COMMUNICATIONS IN SCIENCE AND TECHNOLOGY
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Evaluation of viability and survival of free and maltodextrin microencapsulated *Bifidobacterium animalis* subsp. *animalis* through spray-drying process

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Article history:
Received: 24 July 2023 / Received in revised form: 6 December 2023 / Accepted: 7 December 2023

Abstract

Bifidobacterium animalis subsp. *animalis* is a microorganism integrated into the human intestinal microbiota and performs a probiotic function through mechanisms that promote the absorption of nutrients, the modulation of the immune system, and the production of lactic acid, among other aspects. Microencapsulation using maltodextrin promotes the protection of microorganisms against physical and chemical factors, improving viability over time. *B. animalis* subsp. *animalis* was microencapsulated through spray-drying using maltodextrin. Survival under pH conditions, bile salts, and temperature were evaluated as well as its viability during storage conditions. The viability of the encapsulated agent stored at 25 °C remained high and constant during the first three weeks. The results for free and microencapsulated thermal tolerance showed an important difference among survival percentages of each tested temperature, and microencapsulation showed a protective effect against temperatures like or lower than 55 °C. Regarding pH 2.5 exposure for 3h, there is a survival of 5.38% for free microorganisms in contrast to 11.87% for encapsulated, whereas in a pH 3.5 for 3h, the encapsulated agent showed a survival of 23%. The results obtained from encapsulated cells stressed with a 1g/L concentration of bile salts showed a survival of 19%, while free cells presented a total loss of viability when subjected for 3h at the same concentration. Microencapsulated *Bifidobacterium animalis* subsp. *animalis* demonstrated potential for its use incorporated into foods, but it is necessary to improve viability conditions during storage and survival under gastric stress conditions.

Keywords: Microencapsulation; probiotic bacteria; spray-drying; viability analysis; *Bifidobacterium animalis* subsp. *animalis*

1. Introduction

The presence of viable probiotic cells in the necessary concentrations until consumption is imperative in order to confer a health benefit on the host. The required concentrations may vary among countries due to differing legislation; nonetheless, the minimum amount necessary to achieve any beneficial treatment must be equal to or greater than 10^6 viable probiotic cells per gram or milliliter [1–5]. The viability of probiotic microorganisms is of paramount importance as it is the sole means by which the beneficial effects on the host's health are obtained [6–8]. Therefore, it is crucial for these cells to remain viable during the transit and colonization of the target tissue for probiotic action.

The probiotics' survival is affected by a variety of factors which include pH, oxidative stress, bile salt concentrations, and storage temperature [9–11]. To protect the probiotics during their processing and storage in food products as well as in gastrointestinal transit, innovative technologies such as microencapsulation have been implemented. Microencapsulation technologies allow the incorporation of the probiotic inside an encapsulating material (in the form of dust to food processes) to facilitate their use and to increase the viability of probiotic bacteria and this is a common practice to extend the viability during storage, and under stress conditions like bile salts [12–16].

The spray-drying process is one of several microencapsulation technologies, including the methods of extrusion, lyophilization, emulsion, and freeze-drying, among others [17,18]. Not only is the spray-drying process one of the most used techniques in the food industry [19], but it is also a flexible method, which is performed in easy-to-use equipment that allows the drying of heat-sensitive products without drastically affecting their microbiological quality and viability [20]. Consequently, this technique is widely used for the encapsulation of probiotics, due to its low operating cost and its higher production capacity [21–24]. In addition, maltodextrin is widely used as an encapsulant material for its protective capacity and low cost [20].

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https://doi.org/10.21924/cst.8.2.2023.1239

Diverse studies on species of the genera *Bifidobacterium* and *Lactobacillus* have been carried out using a spray-drying process for microencapsulation, where their viability regarding stress conditions has been tested [25–28]. Of all the known species and subspecies, *B. animalis* subsp. *lactis* has been one of the most used target microorganisms due to its ability to survive the microencapsulation process [29]; however, few studies regarding the survival during storage of microencapsulated *B. animalis* subsp. *animalis* have been performed. In addition, the performance of survival tests of this microorganism during the storage stage and under stress conditions caused by temperature, stomach acids, and bile salts is essential [30,31]. The main novelty of this study corresponds to the analysis of the viability during storage of *B. animalis* subsp. *animalis* encapsulated by spray-drying using maltodextrin, considering the need to establish if there are losses in the concentration of viable cells when using this subspecies, which has not been reported in previous studies about the probiotic viability during storage as a new procedure, since the behavior of this subspecies compared to others such as *lactis*, may be different, making it a better probiotic candidate against gastrointestinal conditions.

2. Materials and Methods

2.1. Biomass production

The inoculum for biomass production was obtained in three stages. First, the bacteria were activated, starting with a vial of the *B. animalis* subsp. *animalis* ATCC 25527 (American Type Culture Collection from Virginia, USA) lyophilized and preserved from 2 °C to 8 °C, rehydrated under the ATCC established conditions, resulting in a massive seeding in the MRS agar (Biomerieux, Spain) supplemented with 0.05% of L-Cysteine; this was incubated for 36 h at 37 °C under anaerobic conditions. After observing bacterial growth and verifying the strain bacterial morphology (Gram-positive bacilli) using the Gram differential stain. Second, the resulting cells were taken, and a suspension was prepared in sterile saline solution (0.9% (w/v) adjusted to the 0.5 McFarland scale standard). After that, an inoculation of this active agent was performed in a pre-inoculum at 10% v/v in MRS broth (Biomerieux, Spain) enriched with L-Cysteine at 0.05% (1.5 liters) (Biomerieux, Spain). This preparation was incubated for 36 h at 37 °C under a constant stirring at 150 rpm in anaerobic conditions. Next, the bacterial viability and growth were verified through optical density measurement (OD, 600 nm) until a value of 1.0 was obtained.

Third, MRS broth containing *B. animalis* subsp. *animalis* was transferred to sterile 50-ml tubes to retrieve the biomass by centrifugation at 5500 rpm for 15 min. Once the total pellet was obtained, three washes with sterile saline solution 0.9% (w/v) were made; for every wash, all supernatant was discarded, and saline solution was again added and centrifuged at 5500 rpm for 10 min. The final product was resuspended in 10 ml peptone water (0.1% (w/v)) (Oxoid, Spain).

Considering the culture conditions of the *B. animalis* subsp. *animalis* for its macroscopic (white, creamy, circular colonies) and microscopic (Gram-positive bacilli) characteristics, the bacterial concentration of probiotics was determined before performing the spray-drying method to obtain the microcapsules. For this purpose, 25% maltodextrin solution and 5% biomass solution were used (two changes) for the microencapsulation process according to the methodology proposed in [32]. Thus, the spread plate method was implemented, performing serial dilutions from 10^1 up to 10^{13}, to obtain a precise bacterial count. This same method was developed to determine the microbial load of the probiotics obtained after the microencapsulation process by spray-drying.

2.2. Preparation of encapsulating material

Encapsulant material was obtained by using a sterile maltodextrin solution (25% w/v), a concentration that allows favorable powder yields [33]; the solution was stirred for one hour on a magnetic stirrer and sterilized by heat at 121 °C in an autoclave.

Finally, the maltodextrin solution was left for one hour at 22 °C, after which the total prepared biomass was added. The solution was prepared using one liter of maltodextrin solution and the inoculum (5%) of fresh, viable biomass of *B. animalis* subsp. *animalis*. To evaluate the total microbial load, a bacterial count was determined by the counting method (surface seeding on MSR agar enriched with L-Cysteine at 0.05%) of the final solution (maltodextrin 25% + biomass active agent 5%). Finally, the number of cells (CFU/ml) was determined.

2.3. B. animalis subsp. animalis microencapsulation by spray-drying process

To perform the microencapsulation, a Mini Spray Dryer B-191 Büchi (Labortechnik AG, Switzerland) was employed. Drying conditions were 80 °C inlet air, with a pump performance of 0.05%, 75% of vacuum performance, with airflow of 700NL/h, and outlet temperature of 48 ± 3 °C [32]. Once the *B. animalis* subsp. *animalis* strain was subjected to drying conditions, the cyclone of the device was employed for their separation and collection. Finally, the total amount (18-20 g) of the microencapsulated material was vacuum packaged in high barrier bags SIN IMP 20 X 30 FL (Fres-co System, USA) and stored at 22± 2 °C room temperature until analysis. The probiotic count by the spread plate method was the control parameter.

2.4. Counting of microencapsulated B. animalis

For *B. animalis* subsp. *animalis* counting in all experiments, the methodology described by [34]) was applied, where one gram of the incorporated sample was diluted in 9 ml peptone water (0.1% (w/v)). Subsequently, it was subjected for one minute to a 180 gravity in vortex agitation and incubated at room temperature for 5 min.

After the rehydration and solubilization of capsules, one ml was taken, and serial dilutions were made using peptone water as a diluent (0.1% (w/v)). Finally, the seeding was made in petri plates by the spread plate method. For this purpose, one ml of each dilution was added to each Petri plate, after that, approx. 20 ml of MRS agar (45 ± 3 °C) enriched with L-Cysteine was added and the petri plate was gently stirred to homogenize the solution. Later, it was left at rest until the agar was solidified

before incubation at 37 °C for 48 h in anaerobic conditions [35].

2.5. Evaluation of the microencapsulated B. animalis subsp. animalis survival rate during storage at 25 °C

As an improvement element in probiotic production to ensure cell viability, the evaluation of the survival rate of the microencapsulated *B. animalis* during storage was proposed. For calculating the average permanence time of the product, with the response variable the count of *B. animalis* in CFU/g, with an initial concentration of 10^9 CFU/g at the beginning of the evaluation; the failure rate (in which, the microorganism reaches a concentration less than a count of $<10^6$ CFU/g, necessary to promote the probiotic action) was tested every week, from the very first production batch (week 0) to week 12 with six replicates by week. The evaluations were performed by the spread plate method explained above.

2.6. Assessment of viability under thermal treatments

To evaluate the tolerance of microencapsulated *B. animalis* subsp. *animalis* to conditions of cooking temperature of different foods, the free and encapsulated probiotic was subjected to three temperature conditions (22, 50, and 80 °C) for 10 min. To perform this assay, one gram of the microencapsulated sample and one ml of the *B. animalis* subsp. *animalis* suspension at 10^9 CFU/ml (concentration higher than the minimum required for the probiotic activity of the microorganism) was taken and each one was added to 9 ml of peptone water (0.1% (w/v)) as a suspension. These samples were subjected to thermal treatments in a water bath. Once the samples were quickly cooled to room temperature (22 ± 2 °C), the seeding was performed by the spread plate method on MRS agar with L-Cysteine (0.05%) and processed with 6 replicates. This analysis was performed at the beginning of the experiment, at 2 weeks, and 10 weeks.

2.7. Evaluation of pH tolerance

To assess the viability of the free and encapsulated probiotic under acid stress, a gastrointestinal pH was simulated by submitting the batches of samples prepared at different pH, adjusted to 9 ml of sterile Phosphate Buffered Saline (PBS) to the levels 2.5, 3.5, and 6.2, by using 1M HCI and NaOH. Measurements were obtained using a pH meter (InoLab, model 7110, Germain). These treatments were subjected to incubation periods of 1, 2, or 3 h at 37°C.

To establish differences in the reduction of the count of *B. animalis* subsp. *animalis* by the effect of pH, the cell viability was evaluated for each treatment by the spread plate method, using MRS agar with L-Cysteine (0.05%), with 6 replicates of each.

2.8. Evaluation of bile salt tolerance

To evaluate the viability of the free and encapsulated probiotic under stress induced by bile salts, gastric juices simulated by a suspension of Bile Salts (OXOID LTDA, Spain) in peptone water (0.1% (w/v)) to a final concentration of 1, 3, and 5 g/l were prepared, with a final volume of 9 ml suspension. Each 9 ml was incubated at 37 °C for 1, 2, and 3 h, according to the proposed treatments.

To establish differences in the reduction of the count of *B. animalis* subsp. *animalis* by the effect of these salts, cell viability was determined by the spread plate method on MRS agar with L-Cysteine (0.05%), with 6 replicates in each treatment.

2.9. Electron microscopy observation

The dimensional and morphological characterization of the microcapsules was conducted in a Tescan Vega 3 LMU Scanning Electron Microscope (Tescan, Czech Republic). The microencapsulated samples were stored in a desiccator to prevent hydration and reduce contamination and later opened in a laminar flow chamber. Before using the Scanning Electron Microscope (SEM), samples were placed in a piece of double-sided carbon tape to fix them on the glass slide. The verification of the capsule structures was made at 500 and 20 µm, as well as the diameter measurements, which were analyzed at 20 µm. These analyses were performed by taking a random sample of 50 microcapsules.

2.10. Statistical analysis

The data were subjected to a nonparametric analysis with the Kruskal Wallis test to compare means among treatments with a significance level of 5%. All the statistical analysis was performed by using the SPSS 15.0 software for Windows (IBM, New York).

3. Results and Discussion

3.1. Evaluation of the microencapsulated B. animalis subsp. animalis viability during storage

Considering the viable probiotic cell count, there was a decrease in *B. animalis* subsp. *animalis* going from 13.16 Log CFU/ml of the solution containing the microorganism before spray-drying to 10.79 Log CFU/g of microencapsulated *B. animalis* subsp. *animalis* after the spray-drying, indicating a 103 logarithmic reduction after spray-drying, presumably due to the temperature supplied in this process.

Judging by the final count (microcapsules) concerning the initial count (feeding solution), the microencapsulation obtained by the spray-drying method works as a physical barrier to the probiotic agents. However, the rate of survival after the encapsulation process can be affected by inlet temperatures higher than 100 °C. Furthermore, the viability of microencapsulated *Bifidobacterium* with sucrose and maltodextrin as the encapsulating solution was evaluated [36]. It was concluded that the air temperature shows an impact on the residual moisture of the bacterial agents causing considerable damage. For this reason, the temperature during the spray-drying method should not be over 80 °C, not only because it has a mild influence on the viability of the biological agent, but also because it favors the content of moisture in a 4-7% range.

Therefore, in this study, an 80 °C inlet temperature (outlet temperature 40 – 45 °C) was used to minimize reducing the

viability of probiotic cells. Nevertheless, for the development of the encapsulation processes, suitable properties for the protection of heat-sensitive probiotics are required. In this study, when working with the encapsulating solution there was evidence of a thermostable protector effect, which provided a barrier during the drying process by spray-drying. All this resulted in a microencapsulated viable cell count with a concentration higher than 10^6. The hydrolyzed starches (also called maltodextrin) have been used as supporting material and encapsulant in numerous studies due to their high solubility in water (up to 75%) low viscosity when in solution, mild flavor, and low cost [20]. Similar results reported a higher efficiency in the spray-drying process, by using only maltodextrin as an encapsulation agent instead of inulin [37].

3.2. Storage stability of the microencapsulated B. animalis subsp. animalis

It is important to obtain high viability of the encapsulated biological agents by the spray-drying technique, but it is even more relevant to maintain the viability of the encapsulated product during a long storage period.

Figure 1 shows that the viability of microencapsulated *B. animalis* subsp. *animalis* diminished during storage at room temperature (22 °C to 25 °C). However, its concentration remained high (> 108 CFU/g), showing a concentration of the microorganism acceptable enough as a probiotic for the first three weeks. After this period, a decrease in bacterial viability was detected until week 12, showing for the last week a count of 4,7x103. This is possibly due to the loss of the integrity of the encapsulant material being the microorganism exposed to environmental conditions, and the subsequent oxidative stress experienced by *B. animalis* subsp. *animalis*, which commonly prevails under anaerobic conditions [38]. The microencapsulated microorganism retains its viability for 3 weeks.

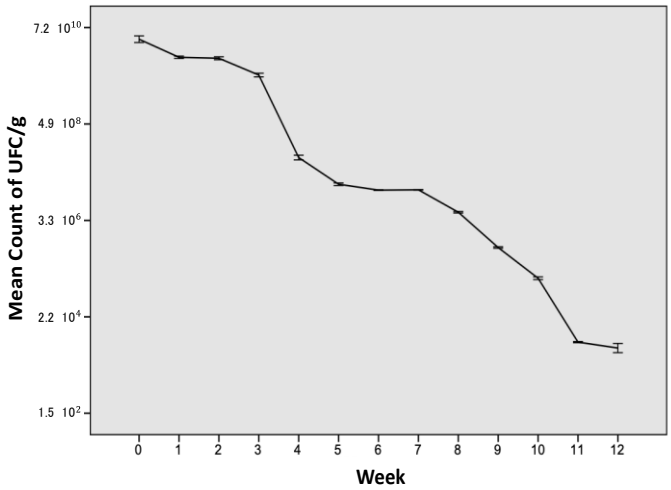

Fig. 1. Microencapsulated *B. animalis* subsp. *animalis* by spray-drying method and stored at 25 °C for 12 weeks. The error bars represent the mean standard deviation of (n = 6 replicates by week)

The results showed that the storage of microencapsulated *B. animalis* subsp. *animalis* in maltodextrin and stored at the temperature of 22 °C to 25 °C range, led to a loss of its viability (see Figure 1). Consequently, the temperature and the encapsulating material are key factors that influence cell viability. Considering that the storage of the commercially available probiotic could be higher than 25 ºC, it would be necessary to evaluate other encapsulating components such as alginate, chitosan, or starch [39], to obtain higher counts than those obtained here to facilitate probiotic action.

3.3. Evaluation of the survival of free and microencapsulated B. animalis subsp. animalis subjected to thermal treatments

One of the main factors that determine the suitability of probiotic bacteria is the ability to tolerate various temperatures employed for food preparation. Studies to evaluate the thermal survival of microencapsulated bacteria with different compositions of the encapsulating material are required. As a result, the values generated in this study can inform future studies that require the production of products made with free and encapsulated *B. animalis* subsp. *animalis* with maltodextrin. The results for the thermal tolerance of free and microencapsulated *B. animalis* subsp. *animalis* (weeks 2 and 10) are shown in Figure 2. In general, there was a significant difference ($p < 0.05$) among the survival percentages of each temperature evaluated. The survival of free and microencapsulated cells diminished as temperature increased. Although it was observed that subjecting the organism without the protection provided by the microcapsules at 55 °C, the percentage of survival only reached 20%, being affected more than 50% of the viability of the microbial agent. On the other hand, the percentage of survival of free (at the beginning of the experiment) and microencapsulated (in week 10 of storage) *B. animalis* subsp. *animalis* was close to zero, when viable cells were subjected to 80 °C, in contrast to the microencapsulated *B. animalis* subsp. *animalis* with 2 weeks of storage which had a survival of 11.87% (Figure 2).

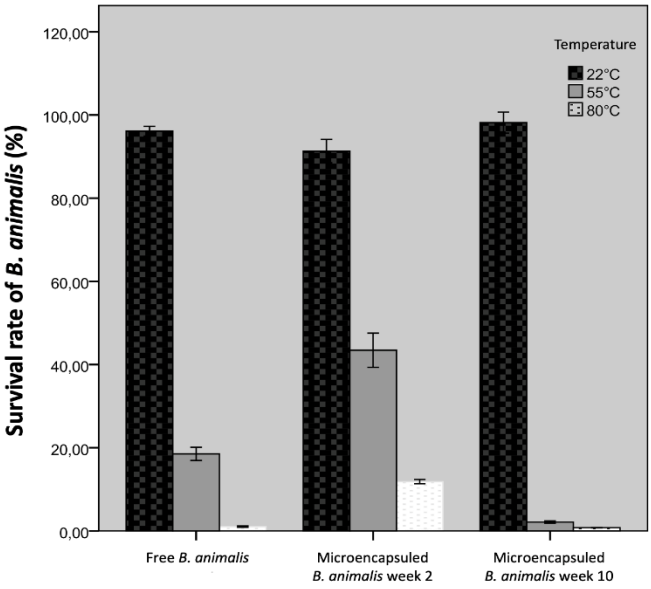

Fig. 2. Results of the survival of the free and microencapsulated *B. animalis* subsp. *animalis* (weeks 2 and 10) after its exposure at 22 °C, 55 °C, and 80 °C for 10 min. Significance of difference between treatments: $p<0.05$ (n=6)

This study demonstrates that microencapsulation presents a protective effect against temperature conditions. However, it is

not recommended for treatments at temperatures exceeding 55 °C or storage for greater than two weeks for microencapsulated and stored *B. animalis* subsp. *animalis* in a period greater than two weeks.

3.4. Evaluation of the survival of free and microencapsulated B. animalis subsp. animalis in bile salts

The high concentration of bile salts in the gastrointestinal system is one of the main barriers to the survival of probiotic organisms [19,40,41]. To evaluate whether the microencapsulation improves the resistance of *B. animalis* to bile salts, free and microencapsulated bacteria were subjected to different concentrations of bile salts (1, 3, and 5 g/l) and incubated for three hours.

The results of the viability of free and microencapsulated *B. animalis* subsp. *animalis* for 3 h of exposure to simulated gastric juices are presented in Figure 3, the encapsulation with maltodextrin had a protective effect on *B. animalis* subsp. *animalis* compared to the free microorganism ($p < 0.05$) under the concentrations of bile salts and incubation time (1, 2, and 3 h) used. Both the viability of free and microencapsulated bacteria decreased as the concentration of bile salts and the exposure time increased; nevertheless, the microencapsulated *B. animalis* subsp. *animalis* had a greater survival rate compared to the free *B. animalis*. Survival ranged from 19% (8.39 Log10 CFU/g) at 1 g/l, and 10% (8.13 Log10 CFU/g) at 5 g/l, as opposed to 17% (1 g/l) and 5% (5 g/l) in free *B. animalis* subsp. *animalis*, for 1 hour of incubation time.

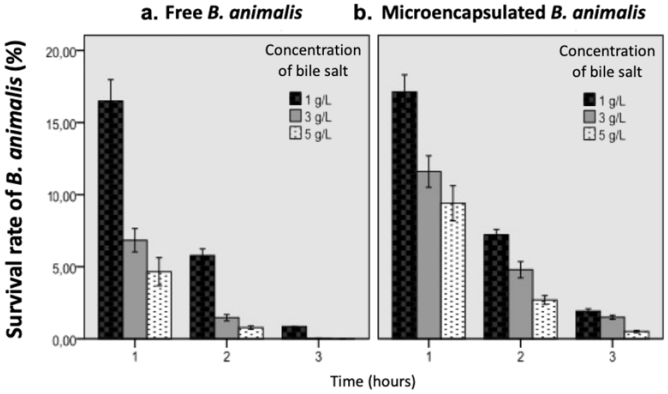

Fig. 3. Survival of (a) free and (b) microencapsulated *B. animalis* subsp. *animalis* to bile salts at 1, 3, and 5 g/l. Significance of difference between treatments: $p<0.05$

In addition, the survival of free cells of *B. animalis* subsp. *animalis* was significantly affected in the in vitro experiments in the presence of different bile salts as time increased, mainly at 2 and 3 h of exposure to concentrations of bile salts of 3 and 5 g/l in contrast to microencapsulated *B. animalis* subsp. *animalis*. This indicates that the free microorganism only showed resilience to low concentrations of bile salts (1 g/l) and for short incubation time (1 hour). Unlike free cells, the encapsulated cells retain a viability of 9% to 5% (considering the existing concentration at the beginning of this experiment), which is equivalent to 8.0 and 7.83 Log10 CFU/g against the concentration of bile salts of 1 and 3 g/l respectively, during 2 h of incubation, showing that under these conditions, the microcapsules provided a barrier between bile salts and the probiotic cells. Consequently, maltodextrin 25% promoted greater protection of microencapsulated *B. animalis* subsp. *animalis*, against gastric stress conditions, compared to the free bacteria.

3.5. Evaluation of the survival of free and microencapsulated B. animalis subsp. animalis against various pH concentrations

A fundamental criterion to consider a microbial agent as a probiotic is its ability to withstand high levels of acid in the intestinal tract. In this system, the secretion of gastric juice prevents frequent colonization of the intestine by many microorganisms transmitted by food [11]. The threshold points to evaluate resistance to the acid conditions were established through pH values 2.5 and 3.5 and an incubation period of 3 h since it simulates the stomach retention time [34,38]. This in vitro simulation is a useful tool to evaluate the survival of probiotic agents in various matrices during the passage through the gastrointestinal tract. The effect of acid conditions of pH on the viability of the free and microencapsulated *B. animalis* subsp. *animalis* is shown in Figure 4.

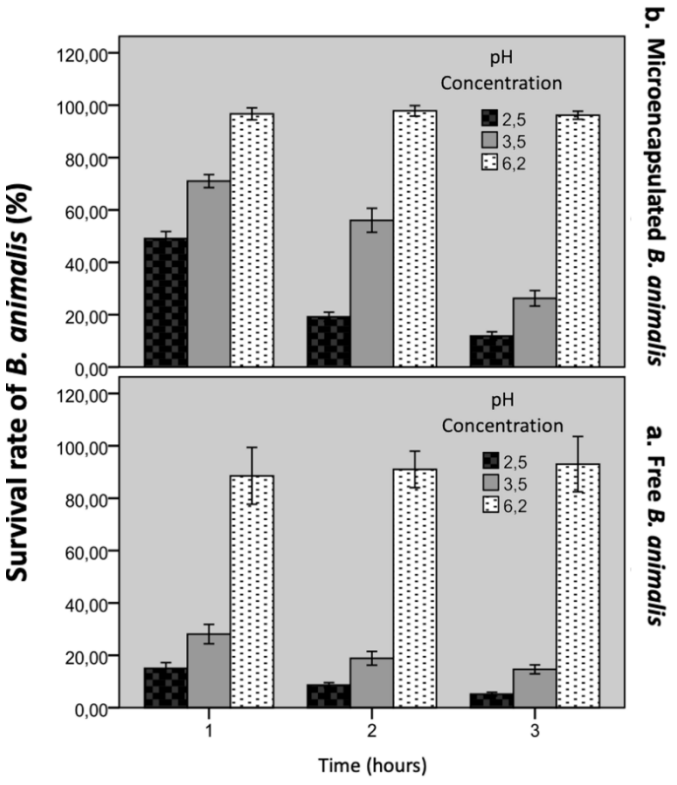

Fig. 4. The results of the survival of free (a) and microencapsulated (b) *B. animalis* subsp. *animalis* after 3 h of exposure to concentrations of pH (2.5, 3.5, and 6.2). Significance of difference between treatments: $p<0.05$

The free biological agent as much as the microencapsulated agent registered a microbial count in each tested concentration. A reduction of both groups exposed to pH 2.5 and 3.5 was generally obtained. However, a constant count above 8 Log10 CFU/g was evidenced for both, when incubated at pH 6.2 (control treatment) for 2 h at 37 °C

The viability of free cells decreased substantially, with an average survival rate of 5.38% (3.041 Log10 CFU/g), when exposed to a pH of 2.5 for 3 h. This reduction was evidenced in the microencapsulated cells. However, the percentage of

survival reached by the organism after 3 h of exposure to pH 2.5 was higher than that observed in the free cells with 11.87% (9.84 Log10 CFU/g). This indicates that encapsulating material was better in protecting the microorganism against acid pH than the free cells. These results are similar to those obtained by (Li et al. 2016) where it was stressed that the survival of the encapsulated cells was much better than that of free bacteria after exposure to simulated gastric juices with pH values of 2.0 and 3.0. It is important to clarify that the pH of the stomach is regularly between 3.5 and 4.0 and this measurement was intended to establish the behavior of the microorganism in an extreme pH such as 2.5, which according to the results would significantly affect the probiotic activity of *B. animalis* subsp. *animalis*.

Similarly, under pH 3.5, a significant decrease in the survival percentage (14.5%) of free *B. animalis* subsp. *animalis* was obtained when a 3 h exposure time was given. On the other hand, after 1 hour of exposure, free *B. animalis* subsp. *animalis* presented a survival of 28.11%, while the microencapsulated *B. animalis* subsp. *animalis* under the same exposure time, had a survival percentage of 71%. This percentage was reduced after 3 h of exposure, with a 23% viability (Log10 4.23 CFU/g). This demonstrates that the encapsulated probiotic under conditions of pH 3.5 meets the minimum requirement of 106 viable probiotic cells per ml. Not surprisingly, the survival rate of either free or microencapsulated *B. animalis* subsp. *animalis* at pH 3.5 depending on the exposure time. This is possible due to a gradual deterioration of the bacteria concerning enzymatic inhibition or effects on solute transport [42].

3.6. Morphology and Size of Microcapsules

The SEM images for *B. animalis* subsp. *animalis* encapsulated by the spray-drying method (Figure 5) confirmed the formation of microcapsules and the absence of free bacteria. The evaluations of the microcapsules exhibited microspheres with smooth forms and a complete structure since fissures or surface ruptures were not evident. However, concavities and indentations were observed on the surface which may be the result of moderate drying temperature (140 °C inlet and outlet 60 °C). Spray-dried particles exhibit a broad size distribution, with an apparent degree of particle aggregation related to outlet temperature, moisture content, and hygroscopicity of the sample; factors that could be affecting their morphology. In this study, the microcapsules exhibited a wide distribution of the spheres which corresponded to a range of sizes from 9 to 22 µm.

4. Conclusion

The concentration of microencapsulated *B. animalis* subsp. *animalis* remained stable for three weeks during storage at room temperature and then had a drastic decline that may be due to the loss of the integrity of the encapsulant material that protected the microorganism. The results for the behavior of thermal tolerance of free and microencapsulated *B. animalis* subsp. *animalis* showed a partial protective effect against temperatures equal to or lower than 55 °C, therefore its incorporation into food products that require heating is not recommended. Regarding survival in the presence of bile salts and low pH, microencapsulation favored the survival of *B. animalis* subsp. *animalis*, mainly under bile salts in a concentration of 1 g/l and pH equal to or greater than 3.5. It was concluded that the microencapsulation of *B. animalis* subsp. *animalis* promotes greater survival against the conditions of temperature, pH, and bile salts compared to free *B. animalis* subsp. *animalis* and maintains a concentration appropriate for probiotic action for three weeks. These results indicate that the evaluated subspecies *B. animalis* subsp. *animalis* is promising for incorporation into foods after microencapsulation, but it is necessary to explore alternatives that improve its viability in storage and its resistance to factors related to gastric stress. The results in this study are similar to others where Maltodextrin was used with other subspecies. Considering the need to increase the survival of *B. animalis* subsp. *animalis* it is pertinent that new studies be focused on optimizing the integrity of the capsule and its influence on the viability of the microencapsulated *B. animalis* subsp. *animalis* during storage.

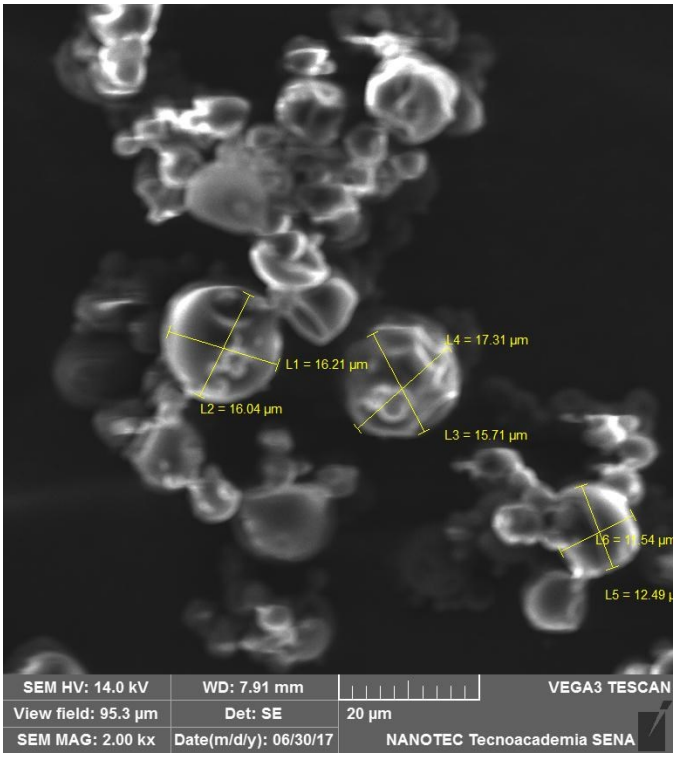

Fig. 5. SEM micrographs of microcapsules containing *B. animalis* subsp. *animalis* obtained by the spray-drying method with 25% of maltodextrin

Acknowledgments

This work was supported by Universidad Católica de Manizales.

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