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Microencapsulation by spray drying of the *Lactobacillus plantarum* P2 for potential application as a probiotic

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Abstract

Probiotics are microorganisms that, when ingested in adequate amounts, confer benefits to the organism. The biggest concerns with these products are storage, stability, and survival of the microorganisms that make up these products. Spray-drying processes are an excellent alternative for drying these products because, through microencapsulation, the stability, survival, and resistance to gastric juice and bile salts are preserved. This work evaluated the influence of entry temperature and arabic gum concentration in the spray drying of *Lactobacillus plantarum* P2. Encapsulated cells were characterized for survival, resistance to gastrointestinal conditions, and particle morphology. Both parameters significantly impacted the spray-drying process. An increase in arabic gum concentration resulted in a higher survival rate, while elevated temperatures caused a reduction in survival rates. As for the resistance response to gastrointestinal conditions, only the quadratic temperature was statistically significant. By scanning electron microscopy, we observed the microencapsulation of the microorganism. An optimum estimated point was reached for drying the P2 strain at an entry temperature of 105°C and an arabic gum concentration of 21%, obtaining a process with a higher survival rate and better resistance to gastrointestinal conditions, reinforcing the potential application of this microorganism as a probiotic.

Keywords: probiotics; microencapsulation; spray drying.

Practical Application: Spray-drying technique to probiotic cell encapsulation.

1 INTRODUCTION

Lactic acid bacteria (LAB) are Gram-positive, facultative anaerobic bacteria found in fermented products with probiotic properties (Lamont et al., 2017; Raman et al., 2022). The *Lactobacillus* genus, including over 150 species like *Lactobacillus plantarum*, shows high probiotic potential and is found in dairy, meat, and vegetables (Sabo et al., 2014; Zago et al., 2011). LAB hold Generally Recognized as Safe (GRAS) status, making them widely used in the food, pharmaceutical, and cosmetic industries (Kavitake et al., 2018; Lamont et al., 2017).

Probiotics provide health benefits when consumed in adequate amounts (10^8–10^9 colony-forming unit [CFU]/day) (Agência Nacional de Vigilância Sanitária [ANVISA], 2018), including reducing gastrointestinal diseases, cholesterol, and diabetes and boosting the immune system (Abid et al., 2018; Rajam & Subramanian, 2022). However, maintaining viability during processing and storage remains challenging (Corpas-Iguarán et al., 2023; Shori, 2015).

Spray drying offers an effective solution for probiotic encapsulation, improving stability, shelf life, and protection from environmental factors (Anselmo et al., 2006; Wang & Zhong, 2024). The process involves droplet formation, solvent evaporation, and powder separation (Stunda-Zujeva et al., 2017). Adjuvants like gum arabic, a water-soluble polysaccharide and prebiotic, are commonly used to enhance stability (Khalid et al., 2014; Tontul & Topuz, 2017).

This study investigates the spray-drying process of *L. plantarum* P2, assessing variables such as inlet temperature and gum arabic concentration. Parameters evaluated include survival rate, particle morphology, and resistance to simulated gastrointestinal conditions.

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1.1 Relevance of the work

Probiotics are microorganisms that provide health benefits to the host when consumed in sufficient quantities. Owing to these advantages and the growing demand for functional foods, the market for probiotic-based products has been expanding. However, a primary challenge in this field lies in ensuring the microorganisms' storage, stability, and viability within these products. In this context, spray drying is an effective method for processing such products. Through microencapsulation, this technique helps maintain the stability and survival of probiotics, enhances their resistance to gastric acid and bile salts, and significantly extends the final product's shelf life during storage.

2 METHODOLOGY

2.1 Probiotic potential lactic bacteria

The bacterium used in this study was provided by the Basic Microbiology Laboratory at the Federal University of São João del Rei, Midwest Campus, Divinópolis-MG. The *L. plantarum* P2 strain was isolated by the research group from the Basic Microbiology Laboratory from artisanal Minas standard cheese.

2.2 Preparation of the culture medium from whey

Raw whey was obtained from a rural producer in the municipality of Itapecerica, MG. After collection, the pH of the whey was adjusted to 4.5 using 1 M HCl and then autoclaved at 121°C for 15 min to denature the proteins. After removing the precipitates by centrifugation at 5000 rpm for 10 min, the pH of the supernatant was adjusted to 6.3 with 1 M NaOH and sterilized again at 121°C for 15 min (Rodrigues et al., 2006).

2.3 Cultivation of L. plantarum *P2*

The bacterium was inoculated on MRS agar plates (1% peptone, 1% meat extract, 0.5% yeast extract, 0.1% Tween 80, 0.2% tri-ammonium citrate, 0.02% magnesium sulfate, 0.01% manganese sulfate, 0.2% dipotassium phosphate, 2% glucose, 0.5% sodium acetate, and 1.5% agar) and incubated for 48 h at 37°C under microaerophilic conditions. The plates were placed in containers with a lit candle to maintain this environment and consume the ambient oxygen. After this incubation period, a pre-inoculum was prepared using whey, in which the bacterium was cultivated for 18 h at 37°C with shaking at 110 rpm. Once the medium became turbid, this inoculum (10% v/v) was added to 2000 mL of whey medium. The incubation was continued for 24 h at 37°C with shaking at 110 rpm (Madhu & Prapulla, 2014).

2.4 Spray drying and experimental design

The bacterial culture was centrifuged at 5000 rpm for 10 min, and the supernatant was discarded. The sediment was resuspended in 100 mL of distilled water and centrifuged again at 5000 rpm for 10 min, and the supernatant was discarded. The sediment was then resuspended in 100 mL of distilled water (Bustamante et al., 2015). Gum arabic, previously prepared and autoclaved at 121°C for 15 min, was added to the solution, which was maintained under constant stirring before spray drying.

Spray drying followed the methodology of Oliveira (2006), with modifications, using a mini spray dryer (MSDi 1.0, Labmaq). The parameters were set as follows: 1.2 mm atomizer nozzle diameter, feed rate of 0.6 L/h, compressed air pressure of 3.5 kgf/cm², and atomization air flow of 40.5 L/min.

Inlet temperature and gum arabic concentration were varied according to the experimental design based on response surface methodology. A central composite rotational design (CCRD) was employed, comprising a complete 2^2 factorial design with four axial points and three central point repetitions, totaling 11 experiments. The independent variable levels and experimental trials are presented in Table S1.

2.5 Characterization of atomized powders

The cultures dried using the spray dryer were characterized by survival rate, resistance to simulated gastrointestinal conditions, and particle morphology, as described below.

2.5.1 Counting and survival rate of the bacteria L. plantarum P2

The colony count was performed using the serial dilution method of the samples with sterile 0.85% NaCl solution, both before and after spray drying. MRS agar plates were incubated at 37°C for 48 h in a microaerophilic system. For the serial dilution before drying, 1 mL of culture was added to 9 mL of saline solution, followed by dilutions up to 10^6 times and plating on MRS medium. For the serial dilution of the atomized powder, 0.1 g of the powder was added to 0.9 mL of saline solution, followed by dilution up to 10^5 times and plating on MRS medium. The survival rate of *L. plantarum* P2 was determined by the ratio of the colony count before and after spray drying (Huang et al., 2017). The survival rate was calculated according to Equation 1:

$$Survival\ (\%) = \frac{N}{N_0} \times 100 \quad (1)$$

Where:

N corresponds to the Log_{10} CFU after spray drying; and

N_0 corresponds to the Log_{10} CFU before spray drying.

2.5.2 Resistance to simulated gastrointestinal conditions

To assess resistance to simulated gastrointestinal conditions, 0.1 g of the atomized powder was added to 4.9 mL of 0.5% NaCl solution at pH 1.5, supplemented with 3 g/L of pepsin, and incubated at 37°C for 2 h with shaking at 150 rpm. Before incubation, an aliquot was taken for serial dilution using 0.85% NaCl. For the serial dilution, 0.1 mL of the sample was added to 0.9 mL of saline solution, and dilutions were made up to 10^5, followed by plating on MRS medium. After 2 h of incubation, the pH was adjusted to 4–5 using an alkaline solution (150 mL of NaOH 1 M, 14 g of $NaH_2PO_4 \cdot 2H_2O$, and 1 L of distilled water), and bile salts were added to the samples at 10 g/L. The samples were then incubated for another 2 h at 37°C with shaking at 150 rpm. Subsequently, the pH

was adjusted to 6–7 using the previously mentioned alkaline solution, and the samples were incubated for another 2 h at 37°C with shaking at 150 rpm. After this, an aliquot of 0.1 mL was added to 0.9 mL of saline solution, followed by dilution up to 10^2 and plating on MRS medium. The plates obtained from the serial dilution were kept in a microaerophilic environment for 48 h at 37°C (Buriti et al., 2010). Resistance to simulated gastrointestinal conditions was calculated according to Equation 2:

$$Gastrointestinal\ resistance\ (\%) = \frac{N_{t6}}{N_{t0}} \times 100 \quad (2)$$

Where:

N_{t6} corresponds to Log_{10} CFU/mL at the final time (6 h); and

N_{t0} corresponds to Log_{10} CFU/mL at the initial time (0 h).

2.5.3 Morphology of the atomized powders

The morphology of the atomized powders was analyzed according to Benchabane et al. (2007), with modifications. The atomized powders were placed on a carbon tape fixed to "stubs" and were coated with a thin layer of gold using a sputtering equipment (BalTec). Scanning electron microscopy (SEM) was performed using the LEO Evo40 XVP scanning electron microscope.

2.6 Statistical analysis

The statistical data analysis was conducted using Statistica 8.0 software. The software was employed for variance analysis (ANOVA), obtaining statistical models, and constructing response surface and contour graphs. The residual error was considered, with a confidence level of 95% for the response variables: survival rate, logarithmic reduction of viable cells, and resistance to simulated gastrointestinal conditions, and a confidence level of 90% for gastrointestinal resistance. The F-test was used to analyze the regression fit. The mathematical adjustment of the coded models was complete.

Statistical models describing the relationship between the independent and dependent variables of the process are indicated by a second-order equation (Equation 3):

$$Y=\beta 0+i=1\Sigma k\beta ixi+i=1\Sigma k\beta iixi2+i<j\Sigma k\beta ijxixj \quad (3)$$

Where:

Y is the response variable;

$x_1, x_2, ..., x_k$ are the coded independent variables;

β_0 is the intercept;

β_i are the linear coefficients;

β_{ii} are the quadratic coefficients; and

β_{ij} are the interaction coefficients.

3. RESULTS AND DISCUSSION

The results obtained from the experimental design of the spray drying of *L. plantarum* P2 cultures are shown in Table 1. The influence of the inlet temperature and the adjuvant concentration on the survival rate during the spray-drying process and the resistance to gastrointestinal conditions after drying were determined.

According to Table 1, it can be observed that the experimentally obtained values are very close to the values estimated from the mathematical models, indicating that the models were able to explain the behavior of the factors on the dependent variables.

The spray-drying process resulted in a survival rate of *L. plantarum* P2 between 60.82 and 91.26%. These values are promising, as the goal of microencapsulation and drying is to maintain the survival of microorganisms. Similarly, in the present study, Arslan et al. (2015), using gum arabic as an encapsulating agent for the spray drying of *Saccharomyces cerevisiae* var. *boulardii*, achieved a survival rate of 84%. Bustamante et al. (2015) found a survival rate of 47–78% in the spray drying of *Lactobacillus acidophilus* La-05 using flaxseed mucilage and protein as an adjuvant. Nunes et al. (2018) obtained a survival

Table 1. Characterization of the spray-dried powders of *L. plantarum* P2 and outlet temperatures.

Assay	Outlet temperature (°C)	Survival rate (estimated rates[a])	Resistance to gastrointestinal conditions (estimated values[a])	Log_{10} CFU/mL after spray drying[b]
A	46.5–52.3	81.69 (83.96)	0.00 (21.89)	1.35×10^8
B	86.5–90.0	69.06 (67.30)	0.00 (14.66)	1.01×10^7
C	52.0–58.3	91.26 (90.20)	0.00 (−1.68)	2.62×10^9
D	83.7–91.1	85.36 (80.24)	28.94 (20.03)	7.05×10^8
E	42.8–46.1	85.33 (83.89)	0.00 (−11.61)	2.19×10^7
F	99.0–102.2	60.82 (65.06)	0.00 (−1.37)	3.38×10^6
G	65.8–71.3	80.53 (79.59)	63.53 (40.37)	5.31×10^8
H	69.4–73.6	89.40 (93.16)	17.33 (27.51)	1.88×10^9
I	70.0–75.6	84.23 (87.33)	29.67 (29.08)	7.78×10^8
J	70.6–74.7	87.12 (87.33)	29.05 (29.08)	6.76×10^9
K	69.4–73.3	90.64 (87.33)	28.52 (29.08)	1.09×10^9

[a]Estimated values from the mathematical models. [b]CFU found in the powder derived from 2000 mL of culture, where the cells were centrifuged, washed, and resuspended in 100 mL of distilled water before entering the spray dryer.

rate of 84.68% using gum arabic to spray dry *L. acidophilus*. Lian et al. (2002) also reported that gum arabic increased the survival of *Bifidobacterium* after spray drying.

The survival rate may be related to the outlet temperature of the spray-drying process, with lower outlet temperatures leading to higher survival rates (Arslan et al., 2015). This can be observed in trial C, which exhibited the highest survival rate (91.26%) with a low outlet temperature of 52.0–58.3°C, while the opposite is observed in trial F, which had the highest outlet temperature of 99.0–102.2°C and the lowest survival rate (60.82%). This phenomenon can be justified by the relationship between outlet and inlet temperatures, where higher inlet temperatures can result in higher outlet temperatures (Benchabane et al., 2007).

After drying, the obtained powders were subjected to gastrointestinal resistance tests, yielding values from 0 to 63.53 Log_{10} CFU/mL. The null values for resistance to gastrointestinal conditions can be explained by the acidic pH and the antimicrobial activity of bile salts, resulting from the detergent-like properties of these salts, which disrupt bacterial membranes, leading to microorganism death (Madureira et al., 2011).

The spray-drying process presented cell counts (CFU) within the recommended range for daily intake by ANVISA, except for trials B, E, and F. Notably, these values were obtained from cultivation in a medium consisting solely of whey, a dairy by-product generated in large quantities. Even when used for producing other derivatives like ricotta, this by-product is often improperly discarded, harming the environment (Dullius et al., 2018). Therefore, producing probiotics from this raw material is of low cost and demonstrates sustainability.

The obtained results were subjected to statistical analysis to assess the significance of the independent variables within the responses. The coefficients of determination, regression, and their respective p-values are presented in Table S2. A significance level of 5% ($p < .05$) was considered for the survival rate of the response variables and a significance level of 10% ($p < .10$) for gastrointestinal resistance.

The results demonstrated that the models obtained for the dependent variables "survival rates" and "resistance to gastrointestinal conditions" were able to explain most of the data observed experimentally. This can be evidenced by the determination coefficients presented in Table S2.

Table S2 shows that temperature and gum arabic concentration were significant for the survival rate, and the quadratic temperature was significant for resistance to gastrointestinal conditions. According to the coefficients observed in Table S2, the higher the temperature, the lower the survival rate. Anekella and Orsat (2013) also found that, when performing the spray-drying process using *L. acidophilus* and *Lactobacillus rhamnosus*, with maltodextrin as the encapsulant, the increase in temperature led to reductions in survival rates. The same was observed by Arslan et al. (2015) in the spray drying of *S. cerevisiae* var. *boulardii* with various encapsulants, including gum arabic. During the drying process, there is significant thermal stress and dehydration, which can lead to the inactivation of microbial cells associated with protein denaturation, resulting in the loss of metabolic activities, membrane destabilization, and possibly the formation of pores and release of intracellular components (Broeckx et al., 2016), thus leading to lower survival rates and greater logarithmic reductions in viable cells.

It was also observed that the higher the gum arabic concentration, the higher the survival rate. In the microencapsulation process, gum arabic forms a film, making the capsule less porous, thereby protecting the microorganism, increasing the survival rate, and decreasing the logarithmic reduction of viable cells (Boscarioli, 2010). The survival of microorganisms during spray drying can be attributed to the strong adhesion to the encapsulating agent (Leone et al., 2017).

Analyzing at a significance level of 10% ($p < .10$), with the aim of understanding the behavior of resistance to gastrointestinal conditions with the factors, it was observed that the quadratic temperature presents a p-value of .06, thus being significant. Since the regression coefficient of the quadratic temperature is negative and the coefficient of the linear temperature is positive, the second-order function would generate a "ridge-" type response surface graph. This indicates that extreme inlet temperature values (maximum and minimum) would lead to low resistance and that median temperatures would result in better resistance to gastrointestinal conditions.

In fact, the implications on resistance to gastrointestinal conditions pointed out by the adjusted model are consistent with the data obtained experimentally. In trials A, C, and E, conducted with lower inlet temperatures, and in trials B, D, and F, with higher inlet temperatures, zero resistance values were observed. On the other hand, in trial D, considerable resistance was observed despite the high inlet temperature. This may have occurred due to the high concentration of the adjuvant, which may have acted by protecting the microorganism.

Additionally, these null values can be justified by the exposure time of the microorganism in the drying chamber, as a low inlet temperature leads to a reduction in the outlet temperature, increasing the drying time and exposing its proteins to a longer duration for denaturation (Arslan et al., 2015). Although high inlet temperatures result in a shorter exposure time of the cellular proteins to denaturation, they cause more severe dehydration and denaturation, leading to considerable cellular damage (Broeckx et al., 2016).

Trials G–K, which used median inlet temperatures, showed considerable resistance values to gastrointestinal conditions, which can be explained by the fact that the exposure time in the drying chamber may have only caused cell weakening, with reduced protein denaturation. From the experimental results, the regression fit was analyzed through the F-test (Table S3).

The response for the survival rate parameter obtained a satisfactory regression fit, with the $F_{calculated}$ (8.76) being more significant than the $F_{tabulated}$ (5.05), thus generating the response surface and contour plot (Figure 1). However, regarding the parameter for resistance to gastrointestinal conditions, it did not statistically achieve a significant regression fit. Therefore, constructing the response surface and contour plot is not recommended.

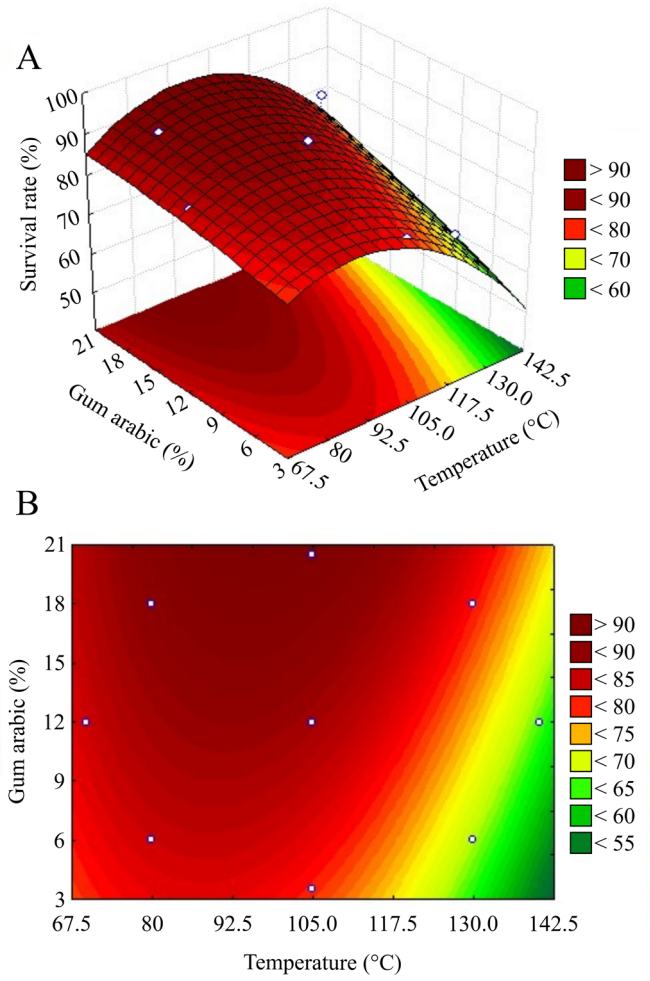

Figure 1. (A) The response surface and (B) contour plot for survival rates.

With the aid of the response surface and contour plot (Figure 1), it is possible to predict the optimal range of temperature and gum arabic concentration that leads to higher survival rates for the spray drying of *L. plantarum* P2. The optimal temperature range was between 88°C and 105°C, and the optimal concentration was between 16 and 21% gum arabic.

To obtain a powder with a higher survival rate, the maximum (105°C and 21% gum arabic) and minimum (88°C and 16% gum arabic) points within the optimal spray-drying parameters studied in this work were analyzed as the best options. According to the mathematical models obtained, the predicted survival rate for the minimum point was 91.12 ± 12.44%, and for the maximum point, it was 93.45 ± 14.53%. Thus, as the found values are close, the optimal drying point is estimated to be at a temperature of 105°C and 21% gum arabic since this temperature, as mentioned earlier, would also lead to better resistance to gastrointestinal conditions, a fundamental characteristic of probiotics.

According to the predicted value, it can be observed that at the established point for the spray-drying process, high survival rates and considerable resistance to gastrointestinal conditions are obtained. To confirm the predicted value, it is necessary to validate the results, meaning that experimental analysis of the drying at the established point of 105°C and 21% gum arabic should be conducted.

For SEM analysis, samples from trials C, E, F, G, H, and I were selected (Figure 2).

When analyzing the images obtained from SEM, it is observed that the formed microcapsules have a rounded, smooth surface with concavities, without cracks or fissures, and of varying sizes. The absence of cracks or fissures indicates greater protection for the microorganism, as the air permeability is minimal (Nunes et al., 2018).

The concavities, referred to as the "flat ball effect," may be correlated with the evaporation of water in the droplet formed in the drying chamber (Bustamante et al., 2015). This effect during spray drying facilitates the release of heat within the particle, thus causing less damage to the microorganisms (Lian et al., 2002). Several drying parameters influence particle morphology, such as atomization pressure, nozzle size, feed flow rate, and temperature (Eckert et al., 2017).

Arslan et al. (2015) observed that lower inlet temperatures led to the formation of larger particles when using gum arabic as an encapsulating agent. The same was observed in the present study, where trials C and E, which had lower drying temperatures (80°C and 70°C, respectively), resulted in larger particles. Conversely, the opposite was observed for trials G and F; higher drying temperatures (105°C and 140°C, respectively) led to the formation of smaller particles. However, trials H and I, which had a drying temperature of 105°C, generated larger particles, possibly due to the higher concentration of the encapsulating agent used in these trials.

The size of the particles is an essential characteristic of products dried by spray drying. Smaller particles ensure greater quality and homogeneity in the formulations to which they will be applied (Nunes et al., 2018).

Since the presence of the microorganism was not observed in the images, it was decided to carry out drying without the use of the encapsulating agent, gum arabic, to confirm microencapsulation. However, upon analyzing the images from this drying process (Figure 3), the presence of the microorganism under study was not observed. This fact can be justified by the use of whey as a culture medium. When washing before the drying process, components of the whey medium may have remained in the suspension, and during the drying process, these components may have acted as encapsulating agents. The literature includes studies mentioning the use of whey as an encapsulating agent, corroborating this hypothesis (Castro-Cislaghi et al., 2012; Eckert et al., 2017).

To verify whether whey could have acted as an encapsulating agent, a drying process without any adjuvant for the microorganism was conducted using MRS medium instead of whey medium. Through the images generated from the electron microscopy of this drying (Figure 4), it is possible to observe the presence of smaller particles, but with a rough characteristic. When these images are magnified at 5000 ×, we can see bacilli

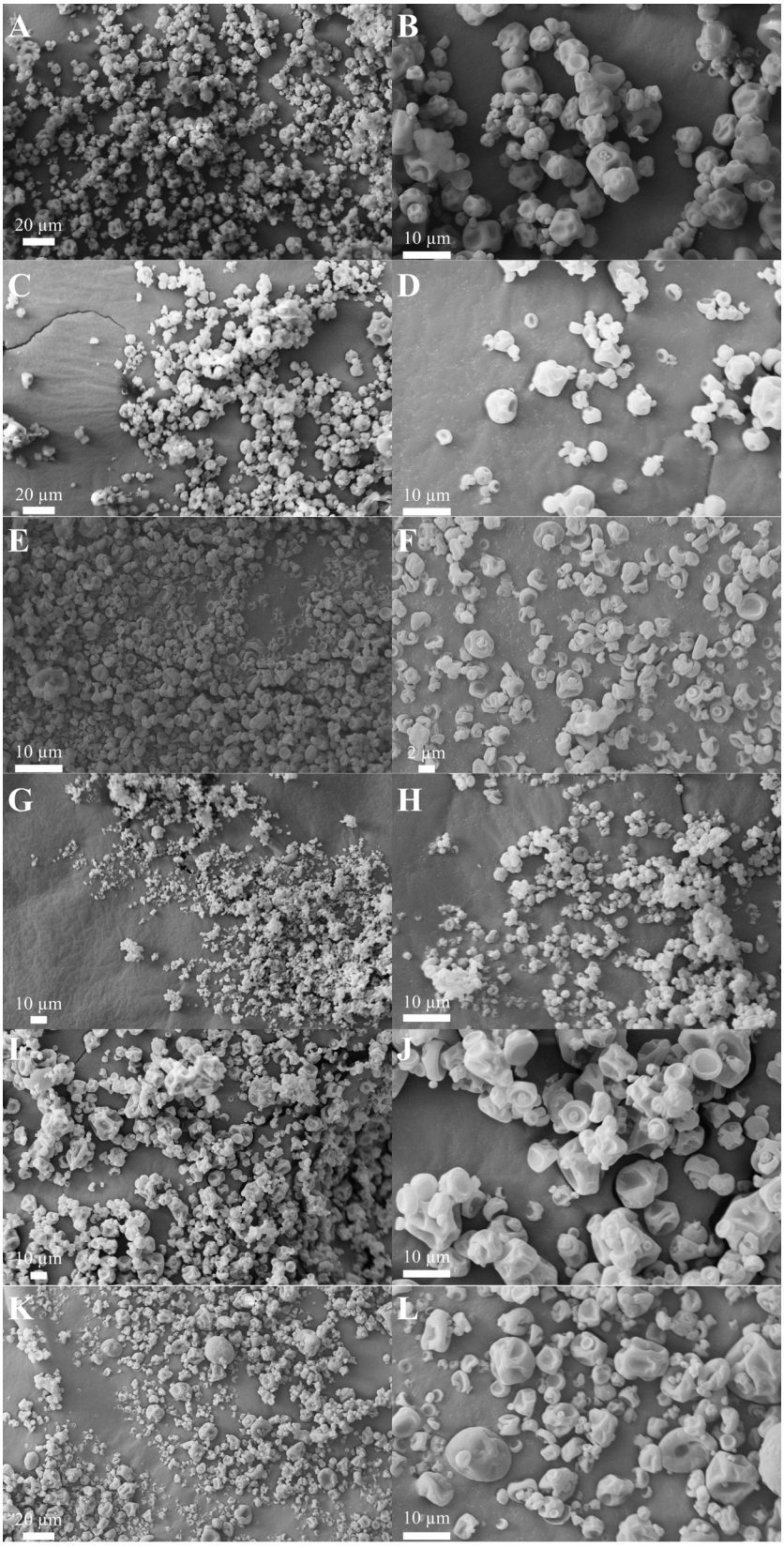

Figure 2. Scanning electron microscopy images of different trials at various temperatures and gum arabic concentrations: (A and B) Trial C (80°C, 18% gum arabic) at 1000 × and 3000 ×, respectively; (C and D) Trial E (70°C, 12% gum arabic) at 1000 × and 3000 ×, respectively; (E and F) Trial F (140°C, 12% gum arabic) at 3000 × and 5000 ×, respectively; (G and H) Trial G (105°C, 1.51% gum arabic) at 1000 × and 3000 ×, respectively; (I and J) Trial H (105°C, 20.48% gum arabic) at 1000 × and 3000 ×, respectively; and (K and L) Trial I (105°C, 12% gum arabic) at 1000 × and 3000 ×, respectively.

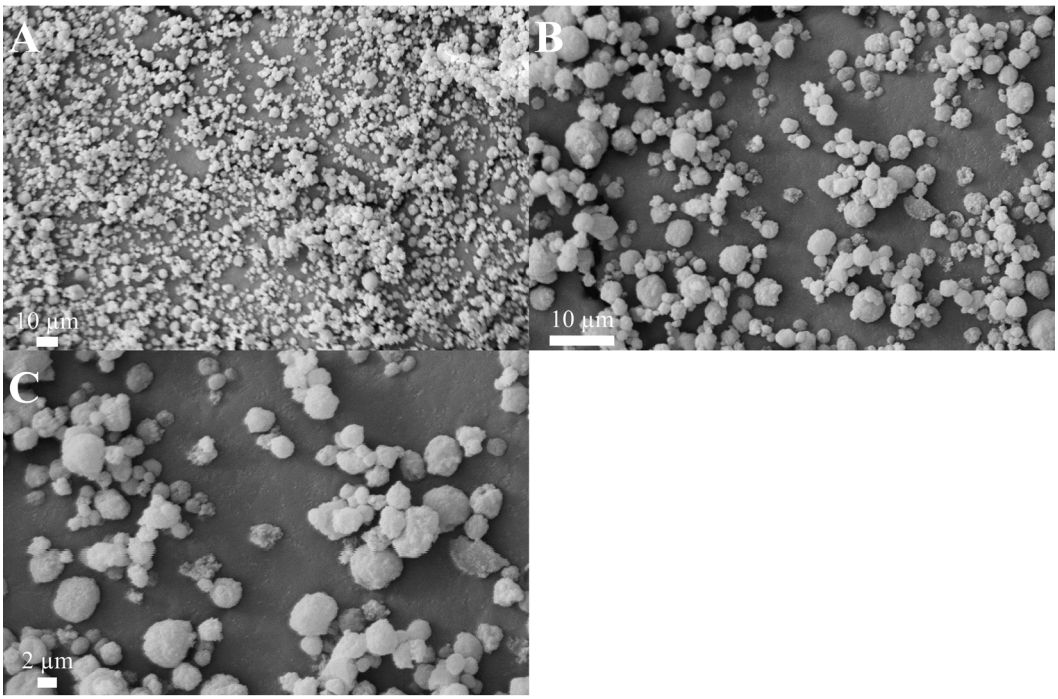

Figure 3. Scanning electron microscopy of drying without gum arabic (70°C). (A) 1000 ×, (B) 3000 ×, and (C) 5000 ×.

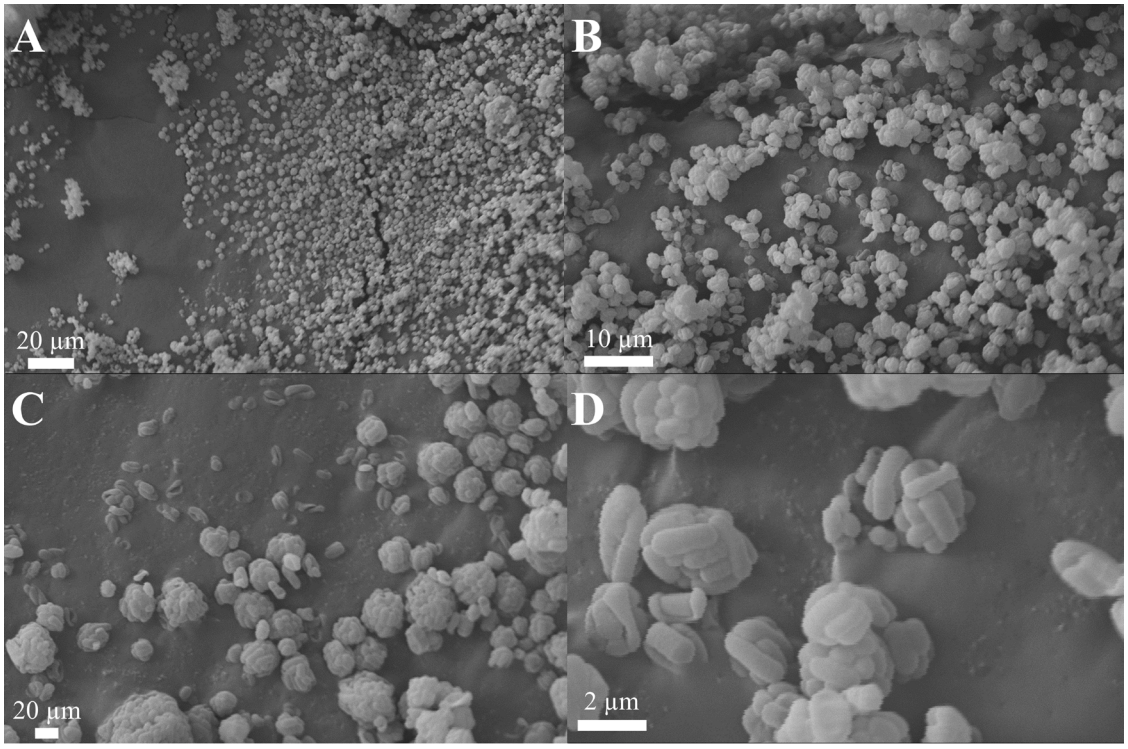

Figure 4. Scanning electron microscopy images of cells grown in MRS medium and dried by spray drying (70°C) without the addition of any adjuvant. (A) 1000×, (B) 3000×, (C) 5000×, (D) 15,000×.

aggregates, indicating the microorganism's presence without microencapsulation. At a magnification of 15,000 ×, we can more clearly observe the shape of the microorganism under study, confirming that both whey and gum arabic acted as encapsulating agents.

4 CONCLUSIONS

Taking into account the obtained results, it is possible to conclude that through the spray drying of *L. plantarum* P2, the survival rate was statistically dependent on temperature and

the concentration of gum arabic, where lower temperatures and higher concentrations of gum arabic led to higher survival rates. Only the quadratic temperature was statistically significant for resistance to gastrointestinal conditions. The micrographs confirmed the microencapsulation of the microorganism.

The overall purpose of this work was to establish the best spray-drying conditions for *L. plantarum* P2. In this context, it can be concluded that, under the analyzed conditions, an estimated optimal drying point exists to obtain a powder with higher survival rates and better resistance to gastrointestinal conditions. However, there is a need to validate the models.

It is also noteworthy that the culture medium used in this process is whey, which is a low-cost medium without additives and supplements, making it environmentally sustainable and reinforcing the significant application potential of this input.

As a perspective for this work, it is suggested that this powder be used in food formulations, followed by in vivo studies to confirm its probiotic activity.

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