












Microencapsulation of Probiotic *Lactobacillus helveticus* with Different Wall Materials by Spray Drying

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Abstract: The present study seeks to evaluate different matrixes (chitosan, gelatin – bloom 189, gelatin – bloom 246, gum Arabic, and maltodextrin) to encapsulate *Lactobacillus helveticus* (LH 091), as well as optimal spray-drying conditions. *L. helveticus* displayed a survival rate close to 89%, with their viability above the minimum level of 10^7 CFU/g at point of delivery, after microencapsulation in a gum Arabic-maltodextrin (1:1) matrix followed by spray-drying (air in at 70 to 80°C, a feed rate of 0.09 mL/min, and a pressure of 0.10 bar). Microcapsules containing probiotic displayed a water activity of 0.494, rounded edges and an average size of 2.6 µm. The differential scanning calorimetry and thermogravimetry pointed to the thermal stability of the microparticles with encapsulated probiotics. These favorable properties of the probiotic microparticles make them suitable for incorporation into functional food.

Keywords: *Lactobacillus helveticus*; gum Arabic; maltodextrin; atomization; microencapsulation.

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1. Introduction

Probiotics are live microorganisms that offer their host various health benefits by maintaining normal intestinal microbiota, inhibiting the adhesion of pathogenic bacteria to the intestinal mucosa, increasing immunity, and lowering cholesterol levels [1-6]. The two genera most commonly used as probiotic microorganisms are *Bifidobacterium* sp. and *Lactobacillus* sp [2,6-11]. However, providing and maintaining the required minimum amount of viable probiotic cells to confer any positive health effects (10^6 to 10^7 CFU/g at a point of delivery) is a great challenge, considering their sensitivity to processing, storage, and shipping. Furthermore, incorporating live probiotic microorganisms into food products also has issues, mostly due to the probiotic's susceptibility to several factors, such as temperature, oxygen, water activity, and osmotic pressure mechanical stress, pH value, etc. [12,13].

Spray-drying is the most potential method, mainly to modify the conditions during spray drying to control both size and shape of the particle. Microencapsulation is a promising method for protecting probiotic cells against the adverse conditions they may face [11-14]. Among many microencapsulation techniques, spray drying is one of the most interesting due to its low costs, large scale potential, high probiotic stability, and low amounts of cellular

degradation during drying if the drying process and formulation parameters have been optimized correctly [15,16]. However, during the spray-drying process, probiotic cells are subjected to heat and dehydration, which might damage the cell's membrane or, in extreme cases, completely inactivate the probiotic's culture. However, this might be controlled by adjusting the drying process' parameters like outlet temperature, feed rate, inlet temperature, drying time [17], and the drying medium.

The use of carbohydrates with high activation energy helps to prevent thermal and oxidative stresses during storage. Among them, maltodextrin is widely used as a coating material due to its non-toxicity, low cost, good solubility, low viscosity even at high solid content, and availability [18]. Furthermore, maltodextrin moderately acts as a prebiotic. Its low emulsifying capacity is preferred only in combination with other carrier agents such as gum Arabic [19,20]. Gum Arabic is a dried exudate obtained from *Acacia senegal* (L.). It consists mainly of higher molecular weight polysaccharides and its salts. When subjected to hydrolysis, it yields arabinose, galactose, rhamnose, and glucuronic acid. At levels of 1 to 10%, gum Arabic acts as a film former, moisture stabilizer, and mouth-feel enhancer [21]. Apart from this, gum Arabic prevents complete dehydration of cell components and stabilizes bacterial cells during drying and storage [22,23].

Lactobacillus helveticus is generally recognized as safe (GRAS). It is used as a starter culture in manufacturing semi-hard cheeses, fermented milk products, or food additives because of its potential antihypertensive effect [24,25]. However rare are the studies in the literature that evaluated the encapsulation of *L. helveticus*. The present study evaluates different wall materials in the probiotic encapsulation by spray-drying and the formed microcapsules' characterization.

2. Materials and Methods

2.1. Probiotics preparation.

A freeze-dried strain of *Lactobacillus helveticus* (LH 091) l was obtained from SACCO Brasil (Campinas, São Paulo, Brazil). To prepare the probiotic culture, different assays were performed to obtain a solution of $10^8 - 10^9$ CFU [26]. Each of the assays was performed as follows:

- Assay 1: Inoculum preparation was performed by adapting what has been described by Silva *et al.* [27]. To do this, the freeze-dried bacterial strain was reactivated in 10 mL of Luria-Betani (LB) broth, incubated in an anaerobic jar for 24 h at $35^\circ\text{C} \pm 1^\circ\text{C}$. After the incubation period, 10 mL of the culture were added to 90 mL of LB broth and incubated once again at $35^\circ\text{C} \pm 1^\circ\text{C}$ for 24 h. Further activation was then performed by adding 100 mL of the inoculum in 500 mL of LB broth and incubating the solution at $35^\circ\text{C} \pm 1^\circ\text{C}$ for 24 h in an anaerobic jar. The culture was then centrifuged (MeD Instrumentos - MPW 351R) at 7000 rpm for 5 min at 5°C . Centrifuged cells were washed twice with a 2% sodium citrate solution and then resuspended in 10 mL of sodium citrate (2%). This solution was then used to determine the amount of the culture's viable cells.

- Assay 2: This assay followed the same procedures described in Assay 1; however, this time, samples were incubated in an anaerobic jar for 48 h at $35^\circ\text{C} \pm 1^\circ\text{C}$.

- Assay 3: In this assay, 1 g of the freeze-dried strain was added to 10 mL of LB broth and incubated in an anaerobic jar for 48 h at $35^\circ\text{C} \pm 1^\circ\text{C}$. After the incubation period, 10 mL of the culture were added to 100 mL of LB broth (final volume of 110 mL) and once again

incubated for 48 h at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Following incubation, the 110 mL of culture were seeded in 2 flasks containing 500 mL of LB broth (total volume of 1.1 L) and were incubated again in an anaerobic jar for 48 h at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The cultures were then centrifuged at 5°C , 7000 rpm for 10 min. Cells were washed twice with a 2% sodium citrate solution and then resuspended in 7 mL of sodium citrate 2%.

- Assay 4: This assay followed the same procedures of Assay 3; however, the initial strain reactivation procedure was performed by adding 1g of freeze-dried cells to 10 mL of de Man, Rogosa and Sharpe (MRS) broth and incubating at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 60 h in an anaerobic jar. The solution was then transferred to 100 mL of MRS broth (total volume of 110 mL) and incubated at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 60 h.

- Assay 5: During this assay, 1 g of freeze-dried cells were reactivated in 10 mL of MRS broth, incubated in an anaerobic jar at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 48 h. The culture was then centrifuged at 5°C , 4000 rpm for 15 min, washed with peptone water and suspended in 10 mL of MRS broth. The cell suspension was then used to produce more biomass, with samples of 0.1 mL taken every 48 h to be inoculated in 10 mL of MRS Broth, with the previous culture being centrifuged at 4000 rpm, for 15 min at 5°C , washed with peptone water, resuspended in 10 mL of RMS both and then submitted to the counting of viable cells.

- Assay 6: The freeze-dried strain (1 g) as reactivated in 10 mL of MRS broth, followed by dilution and counting of viable cells.

2.2. Preparation of the encapsulating matrix.

The polymers to be used as the encapsulation matrix were obtained from different retailers. Gum Arabic was obtained from Dinâmica Química Contemporânea (São Paulo, Brasil); maltodextrin and starch came from Ingredion (Mogi Guaçu, Brasil); gelatin was produced by Gelnex (Itá, Brasil); chitosan and trehalose were acquired from Purifarma (São Paulo, Brazil). Different treatments were conducted using these materials, seeking to determine the best encapsulating matrix:

- Treatment 1: Chitosan (100%);
- Treatment 2: Gelatin, 189 bloom (100%);
- Treatment 3: Gelatin, 246 bloom (100%);
- Treatment 4: Gum Arabic (100%);
- Treatment 5: Maltodextrin (100%);
- Treatment 6: Maltodextrin (70%), gum Arabic (30%);
- Treatment 7: Maltodextrin (60%), gum Arabic (40%);
- Treatment 8: Maltodextrin (50%), gum Arabic (50%).

All matrixes (Treatments 1 to 8) were tested using commercial white chocolate. After melting and retempering, the encapsulating agent was added in concentrations of 3, 5, and 7% (m/m). The chocolate molding was done in an acrylic container, which was then cooled at $5^{\circ}\text{C} - 7^{\circ}\text{C}$ for 20 min. The chocolate bars were wrapped in tinfoil and stored at 20°C for 7 days, allowing for stable β crystals.

The samples were submitted to sensory analysis, where 10 g of chocolate samples were offered to non-trained tasters ($n = 25$), in plastic containers coded by three-digit numbers, alongside an evaluation sheet for global impression (acceptability) – Hedonic scale test, 9-point-structured scale (1 – Dislike extremely; 9 – Like extremely), according to the methods described by Dutcosk [28]. In between samples, the tasters used room temperature mineral water and a cracker to clean their palate.

Before the sensory analysis took place, the project was submitted to the ethics committee approval, registered as 01804818.3.0000.5351. Participants also signed a consent form. They declare that they willingly chose to participate in the sensory evaluation and allow the test results to be published.

2.3. Encapsulation and spray-drying.

Before the spray-drying process started, the drying apparatus (SD05, Lab Plant) was sanitized using ultrapure water at 150°C for 10 min. The equipment's needle was also sanitized by being immersed in a 70% ethanol solution for 30 min. Initial probiotic encapsulation tests (Assays 1, 2, 3, 4, and 5) were performed using 24 g of encapsulating matrix. The matrix was hydrated with 100 mL of sodium phosphate buffer (pH 7.0) for 2 h under magnetic stirring. After the hydration period, 1 g of probiotic cells (10^8 - 10^9 CFU/g) were added to the matrix and stirred for 10 min. The solution containing the matrix, probiotic cells, and the buffer was pumped (pneumatic injector orifice diameter of 0.5 mm) at a flow rate of 0.09 mL/min, a pressure of 0.08 – 0.12 bar, and drying chamber inlet temperatures of 70, 80, 90, 100, and 110°C.

When using the freeze-dried microorganism (Assay 6), the same concentration of encapsulating agent and hydration conditions previously described was used. After the matrix's hydration, different amounts of freeze-dried probiotic cells were added (4, 5, 9, and 18 g). The probiotic/matrix/buffer solution was stirred for 10 min before being spray-dried under the same conditions as previously described.

The microcapsules from the spray-drying process were evaluated regarding the number of probiotic cells, water activity, moisture, and, for the maximized parameters, morphology (scanning electron microscopy), thermogravimetric analysis (TG), and differential scanning calorimetry (DSC).

2.4. Analytical determinations.

2.4.1. Water activity (Aw).

Water activity determination was performed in Aqualad CX-2 Water Activity – System. The system was calibrated using distilled water and a NaCl solution (0.819 aw). After the system stabilized, readings of aw/T°C of the samples were performed.

2.4.2. Moisture.

The samples' moisture was determined via infra-red drying in a Mettler LTJ (Brazil) apparatus.

2.4.3. Differential scanning calorimetry (DSC).

The microcapsules were analyzed in a DSC-60 (Shimadzu, Kyoto, Japan). Samples of approximately 5 mg were placed in sealed aluminum pans and submitted to a temperature cycle of 30 to 300°C (rate of 10°C/min) under a nitrogen atmosphere (150 mL/min).

2.4.4. Thermogravimetric analysis (TGA).

TGA analysis was performed in a DTG-60 (Shimadzu, Kyoto, Japan). Samples of approximately 7 mg underwent a temperature cycle of 20 to 300°C at a rate of 10°C/min, under a nitrogen atmosphere (100 mL/min).

2.4.5. Scanning electron microscopy (SEM).

The encapsulated samples' morphology was analyzed by scanning electron microscopy (SEM) technique using a ZEISS/EVO LS 25 model microscope (ZEISS instrument, Germany) with a magnification of 107, 5,000, and 10,000 times and a voltage of 10 kV. The samples were fixed on metallic support, using double-sided carbon adhesive tape, and covered with a thin layer of gold under vacuum. Particle size was measured by the Size Meter software (version 1.1) using at least 50 particles for each experiment. In contrast, the software Statistica® 5.0 was used to calculate the particle size distribution.

2.5. Statistical evaluation.

The results ($n = 3$) were statistically treated via analysis of variance (ANOVA), with means being compared by Tukey test and/or Student's *t*-test ($p < 0.05$), using the software STATISTICA 7.0 (Statsoft Inc, USA).

3. Results and Discussion

3.1. Encapsulating matrix.

Table 1 shows the treatments performed with different encapsulating matrixes and different concentrations (3, 5, and 7%) in commercial white chocolate samples and the mean score given by the sensory evaluating panel using the 9-point hedonic scale.

Table 1. Mean scores of white chocolate samples were added with 3, 5, and 7% of each encapsulating matrix.

Encapsulating matrix	Taster's score*		
	3%	5%	7%
Treatment 1 – Chitosan (100%)	7.2 ^{cA}	7.0 ^{cA}	6.5 ^{dB}
Treatment 2 – Gelatin 189 bloom (100%)	6.5 ^{dA}	6.0 ^{bB}	5.8 ^{eB}
Treatment 3 – Gelatina 246 bloom (100%)	6.7 ^{dA}	6.2 ^{bcB}	6.0 ^{eB}
Treatment 4 – Gum Arabic (100%)	8.0 ^{bA}	8.0 ^{bA}	6.8 ^{cB}
Treatment 5 – Maltodextrin (100%)	8.2 ^{abA}	8.0 ^{bB}	7.2 ^{bC}
Treatment 6 – Maltodextrin (70%), gum Arabic (30%)	8.0 ^{bA}	7.9 ^{bA}	6.5 ^{dB}
Treatment 7 – Maltodextrin (60%), gum Arabic (40%)	7.6 ^{bcA}	7.5 ^{cA}	7.0 ^{bcB}
Treatment 8 – Maltodextrin (50 %), gum Arabic (50%)	8.6 ^{aA}	8.5 ^{aA}	8.1 ^{aB}

*Means followed by identical lowercase/uppercase letters in lines/rows indicate no significant difference at 95% confidence level (Tukey test, $p < 0.05$). Hedonic scale: 1 – dislike extremely; 9 – like extremely.

Chitosan (Treatment 1) and gelatin (Treatments 2 and 3) displayed a negative influence ($p < 0.05$) on the chocolate sample's global evaluation. It was observed that these treatments also altered the chocolate's color, and some panelists described the samples as having a grainy texture, akin to sand.

Gum Arabic (Treatment 4) and maltodextrin (Treatment 5) behaved similarly, with no major changes on the sample's overall acceptability ($p > 0.05$). Concentrations of 3 and 5% (m/m) of encapsulating agents were the ones to cause the lowest amount of changes in the sample's overall evaluation by the panelists.

Based on the data found during these initial tests, the encapsulating matrix composed of 50% gum Arabic and 50% maltodextrin (Treatment 8) was chosen to be used in further spray-drying encapsulation assays in a concentration of 5% (m/m).

3.2. Encapsulation efficiency of spray-dried probiotic powder.

Table 2 lists the different drying temperatures and the probiotic's viability after being encapsulated in a gum Arabic-maltodextrin (1:1) matrix. The highest survivability rates for *L. helveticus* were observed at temperatures of 70 and 80°C (88.93% and 66.7%, respectively). However, microcapsules that have been dried at temperatures above 80°C do not provide the minimum necessary amount of viable probiotic cells to successfully affect the host (10^7 CFU/g at a point of delivery) [26].

Table 2. Spray-drying temperatures and cellular viability of probiotic cells encapsulated in a gum Arabic-maltodextrin matrix (1:1)

Air inlet temperature (°C)	Air outlet temperature (°C)	Cell count before drying (log CFU/g)	Cell count after drying (log CFU/g)	Survivability rate (%)*
70	44	9,40 ± 0,08	8,36 ± 0,05	88,93 ^a ± 0,174
80	51	9,00 ± 0,08	6,00 ± 0,12	66,7 ^b ± 0,205
90	56	9,04 ± 0,08	4,00 ± 0,10	44,2 ± 0,210
100	59	9,30 ± 0,08	3,00 ± 0,10	32,3 ± 0,190
110	61	9,20 ± 0,08	0	0

*Means followed by identical letters indicate no significant difference at a 95% confidence level (Tukey test, $p < 0.05$).

The viability of microencapsulated probiotics that have been exposed to thermal treatment relies on many factors, such as using an encapsulating technique that can be adapted to the matrixes to be used in the process [29].

Arepally and Goswami [21] studied the effects of air inlet temperature and Arabic gum concentration on spray-dried microcapsules of *Lactobacillus Acidophilus* (NCDC 016) and have obtained an initial viable cell count in all of their treatments, ranging from 10.81 to 11.36 log CFU/g at a temperature of 130°C. They have also noted that by increasing the air inlet temperature, cell viability would be reduced from 9.97 to 7.3 log CFU/g. This reduction in cell viability might have been caused due to injuries such as DNA and RNA denaturation, cell membrane dehydration, or cell rupture and collapse due to the sudden removal of water from inside the cells. The major drawback of the spray drying method for probiotic microencapsulation is the limited survival of the probiotics due to the mechanical stress and heat treatment involved [30].

In a study done by Nunes *et al.* [31], the viability of microparticles containing *L. acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced in different drying temperatures (110 to 140°C) was also negatively impacted by the increase in temperature. Similar results were also reported by Bustamante *et al.* [32] using chia seed mucilage extracted via spray-drying using different temperatures (110 and 140°C). Arslan *et al.* [33] reported that an increase in the air inlet's temperature resulted in reduced viability and lower survivability rates of *Saccharomyces cerevisiae* var. bouldardi. Fávaro-Trindade and Grosso [34] and Lian [35], reported that different microorganism strains might have different temperature tolerance thresholds for spray-drying.

Table 3 describes the assays performed to prepare the inoculum of *L. helveticus*, the counting of live cells before and after spray-drying at 70°C, and the survival rate of cells encapsulated on a matrix of gum Arabic-maltodextrin (1:1).

The number of probiotic cells was counted before and after the inoculums underwent the spray-drying process. During the inoculation of *L. helveticus*, cell counts of 9.40 and 9.04 log CFU/g were found for assays 3 and 4, respectively. When using freeze-dried cells (Assay 6), an initial cell count of 10.3 log CFU/g was found. The microcapsules found in assays 3, 4, and 6, after spray-drying at 70°C, displayed cell numbers above 8 log CFU/g, and were the ones with the highest survivability rates (88.93, 88.38, and 90.48%, respectively).

Table 3. Survival rate of encapsulated probiotic cells from different inoculum, after spray-drying at 70°C.

Assay	Cell count before drying (log UFC/g)	Cell count after drying (log UFC/g)	Survivability rate (%) [*]
1 – LB broth/24 h incubation	8.30 ± 0.08	7.30 ± 0.08	87.95 ^c ± 0.169
2 – LB broth/48 h incubation	8.00 ± 0.08	6.00 ± 0.12	75.0 ^d ± 0.205
3 – Culture split/LB broth/48 h	9.40 ± 0.08	8.36 ± 0.05	88.93 ^b ± 0.174
4 – MRS broth/60 h incubation	9.04 ± 0.08	8.08 ± 0.05	89.38 ^b ± 0.069
5 – MRS broth/48 h incubation	8.08 ± 0.04	7.04 ± 0.03	87.12 ^c ± 0.032
6 – Freeze dried cells/MRS broth	10.30 ± 0.08	9.30 ± 0.07	90.48 ^a ± 0.049

^{*}Means followed by identical letters indicate no significant difference at a 95% confidence level (Tukey test, p<0.05).

Other than the minimal established number of viable cells added to a product, the encapsulating matrix is essential to guarantee that the cells are adequately protected during the drying process. Arslan *et al.* [33], when using gum Arabic as an encapsulating agent for spray-drying of *S. cerevisiae*, could obtain a survivability rate of 84%. Nunes *et al.* [31] found a survivability rate of 84.61% for *L. acidophilus* when using gum Arabic and drying temperature of 130°C. Lian [35] has also reported that gum Arabic could increase the survivability rate of *Bifidobacterium* after spray-drying at 130°C.

3.3. Microcapsule characteristics.

The microcapsules of gum Arabic and maltodextrin (1:1), dried at 70°C, were characterized regarding their water activity, humidity, and morphology. Furthermore, they were evaluated via differential scanning calorimetry (DSC) and submitted to thermogravimetric analysis (TG). Table 4 shows the results found for water activity and humidity of the microcapsules obtained from Assays 3 and 6 (Table 3), as they were the ones with the highest survivability rates after drying.

The microparticles' water activity and moisture are critical parameters on the stability of the probiotic cells. They may also play a role in the probiotic's viability during storage [36].

All the evaluated microparticles displayed A_w values below 0.50 (Table 4). According to Fávaro-Trindade *et al.* [37] and Kumar *et al.* [38], this would positively impact the microcapsules' stability, for there is less water available be used in a biochemical reaction, thus, increasing their stability during storage. Regarding moisture, it can be noted that the samples remained at or below 13%, something that is inversely proportional to the temperature used during the spray-drying process. However, since it is a probiotic that is being encapsulated, temperatures over 90°C greatly impact the microorganism's survivability. Furthermore, while the recommended humidity for microcapsules should be around 10% [36], the probiotic's stability is greatly influenced by the product's sorption properties where the microcapsules will be added.

Table 4. Spray-drying conditions and water activity (A_w) and moisture (%) for probiotic microcapsules (Assays 3 and 6).

Assay	Air inlet temperature (°C)	Air outlet temperature (°C)	Moisture* (%)	A_w *
3	70	44	13.20 ^a ± 0.10	0.492 ^a ± 0.0020
6	70	44	13.30 ^a ± 0.10	0.494 ^a ± 0.0017
3	80	51	12.70 ^a ± 0.78	0.436 ^d ± 0.0006
6	80	51	12.50 ^a ± 0.89	0.426 ^e ± 0.0225
3	90	56	10.63 ^b ± 0.06	0.445 ^c ± 0.0006
6	90	56	10.60 ^b ± 0.17	0.462 ^b ± 0.0239
3	100	59	10.67 ^b ± 0.06	0.422 ^e ± 0.0010
6	100	59	10.57 ^b ± 0.06	0.427 ^e ± 0.0025
3	110	61	9.40 ^c ± 0.10	0.402 ^f ± 0.0060
6	110	61	9.33 ^c ± 0.15	0.405 ^f ± 0.0002

*Spray-drying operation conditions: flow rate – 0.09 mL/min; atomization pressure – 0.08 to 0.12 bar; encapsulating agent gum Arabic (50%), maltodextrin (50%).

** Means followed by identical letters indicate no significant difference at a 95% confidence level (Tukey test, $p < 0.05$).

Focusing on the a_w values found (Table 4), it can be observed that water activity decreases as the temperature increases. This might be caused due to gum Arabic's high affinity for water, as well as its highly ramified chain [39]. Arepally and Goswami [21] have observed something similar as well, as they have reported that the use of 10% of gum Arabic significantly reduced their microcapsule's a_w (0.33) when compared to ones without gum Arabic (0.50) and dried at 130°C. Nunes *et al.* [31] have encapsulated probiotics in a matrix composed of gum Arabic (8 g), maltodextrin (2 g), glycerol (1.9 mL), and tween 80 (0.1 mL). They have observed an a_w value of 0.289 in microcapsules containing *L. acidophilus* La-5e, and 0.228 when encapsulating *Bifidobacterium* Bb-12. Both microcapsule types were spray-dried at 110°C

Figure 1 shows the thermogram found during the differential scanning calorimetry (DSC) of the maltodextrin-gum Arabic microcapsules with and without *L. helveticus*, dried at 70°C.

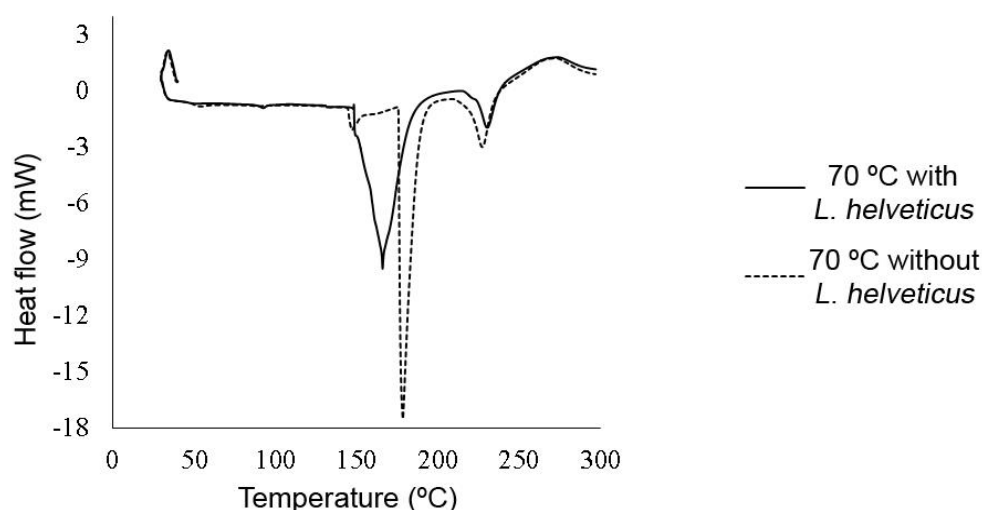


Figure 1. Thermogram of differential scanning calorimetry (DSC) of microcapsules made of gum Arabic – maltodextrin, with and without *L. helveticus*, dried at 70°C.

Observing Figure 1, it is possible to notice the presence of two endothermic peaks related to the melting temperature of the crystalline solid (T_m). The peak at 165°C refers to the sample containing *L. helveticus*, while the one at 178°C comes from microcapsules without the

probiotic. The addition of probiotic cells to the microcapsules drastically reduces the complex's melting point; however, this difference will not be relevant to the product in which the microcapsules will be added (chocolate).

Figure 2 presents thermograms found during thermogravimetric analysis of the microcapsules, with and without probiotic cells, spray-dried at 70°C.

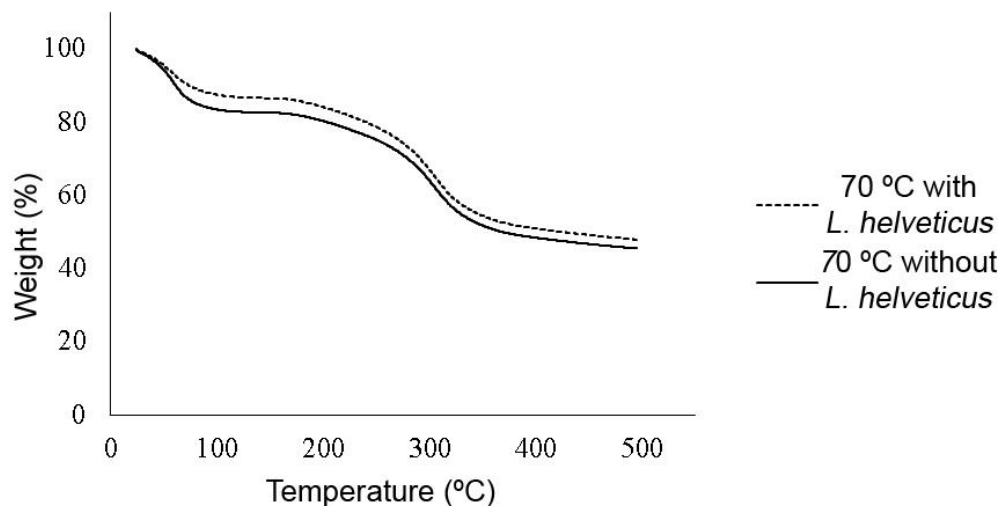


Figure 2. TGA thermogram for microcapsules made of gum Arabic – maltodextrin, with and without *L. helveticus* cells, spray-dried at 70°C.

By observing Figure 2, it is possible to notice that the loss of mass behavior between the samples was very similar. The curves show an initial loss of approximately 15%, related to the phosphate buffer's humidity, which corroborates with the results found via infra-red analysis (Table 4) in the range of 90 to 95°C. Another loss of mass is also evident at about 50%, related to the encapsulating agent and the probiotic cells, along with the temperature range of 160 to 350°C. Above 350°C, no other changes were observed. According to the studies were done by Nunes *et al.* [31], gum Arabic was considered the material with the highest thermal stability when compared to starches in general, for when modified starches replaced gum

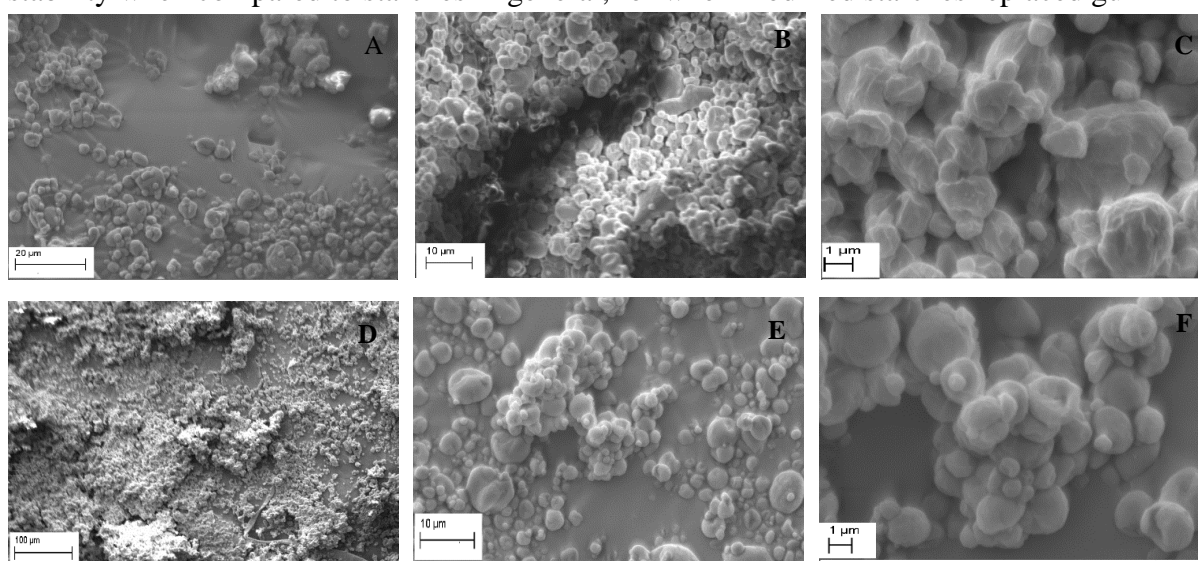


Figure 3. Electronic photomicrographs of gum Arabic – maltodextrin microcapsules containing *L. helveticus* (microcapsule clusters of 20 μm – A; 10 μm – B; 1 μm – C) and without probiotic cells (100 μm – D; 10 μm – E; 1 μm – F).

Arabic, the microparticle's thermal stability was reduced.

The gum Arabic-maltodextrin microcapsules containing *L. helveticus* displayed a round shape, even particle size, no cracks, and few hollow pockets or flat areas (Figure 3), typical of spray-dried products [32]. These concavities or flattened areas usually come from high drying temperatures. They are related to the material used as a matrix [35].

It is also possible to see clusters of microparticles outside the spheres. When comparing the microcapsules containing probiotic cells with the ones without, they did not differ. It was possible to see the complete envelopment of the probiotic cells by the matrix (Figure 3), in pictures with a zoom of 20.000, 10.000, and 1.000 times, it was possible to note a honeycomb-like pattern that traps the probiotic cells in the matrix, which suggests the existence of some form of physical protection for the cells by the encapsulating materials (gum Arabic – maltodextrin).

The particle's average size for the ones containing probiotic cells and the ones without were 2.60 μm (± 0.60) and 3.04 μm (± 0.81), respectively. According to Kurozawa, Park, and Hubinger [40], higher concentrations of encapsulating agents promote an increase in particle size. Still, it is worth noting that the microparticles obtained via spray-drying had a small size and an even, spherical shape, which might help its incorporation on different food products due to the small surface tension between the microparticle and the food matrix, allowing for better fluidity and flow for the material [41].

4. Conclusions

This study presents a simple method for encapsulating the probiotic bacteria *Lactobacillus helveticus* in gum Arabic – maltodextrin microparticles via spray-drying. The optimization of the process and formulation parameters resulted in the production of novel probiotic microparticles with preserved stability and viability of probiotic cells above the minimum level of 10^7 CFU/g at point of delivery, after microencapsulation. Also, the conditions and composition used in the present study created microparticles with acceptable physicochemical properties for further incorporation into food products.

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Conflicts of Interest

The authors declare no conflict of interest.

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