastrointestinal tract, the ileum (pH 7.5), the microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) continued to show a significant increase ($P<0.05$) in the number of viable cells (Table 3). As the pH was rising, the number of bacterial cells was increasing. The acid conditions of the stomach cause a dormant state in the bacterial cells, as the pH goes up they regain their growth (MOUMITA et al., 2017). The microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) after exposure to simulated gastrointestinal tract conditions presented reductions of 1.72 and 2.04log cycles, respectively. Therefore, the *Lactobacillus acidophilus* La-5 (ML) microparticles were more resistant to simulated gastrointestinal conditions than *Bifidobacterium* Bb-12 (MB). These results differ from those reported by PEDROSO et al. (2012) who reported greater gastrointestinal survival for *Bifidobacterium lactis* compared to *Lactobacillus acidophilus*. According to GOMES & MALCATA (1999) and KÕLL et al. (2008), there is a variation in the ability of *Bifidobacterium* and *Lactobacillus acidophilus* to resist acid and bile conditions. These authors further reported that these properties are specific to strains and species.

Stability of microparticles during storage at different temperatures

Table 4 shows the viability of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles at room temperature (25°C), below freezing (-18°C) and under refrigeration (7°C). Room temperature (25°C) was the most damaging to the viability of *Lactobacillus acidhopilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles, promoting reductions after 120 days' storage between 3.82 and 3.51logs CFU/g^{-1}. HUANG et al. (2017) microencapsulated by spray drying *Lactobacillus casei* BL23 and *Propionibacterium freudenreichii* TG P20 and verified that storage at room temperature (25°C) resulted in greater viability loss. KOTULA (2008) reported that storage of probiotic powders above refrigeration temperatures increases rates of bacterial metabolism, which can lead to the accumulation of toxic residues and lead to a reduction in viability.

For freezing and refrigeration temperatures, microparticles presented losses of 2.81 and 2.22log cycles for *Lactobacillus acidophilus* La-5 (ML) and 2.42 and 2.12 for *Bifidobacterium* Bb-12 (MB), respectively. Thus, the refrigeration temperature promoted the greatest viability during storage for 120 days for both studied microparticles. OLIVEIRA et al. (2007) showed that *L. acidophilus* exhibited greater viability at a storage temperature of 7°C, thus reporting similar results. Among microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB), it is possible to observe that for the different evaluated temperatures, *Bifidobacterium* Bb-12 (MB) presented the smallest reductions during the 120 days of storage. However, considering the minimum level of 10^6logs CFU/g^{-1} (TALWALKAR et al., 2004), *Bifidobacterium* Bb-12 (MB) microparticles had a shelf life of only 60 days at 25°C and 105 days at -18°C while the *Lactobacillus acidophilus* La-5 (ML) microparticles remained viable throughout the storage period at all studied temperatures. Similar results were reported by PEDROSO et al. (2012) who microencapsulated *Bifidobacterium lactis* and *Lactobacillus acidophilus* using spray-chilling. However, BUSTAMANTE et al. (2017) found greater viability for *Bifidobacterium infantis* in comparison to *Lactobacillus plantarum* incorporated in instant juice powder stored at 4°C for 45 days. According to MARTIN et al. (2015) different

Table 4 – Effect of room temperature (25°C), freezing (−18°C), and refrigeration (7°C) on the viability of microparticles containing *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at inlet temperature of 130 °C in the spray dryer during storage for 120 days.

Temperature	----------Room (25°C) ----------	
Treatments Time (Days)	ML (*Lactobacillus acidophilus* La-5)	MB (*Bifidobacterium* Bb-12)
0	9.92 ± 0.10^{aA}	8.25 ± 014^{aB}
15	8.94 ± 0.02^{bA}	7.79 ± 0.02^{bB}
30	8.68 ± 0.08^{cA}	7.23 ± 0.08^{cB}
45	8.16 ± 0.15^{dA}	6.82 ± 0.15^{dB}
60	7.86 ± 0.05^{eA}	6.37 ± 0.06^{eB}
75	7.15 ± 0.09^{fA}	5.99 ± 0.10^{efB}
90	6.63 ± 0.06^{gA}	5.75 ± 0.12^{fB}
105	6.41 ± 0.08^{gA}	5.34 ± 0.13^{gB}
120	6.10 ± 0.10^{hA}	4.74 ± 0.13^{hB}
Temperature	----------Freezing (-18 °C)----------	
Treatments Time (Days)	ML (*Lactobacillus acidophilus* La-5)	MB (*Bifidobacterium* Bb-12)
0	9.92 ± 0.10^{aA}	8.25 ± 014^{aB}
15	8.96 ± 0.08^{bA}	7.92 ± 0.03^{bB}
30	8.68 ± 0.07^{cA}	7.59 ± 0.02^{cB}
45	8.26 ± 0.03^{dA}	7.23 ± 0.08^{dB}
60	7.95 ± 0.06^{eA}	6.93 ± 0.10^{eB}
75	7.65 ± 0.04^{fA}	6.77 ± 0.04^{eB}
90	7.43 ± 0.10^{fA}	6.43 ± 0.09^{fB}
105	7.23 ± 0.11^{gA}	6.12 ± 0.12^{gB}
120	7.11 ± 0.10^{hA}	5.83 ± 0.12^{gB}
Temperature	----------Refrigeration (7°C)----------	
Treatments Time (Days)	ML (*Lactobacillus acidophilus* La-5)	MB (*Bifidobacterium* Bb-12)
0	9.92 ± 0.10^{aA}	8.25 ± 014^{aB}
15	9.35 ± 0.14^{bA}	8.10 ± 0.08^{aB}
30	9.01 ± 0.03^{cA}	7.80 ± 0.09^{bB}
45	8.91 ± 0.04^{cA}	7.56 ± 0.09^{bB}
60	8.67 ± 0.05^{dA}	7.18 ± 0.14^{cB}
75	8.38 ± 0.09^{eA}	6.85 ± 0.03^{dB}
90	8.12 ± 0.10^{fA}	6.58 ± 0.06^{eB}
105	7.89 ± 0.12^{fA}	6.22 ± 0.08^{fB}
120	7.56 ± 0.11^{gA}	6.08 ± 0.08^{fB}

ML: microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Lactobacillus acidophilus* La-5; MB: microparticles produced with 8g of gum arabic, 2g of maltodextrin, 1.9mL of glycerol, 0.1mL of tween 80 and *Bifidobacterium* Bb-12. Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by the Tukey test at 5% significance. Means found in triplicate.

probiotic strains present distinct abilities to resist environmental conditions such as oxygen, pH, light and temperature. In addition, the conditions of the microencapsulation process are of great importance for the microorganisms to remain viable during their storage (OLIVEIRA et al., 2007).

CONCLUSION

The inlet temperature of 130°C in the spray dryer promoted the highest viability and encapsulation efficiency for the *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles, submitted to different drying temperatures. The *Lactobacillus acidophilus* La-5 (ML) microparticles showed greater viability when exposed to thermal treatments and gastrointestinal simulation. In the storage viability for 120 days, the refrigeration temperature (7°C) was the one that maintained the highest viability for both produced microparticles. However, only the microparticles of *Lactobacillus acidophilus* La-5 (ML) maintained their

counts higher than 6log CFU/g^{-1} at all temperatures that were studied (25, -18 and 7°C). *Bifidobacterium* Bb-12 (MB) microparticles had a 60 days shelf life at 25°C and 105 days at -18°C, thus demonstrating that they could be applied to food products with shorter shelf life. Among the studied microparticles, *Lactobacillus acidophilus* La-5 (ML) showed greater viability and resistance under the conditions evaluated in this research or study.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish results.

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