

## ORIGINAL ARTICLE

# Effect of storage and stress conditions on the counts of *Bifidobacterium animalis* microencapsulated and incorporated in plantain flour

*Efeito das condições de armazenamento e estresse na contagem de *Bifidobacterium animalis* microencapsulada e incorporada à farinha de banana-da-terra*

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## Abstract

The probiotic activity in the intestinal microbiota depends on its survival during food storage and its passage through the gastrointestinal tract. This study aimed to evaluate the effect of storage and stress conditions such as temperature, pH and bile salts on the viability of *Bifidobacterium animalis* microencapsulated and incorporated in plantain flour. Between days 21 and 28, the failure percentage decreased from 93% to 27%. The mean counts of *B. animalis* were statistically different with change of temperature, pH and bile salt concentration. For the temperature, the counts obtained at 50 °C and 80 °C decreased by 60.1% and 90.2%, respectively. Likewise, at pH 2.5 showed a over 90% survival reduction during 60 min; whilst at pH 3.5 during 60 min the survivals were less than 50%. Finally, the counts achieved using 1 g/L of bile salts were higher than those obtained at 3 and 5 g/L. The results indicate the need to evaluate other capsular components to improve the survival of *B. animalis* microencapsulated and incorporated in plantain flour.

**Keywords:** Functional food; Microencapsulation; Plantain flour; Probiotics; Spray dry; Survival.

## Resumo

A atividade probiótica na microbiota intestinal depende de sua sobrevivência, não apenas durante o armazenamento dos alimentos, mas também ao passar pelo trato gastrointestinal. Este estudo visou avaliar o efeito das condições de armazenamento e estresse, como temperatura, pH e sais biliares, na viabilidade de *Bifidobacterium animalis* microencapsulado e incorporado à farinha de plátano. Entre os dias 21 e 28, o percentual de falhas diminuiu de 93% para 27%. As contagens médias de *B. animalis* foram estatisticamente diferentes ao variar a temperatura, o



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pH e a concentração de sais biliares. Em relação à temperatura, as contagens obtidas a 50 °C e 80 °C diminuíram 60,1% e 90,2%, respectivamente. Da mesma forma, pH 2,5 teve redução superior a 90% a partir dos 60 minutos, enquanto com pH 3,5, durante 60 minutos, as sobrevivências foram inferiores a 50%. Finalmente, as contagens obtidas com 1 g/L de sais biliares foram superiores às obtidas com 3 e 5 g/L. Os resultados indicam a necessidade de avaliar outros componentes capsulares para melhorar a sobrevivência de *B. animalis* microencapsulado e incorporado à farinha de plátano.

**Palavras-chave:** Alimentos funcionais; Microencapsulação; Farinha de plátano; Probióticos; Secagem por aspersão; Sobrevivência.

## 1 Introduction

Plantain (*Musa paradisiaca*) is grown in Colombia in an area reserved for cultivation, which corresponds to 590,000 ha (Martínez et al., 2015). This fruit represents 12% of the total weight of this plant (Gañán et al., 2008). Bearing in mind that plantain is an essential daily diet product and it occupies a relevant place in the national economy, this study aimed at the production of plantain flour, incorporating a microencapsulated probiotic as an alternative to contribute to the improvement of productive efficiency in this value chain.

Probiotics are relevant in the functional food industry and have the capacity to incorporate themselves into the intestinal microbiota, benefiting human health (Champagne et al., 2018; Ranadheera et al., 2018). These lactic acid producers microorganisms are mainly from the *Lactobacillus* and *Bifidobacterium* genera (Du Toit et al., 2013; Casarotti & Penna, 2015; Coghetto et al., 2016). The probiotics survival can be enhanced by microencapsulation, based on the atomization of the microorganism, and its subsequent coating with a protective material, performed in a drying chamber with hot air to remove water in a short time (Fritzen-Freire et al., 2013).

The microencapsulation of *B. animalis* has been studied in dairy products, including goat milk (Ranadheera et al., 2015), ice cream (Silva et al., 2015), fermented milk (Bogsan et al., 2014; Rodrigues et al., 2014), whey (Casarotti & Penna, 2015; Rodrigues et al., 2011), and yogurt (Akalin et al., 2007). Studies on plantain flour are not known, showing a remarkable and novel aspect of this research. It is noteworthy that a probiotic food must have a count equal to or higher than  $10^6$  CFU/g (Ranadheera et al., 2015). Furthermore, there are evaluative referents of the survival of *B. animalis* during the storage (Rodrigues et al., 2011), heat stress (Du Toit et al., 2013; Fritzen-Freire et al., 2013), and conditions similar to those that the microorganism faces when going through the gastrointestinal tract (Silva et al., 2015), which are essential to its functionality after preparation and consumption of the food.

We evaluated the effect of storage and stress conditions such as temperature, bile salts, and pH on the viability of *B. animalis* microencapsulated and incorporated in fortified instant plantain flour. Additionally, the diameter of the microcapsules was analyzed by scanning electron microscopy (SEM).

## 2 Material and methods

### 2.1 Drying, extrusion and milling procedure for preparation of plantain flour

Initially, we selected plantain of the Dominico-Hartón (*Musa* AAB Simmonds) variety, in stage one of ripening. Then, the plantains were washed with potable water and its disinfection with a commercial agent (Dioxy San at 0.0025%, prepared by mixing 2.5 mL of the disinfectant and 1000 mL of potable water). These units were then subjected to blanching, with an internal temperature of 72 °C for 30 min, and manually separation of the husk adhered to the pulp using a knife. Subsequently, the pulp was chopped, obtaining units in slices of approximately 4 mm, which were later cut into 4 equal parts. The generated pieces were

dehydrated, using hot air drying under forced convection at 80 °C for 5 hours, bringing the product to a moisture between 15 and 25%. Later, the dried plantain was milled, with a particle diameter between meshes number 2 and 4 (U.S. STD. Sieve, of 2 and 4.76 mm, respectively). An EX0113 equipment was used for the extrusion process, at 40 °C and screw speed of 800×g, bringing the product to a moisture of 15-25% in dry weight. Finally, the material was milled in a domestic mill and the flour was stored under vacuum and at room temperature until its use (Montoya et al., 2016).

## 2.2 Preparation of the biomass of *B. animalis* and the encapsulating solution

The strain of *B. animalis* ATCC 2557 was isolated in Man, Rogosa and Sharpe (MRS) agar, supplemented with 0.05% of L-cysteine and incubated at  $35 \pm 2$  °C under anaerobic conditions. An isolated colony from *B. animalis* was inoculated into a flask containing 50 mL of MRS broth supplemented with 0.05% of L-cysteine, which remained under constant stirring at 200×g for 48 hours at  $35 \pm 2$  °C. Subsequently, the total volume of this preculture was added to a flask containing 500 mL of MRS broth supplemented with 0.05% L-cysteine, which was subjected to incubation time for 48 hours at  $35 \pm 2$  °C under constant stirring at 200×g. The biomass was obtained by centrifugation at 5500×g for 15 min, followed by discard of the supernatant and subsequent collection of the pellet in sterile 50 mL Falcon tubes. The obtained biomass was washed three times, subjecting the microorganism in inulin solution, which was centrifuged for 10 min at 5500×g (Rodríguez-Barona et al., 2012).

For the encapsulating solution, 250 g of maltodextrin were added to a bottle containing 1000 mL of distilled water, which, immediately was placed on a magnetic stirrer for one hour. Later, the bottle with the solution was sterilized at 121 °C in an autoclave for 15 min. Finally, when the maltodextrin solution was at room temperature, the biomass previously recovered was added, with a proportion of 5% of biomass in wet weight, with regard to the weight represented by the solution containing the encapsulant compound (Rodríguez-Barona et al., 2012).

## 2.3 Microencapsulation of the microbial suspension and incorporation in plantain flour

The suspension of maltodextrin and biomass of *B. animalis* was spray dried in the Buchi-290 equipment (Flawil, Switzerland). Using an atomized vacuum pump, this suspension was transported through a sterile hose to the drying chamber of the equipment. An aspiration of 75%, compressed air of 0.05 Mpa, temperature of 80 °C at the inlet and 40 °C to 43 °C at the outlet was supplied. Before entering to the drying equipment, the suspension was constantly stirred at 50×g, using a magnetic stirrer. The material obtained (18-20 g) was collected in a sterile aluminum packing, which was subsequently subjected to vacuum (Rodríguez-Barona et al., 2012).

The encapsulation efficiency (EE) was calculated according to  $EE\% = (N / N_0) \times 100$ ; where N is the number of viable cells ( $\log \text{CFU g}^{-1}$ ) released from the microparticles and  $N_0$  is the number of free viable cells ( $\log \text{CFU g}^{-1}$ ) in the feed solution before the encapsulation process. EE corresponded to 82%.

For the incorporation into the food matrix, 1 g of the maltodextrin capsules containing *B. animalis* and 10 g of the plantain flour were weighed in a 50 mL Falcon tube, which was subsequently homogenized using a Vortex shaker for 5 min at 200×g. This process was performed successively for incorporation of the maltodextrin capsules containing *B. animalis* in the product plantain flour. The homogeneity of maltodextrin capsules containing *B. animalis* was verified through a Relative Standard Deviation (RSD) of 11%, regarding the mean count of  $20.5 \times 10^9$  CFU/g, when analyzing 15 repetitions. Finally, the different treatments of the proposed experiments were arranged.

## 2.4 Analysis of *B. animalis* by counting on plate

For the analysis of the microorganism in the biomass and maltodextrin suspension, 1 mL of sample and 9 mL of buffered peptonated water were mixed, producing the first dilution, from which the serial dilutions necessary to facilitate the counting of this probiotic were made. Similarly, for the estimation in the product, the mixture of 0.1 g of the sample (*B. animalis* microencapsulated or plantain flour with this microorganism incorporated) with 1 mL of peptonated water was considered, generating the suspension from which serial dilutions were subsequently performed. Then, from the dilutions considered, aliquots of 0.1 mL were inoculated in duplicate on the surface of MRS agar supplemented with 0.05% L-cysteine. Finally, incubation was performed at 37 °C for 72 hours, inside an anaerobic polycarbonate jars with anaerobic indicator sachets (Fritzen-Freire et al., 2012).

## 2.5 Experiments performed to establish the effect of storage and stress conditions

### 2.5.1 Viability of *B. animalis* during the storage

From a production batch obtained, we performed weekly *B. animalis* analyses, starting on day 1 to day 84, with 15 repetitions for each sample unit stored at 22 °C and 80% RH in Falcon tubes with a capacity of 20 mL. From these counts, the percentage of sample units with counts equal to or higher than 1.0E6, recommended in the resolution 288, was obtained (Colombia, 2008). The reliability percentage has been applied in food to set the time limit until which a particular attribute is fulfilled (Corpas & Tapasco, 2013).

### 2.5.2 Viability of *B. animalis* using different temperatures

According to Fritzen-Freire et al. (2013), the suspension in buffered peptonated water of the plantain flour with the incorporated microorganism remained 10 min with stirring at 50×g using different temperatures (22 °C, 50 °C, and 80 °C). Then, we performed the analysis to obtain the count of *B. animalis*. These analyzes were performed in sextuplicate and an experimental design of a factor was used. In addition, a control analysis of the *B. animalis* count in the product at room temperature was performed, without the stirring to which underwent treatments aforementioned.

### 2.5.3 Viability of *B. animalis* at different pH

10 g of the product with the incorporated microorganism was diluted in 90 mL buffered peptonated water, with adjustment to three pH values (2.5, 3.5, and 6.2), performing analysis of these in sextuplicate, for three contact times (60, 120, and 180 min). The pH was adjusted with 37% HCL and 0.1 N NaOH (Rather et al., 2017). We applied the design of two factors, pH and contact time, applied to establish differences in the count of *B. animalis* among the treatments. Furthermore, we performed a control count of *B. animalis* in the product, with pH of 6.2 and immediate analysis, that is, without contact times.

### 2.5.4 Viability of *B. animalis* in the presence of bile salts

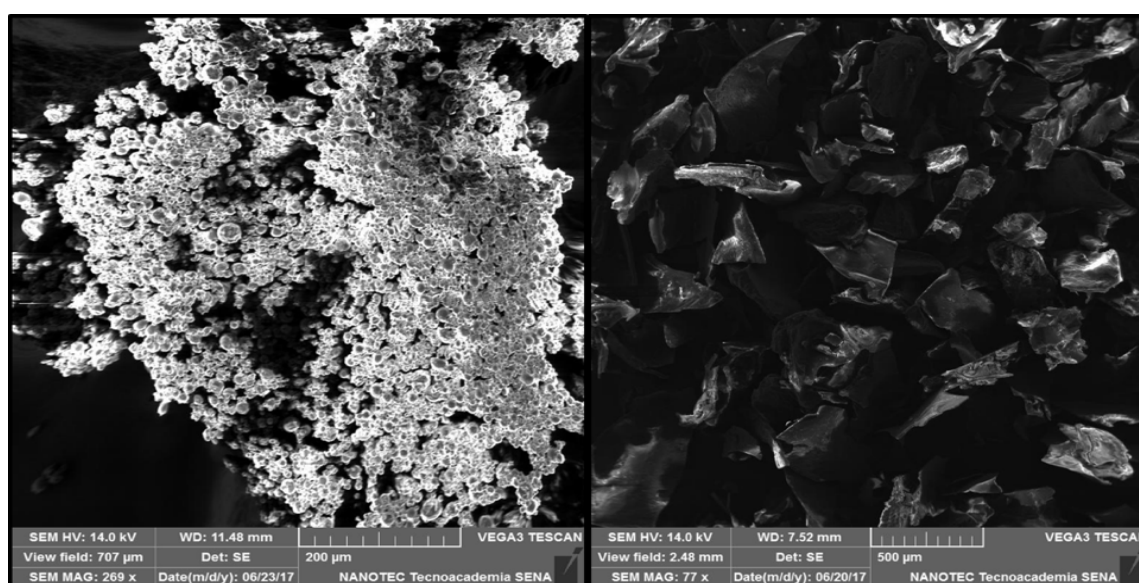
According to Rather et al. (2017), the sample units of plantain flour with the incorporated probiotic were analyzed in sextuplicate, after reconstitution in buffered peptonated water and adjust to three concentrations of bile salts (1, 3, and 5 g/L) and three contact times (60, 120, and 180 min). The design of two factors, bile salts concentration and contact time, was applied to establish their influence on the *B. animalis* count. Likewise, the percentage of decrease of *B. animalis* in the treatments was determined, from a control count without contact of bile salts.

Viability analyzes at different temperatures, pH, and bile salt concentrations were made after product storage time between four and five weeks at 22 °C and 80% RH.

### 3 Results and discussion

#### 3.1 Microscopic characteristics of the maltodextrin capsules

The microscopic characteristics of both the maltodextrin capsules containing *B. animalis* and the plantain flour were observed by SEM. Figure 1 (left) shows a cluster of microcapsules, with similar diameter and external structure. Furthermore, plantain flour was observed with a bigger size than the microcapsules and with irregular structure that nevertheless, had homogeneity in their general dimensions (Figure 1, right). Immediately after microencapsulation, areas between 61 and 336  $\mu\text{m}^2$  were obtained, while in the final measurement the areas were between 16 and 39  $\mu\text{m}^2$ . Likewise, after microencapsulation the capsules had semicircular morphology, with slight concavities on the sides; while from the fourth week of storage the capsules had irregular surface and exposed content. Previous studies show capsules with smaller mean diameters (Feng et al., 2018; Ramos et al., 2018), depending on the encapsulation method used.

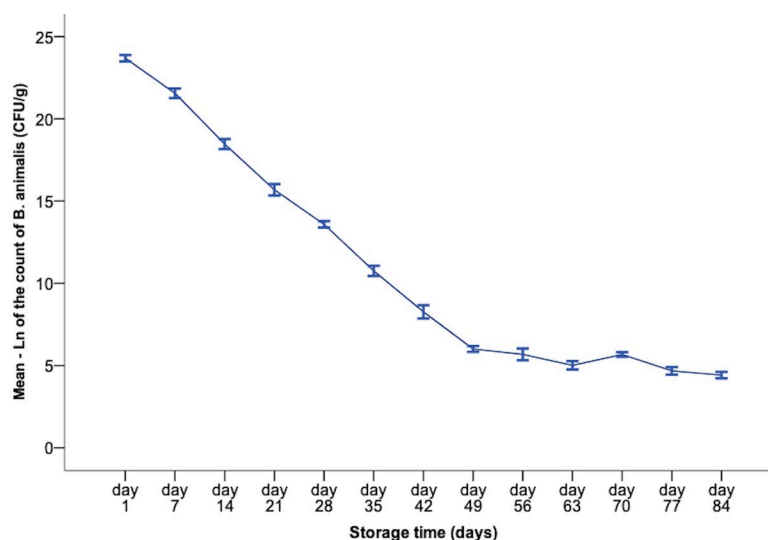


**Figure 1.** SEM micrograph of *B. animalis* microencapsulated by spray drying (left) and of the plantain flour (right).

#### 3.2 Viability during the storage of *B. animalis* microencapsulated and incorporated in plantain flour

Figure 2 shows the behavior of the *B. animalis* count, transformed to its Ln for a clearer and esthetic visual appreciation. In general, there was a decrease in the count of this microorganism during the storage. After microencapsulation, there was a mean concentration of 20.5E9 CFU/g, with decrease of 1 logarithmic cycle per week, reaching a mean of 7.4E5 CFU/g on day 21, where the content of *B. animalis* per gram of the product in 93% of the sample units analyzed was higher than that required by the respective legislation in Colombia. Contrastingly, on day 28 only 27% of the samples analyzed complied the aforementioned criteria, with a mean concentration of 8.5E4. From day 35, all the samples had counts lower than 1.0E6 CFU/g.





**Figure 2.** Counting during the storage of *B. animalis* microencapsulated and incorporated in plantain flour.

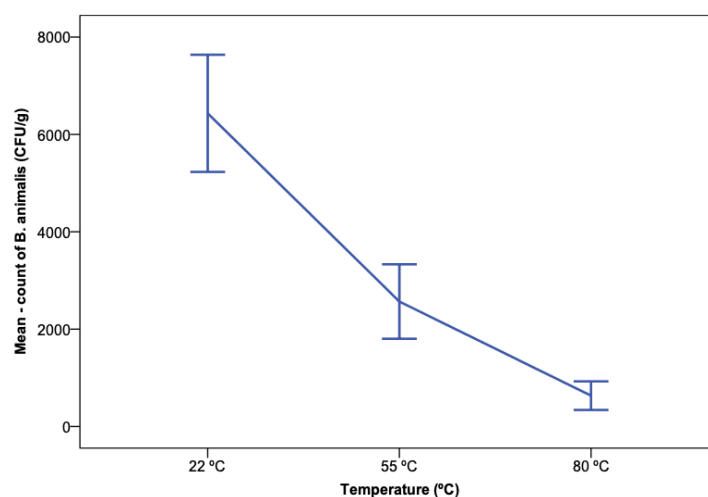
Among the main factors that could have affected the viability of *B. animalis* are the encapsulation method and the type of encapsulant material used. In the case of *L. salivarius* microencapsulated by freeze drying with alginate microgels and alginate-gelatin, we observed a decrease in cell viability of 2.4 log and 1.7 log, respectively, after 5 weeks under wet condition (Yao et al., 2017). Likewise, at 25 °C/60% RH during a storage period of 42 days, the reduction of *L. rhamnosus* microencapsulated with a material based on alginate, lecithin and starch, was between 1.23 and 2.66 log in different formulations tested (Huq et al., 2017). The microencapsulation by spray drying of *L. plantarum* and *L. casei* based on soybeans at 25 °C/60% RH also showed a significant loss of viability after 2 months, which was improved using inulin or oligosaccharides as a wall material (González-Ferrero et al., 2018). These results also show the influence of storage temperature on the reduction of probiotics.

Consistent with the above, it has been established that refrigeration can improve the survival of the probiotics during the storage. The study of the effect of storage temperature on *Bifidobacterium* Bb12 inside of microcapsules based on casein showed a higher rate of inactivation at 25 °C, compared to that obtained at 4 °C, where a decrease of about 1 logarithmic cycle was obtained (Heidebach et al., 2010). Furthermore, the storage of *L. plantarum* in refrigeration for 21 days, microencapsulated into a sodium alginate matrix showed survivals close to 9 log (Coghetto et al., 2016). On the other hand, the decrease denoted in this study could be related to the cellular detriment by metabolic reactions such as the oxidation of fatty acids, which produced protein denaturation and phospholipid degradation (Huq et al., 2017). In particular, bacterial storage is related to an increase in the ratio between saturated and unsaturated fatty acids, due to the lipid oxidation, producing free radicals that damage the DNA and cell membranes (Albadran et al., 2015).

### 3.3 Effect of temperature on the count of *B. animalis* microencapsulated and incorporated in plantain flour

Regarding the mean control count, at 22 °C the mean represented a recovery of 100%. At 55 °C, the mean count meant a reduction of 60.1%. In addition, the mean count of *B. animalis* at 80 °C indicated a reduction of 90.2% (Figure 3). The analysis of variance performed later indicated statistical differences between the evaluated treatments ( $p = 0.00$ ). Furthermore, the multiple comparison Tukey's test showed that the mean counts of *B. animalis* were statistically lower when increasing the temperature. Based on the results obtained, the plantain flour with *B. animalis* incorporated inside of microcapsules must be prepared without the supply of temperatures equal to or higher than 50 °C. Our results corroborate with those of another study (Arslan-Tontul &

Erbas, 2017), where the exposure for 30 min of *S. boulardii*, *L. acidophilus* and *B. bifidum* at 80 °C produced survival rates close to 50%; while at 50 °C, around 90% of these microorganisms survived.

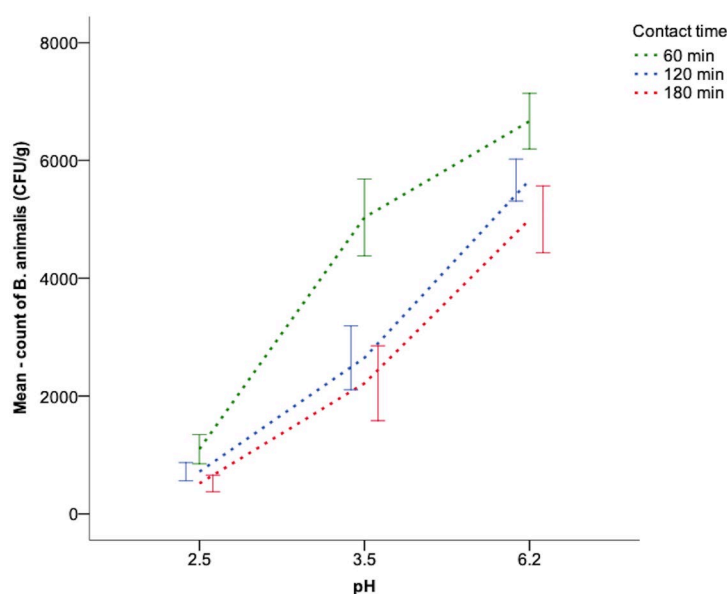


**Figure 3.** Counting of *B. animalis* microencapsulated and incorporated in plantain flour, subjected to different preparation temperatures.

The protection provided by encapsulation against thermal exposure depends on the temperature and time supplied; however, the composition of the capsular structure also constitutes a differential factor for the protection of the probiotic. As a precedent of this, the thermal supply at 63 °C for 15 and 30 min showed significantly lower reductions of *Lactobacillus salivarius* microencapsulated with alginate-gelatin microgels, compared to this microorganism without capsular protection (Yao et al., 2017). In addition, a study performed with *L. acidophilus* showed that the microencapsulated cells by extrusion inside the alginate spheres with double chitosan coat and subjected to 70 °C for 60 min had higher survival rate than the cells with simple coat (Jantarathin et al., 2017). Furthermore, *L. plantarum* and *L. casei* microencapsulated by freeze drying with alginate double layer coat showed a decrease close to 1.0 and 3.0 log, after exposure to 75 °C for 1 and 10 min, respectively (Rather et al., 2017). These precedents demonstrate the influence of the composition of the capsular structure on the protection of probiotic bacteria.

### 3.4 Effect of pH on the count of *B. animalis* microencapsulated and incorporated in plantain flour

Independent of the pH used, the counts invariably decreased when increasing the contact time. Considering the mean control count, the *B. animalis* count was lower at pH 2.5 compared to those obtained in the other pH used. Furthermore, we highlight a decrease of 82.8% after 60 min of exposure to pH 2.5, compared to a decrease of 21.4% at pH 3.5 in the same contact time. Another noteworthy aspect is that at pH 6.2 there was a slight decrease in the *B. animalis* count to a longer time, reaching 21.9% after 180 min (Figure 4). The analysis of variance determined the existence of interaction between the evaluated factors pH and contact time ( $p = 0.00$ ). Finally, the multiple comparison Tukey's test evidenced that the mean values were statistically different when changing pH ( $p = 0.00$  among all treatments) and contact time ( $p = 0.00$  for 60 min vs 120 min;  $p = 0.004$  for 120 min vs 180 min). These results indicate the limited efficiency of the capsular structure to protect this microorganism from the diffusion of acidic molecules that cause physical deterioration and disruption of the biological processes in the bacterial cell.



**Figure 4.** Counting of *B. animalis* microencapsulated and incorporated in plantain flour, subjected to different pH.

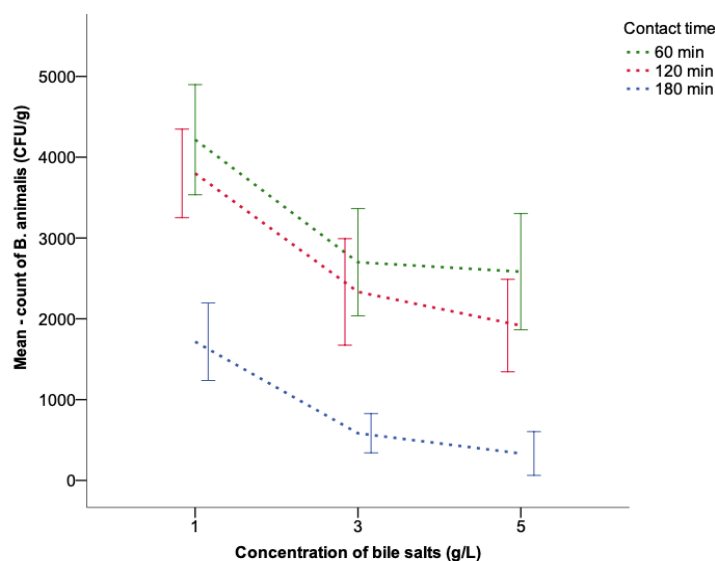
Another aspect susceptible of biological interpretation is that at pH 6.2 the count of *B. animalis* had a reduction directly proportional to the contact time. This allows inferring about the concomitant existence of a degree of deterioration of *B. animalis* at the time of the experiment, as well as a loss of integrity in the maltodextrin capsule, factors that facilitated the subsequent affection of the microorganism by osmotic stress through the time during the experiment. The argument of a progressive loss in the integrity of *B. animalis* is supported by the drastic decline occurred in the analysis of the viability during the storage of this microorganism. Compared to the results obtained, other studies show reductions of between 1 and 3 logarithms when microencapsulated strains were in the presence of simulated gastric fluids, during different exposure times (Coghetto et al., 2016; Yao et al., 2017; Huq et al., 2017). The destruction of probiotic microorganisms in the stomach has been related not only to the level of acidity but also to the destruction of the membrane proteins by enzymatic action (Huq et al., 2017), in specific cases such as pepsin and pancreatin, which cause lysis of the bacterial wall (Coghetto et al., 2016). Consequently, a further study could include this factor to establish its effect, concomitant with acidic pH, on the viability of *B. animalis*.

### 3.5 Effect of bile salts on the count of *B. animalis* microencapsulated and incorporated in plantain flour

Based on the mean control count, a significantly higher count of *B. animalis* was obtained when using 1 g/L of bile salts, with a maximum decrease of 70.7% in a contact time of 180 min, compared to the mean counts obtained with 3 and 5 g/L, concentrations that resulted in reductions of 90.1% and 94.3%, respectively. Furthermore, in the presence of 1 g/L of bile salts, the average reductions were 27.8% and 35.2% in the contact times of 60 and 120 min, respectively. Likewise, with all concentrations of bile salts tested against *B. animalis*, the reductions were appreciably higher when there was a contact time of 180 min, compared to those obtained in shorter times (Figure 5). The analysis of variance applied indicated the association of the *B. animalis* count with the bile salts concentration and contact time ( $p = 0.000$ ) without evidence of interaction between these factors ( $p = 0.969$ ). In addition, the Tukey's test showed that the counts in the presence of 1 g/L of bile salts were statistically higher than those obtained with higher concentrations ( $p = 0.000$ ) and that the counts obtained with 3 and 5 g/L were statistically similar ( $p = 0.710$ ). On the other hand, the counts were significantly lower with a contact time of 180 min, compared to the other times used, whereas there were no statistical differences when comparing the counts at 60 and 120 min ( $p = 0.309$ ).



The transit of the probiotic through the intestinal tract implies, among other aspects, the need to survive in bile salt concentrations close to 1% for 60 to 180 min, conditions under which, *B. animalis* was able to maintain counts similar to those obtained without exposure to bile salts. However, concentrations or times greater than these would probably affect the probiotic capacity of this microorganism.



**Figure 5.** Counting of *B. animalis* microencapsulated and incorporated in plantain flour, in the presence of bile salts.

It has been indicated that the survival of probiotics under gastrointestinal conditions is related to the impermeability and stability of microcapsules (Coghetto et al., 2016). Furthermore, the hydrophilic material is more easily degraded in the presence of bile salts (Arslan-Tontul & Erbas, 2017). In the case of maltodextrin, being an amphipathic polymeric component, the prolonged contact time implies a greater possibility of loss of capsular integrity. This explains why the exposure to bile salts, independent of the concentration supplied, promoted a gradual detrimental effect on the capsule, causing drastic decreases of *B. animalis* when a time of 180 min was used.

Considering that plantain flour is a food matrix with low  $a_w$ , the use of another type of encapsulant could be explored. For example, those based on microgels and microbeads or alternatively, a combination of encapsulant materials of an appropriate nature to prevent deterioration of the capsular integrity, to allow the protection of the probiotic during the storage. In addition, considering that the temperature conditions in microencapsulation can affect the survival capacity during the storage of the microencapsulated microorganism, it would be appropriate to promote the improvement of the viability of *B. animalis* from the effect of controlled stress conditions, of oxidative, thermal, and metabolic nature, prior to the microencapsulation process.

Our findings support some of these considerations. The first aspect is related to the intrinsic survival capacity of the ATCC 2557 strain of *B. animalis* to the factors evaluated; therefore, it would be pertinent to compare their survival capacity against strains of *B. animalis* isolated of the gastrointestinal tract, as well as establish the respective metagenomic understanding. Another aspect is the importance of exploring the integration of prebiotic formulations that include oligosaccharides, to improve the viability of the probiotic in the product (Langa et al., 2019). Likewise, although the in vitro evaluation of the survival capacity against the variables used allows inferring about the probiotic potential of the microorganism, it is necessary to explore simulation models that facilitate an approach to the conditions of the gastrointestinal tract (Silva et al., 2015), and contemplate the influence of other variables such as the content and type of nutrients, the osmotic pressure, and the presence and activity of other microorganisms.

Regarding the consumer perception, some studies have demonstrated that sensory characteristics of the probiotic product are related to willingness, consumption motivations, and buyers' profile (Cruz et al., 2013; Pinto et al., 2018). This implies the need of performing sensory test to the plantain flour with the probiotic incorporated, in order to identify the market opportunities, consider changes in the formulation, and include the characteristics associated with the product in marketing strategies.

## 4 Conclusions

*B. animalis* microencapsulated and incorporated in plantain flour, survived on this product for 3 weeks at the recommended concentration to its probiotic activity. Furthermore, at pH lower than 3.5 and concentrations of bile salts greater than 1 g/L the counts of *B. animalis* had significant reduction after 120 minutes of exposure, which is related to the loss of capsular integrity. Due to the susceptibility of the encapsulated microorganism, we do not recommend heating the product during its preparation.

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