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Optimization of bioprocesses of *Lactiplantibacillus plantarum* UFSJP2 with antioxidant activity and its viability in graviola (*Annona muricata*) sorbet

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Abstract

Functional foods within a varied diet at effective dosages may confer health benefits that surpass the scope of basic nutrition, with probiotics assuming a significant role in this domain. The use of sorbet enriched with probiotics is an alternative to dairy products. However, the viable bioprocessing for probiotic production presents substantial challenges. The aim of this study was to optimize the bioprocesses of *Lactiplantibacillus plantarum* UFSJP2 and to evaluate its antioxidant activity and viability in graviola (*Annona muricata*) sorbet. The optimized production of UFSJP2 biomass was 787% greater than non-optimized processes, resulting in a yield of 3.7 x 10^{10} CFU.mL^{-1} under conditions comprising 10.55 g.L^{-1} yeast extract, 10 g.L^{-1} peptone, and 0.16 g.L^{-1} manganese sulfate. The aggregated spherical structure observed in SEM may have a certain protective effect. Antioxidant activity assays revealed efficacies of 79% and 46% for intact and lysed cells, respectively. The survival rate of the UFSJP2 strain in the presence of hydrogen peroxide was observed at 77.5% (1.0 mM for 8 h). Notably, there was no diminution in the number of viable cells of UFSJP2 in graviola sorbet in 30 days. These results underscore a cost-effective strategy for probiotic production and for enhancing viability within plant-based matrices.

Keywords: probiotics; bioprocess; non-dairy.

Practical application: Graviola sorbet is a vehicle for the delivery of probiotics with antioxidant activity.

1 INTRODUCTION

Since the 1980s, the term "functional foods" has been used. Most countries' definitions are often misunderstood because they are regulated but not legally recognized, resulting in no proper definition (Ye et al., 2018). One of the definitions is presented by Granato et al. (2017), in which functional foods are industrially processed or natural foods that, when regularly consumed within a diverse diet at efficacious levels, have potentially positive effects on health beyond essential nutrition. To be functional, food should be validated in intervention trials to comply with the regulations in each country, e.g., the Brazilian Health Regulatory Agency in Brazil (Brown et al., 2018; Cassidy et al., 2018).

Probiotics have special attention among those components, have been considered functional food (Begum et al., 2017), mainly belong to Gram-positive bacteria, have a natural habitat in the human gut, and may alleviate gastrointestinal dysbiosis, lower serum cholesterol, ameliorate cancer, and prevent allergic and autoimmune disorders (Hajavi et al., 2019). Studies have demonstrated that *Lactobacillus*, *Lactiplantibacillus*, *Bacillus, Bifidobacterium*, *Enterococcus, Saccharomyces,* and *Streptococcus* are the most popular probiotic genus sources. Their application as probiotics is extensive in fermented food products, non-fermented food items, and functional and nutraceutical dietary supplements (Di Cerbo et al., 2016; Zheng et al., 2020).

The increase in veganism, lactose intolerance, and/or hypercholesterolemic individuals have been demanding changes to products with probiotics and dairy products (yogurt and other fermented products) (Nguyen et al., 2019). Lactose intolerance is a genetically determined β-galactosidase deficiency resulting in the incapacity to hydrolyze lactose into glucose and galactose monosaccharides (Oak & Jha, 2019). Thus, the avoidance of products derived from

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animals has become an increasing trend in modern lifestyles. Many consumers demand plant-based milk alternatives for sustainability, health-related, lifestyle, and dietary reasons or broader social or political concerns, resulting in abundant products based on nuts, seeds, or beans (Ploll et al., 2020; Tangyu et al., 2019).

Diversifying the non-dairy matrix to deliver probiotics is a trend in the food industry. Using fruit-based sorbets as a carrier of functional ingredients could improve health benefits. Among the functional products such as breads, berries, and vegetables, frozen foods such as sorbets consumed by global populations are gaining attention (Williams et al., 2023). Sorbets are considered a functional food and are one commercial method used to provide health benefits, extend food storability, preserve nutrients, and give palatability to the consumer (Bahramparvar & Mazaheri Tehrani, 2011; Williams et al., 2020).

Biomass production of probiotics on an economically viable large scale is a challenge, and the use of cheese whey has been considered since it is a valuable alternative to commercial media to produce lactic acid bacterium (LAB) biomass (Colares et al., 2021; Pescuma et al., 2015). LABs are fastidious microorganisms that need many elements to grow, such as carbohydrates, amino acids, lipids, and salts, and the use of commercial media is not the industry's interest due to the high cost (Ficoseco et al., 2018). Associating the use of food waste as industrial by-products has environmental and economic problems, and the circular economy could be an alternative for energy source during fermentation (Colares et al., 2021).

One of the tropical fruits with excellent components to improve health is *Annona muricata*, commonly known as graviola, with a phytochemical composition of the extracts of a high concentration of secondary class metabolite compounds, such as alkaloids, coumarins, flavonoids, saponins, terpenoids, and other lactones, anthraquinones, tannins, cardiac glucosides, phenols, and phytosterols (Gavamukulya et al., 2014). Its composition is responsible for anticancer, antioxidant, antimicrobial, anti-inflammatory, and other health benefits (Errayes et al., 2020).

This work aimed to optimize the bioprocesses of *Lactiplantibacillus plantarum* UFSJP2 and evaluate its antioxidant activity and viability in graviola (*A. muricata*) sorbet.

2 MATERIALS AND METHODS

2.1 Materials

The materials used in this study were yeast extract (Neogen, Lansing, USA); $MnSO_4$ (Dinamica, Indaiatuba, Brazil); $MgSO_4$ (Isofar, Duque de Caxias, Brazil); MRS agar (Sigma-Aldrich, Saint Louis, USA); MRS broth (Difco, Franklin Lakes, USA); saline solution and peptone (Kasvi, São José dos Pinhais, Brazil). Laticínios Curral de Minas Ltda gently provided the whey.

2.2 Microorganisms and maintenance

L. plantarum UFSJP2, a LAB isolated from artisanal minas cheese, was used in this study (Guimarães et al., 2020). The LAB strain was maintained in MRS with 20% glycerol at -80°C and sub-cultured in MRS broth at 37°C for 24 h right before every experimental procedure.

2.3 Culture media and growth conditions

To obtain the pre-inoculum, the *L. plantarum* UFSJP2 strain was cultured in an Erlenmeyer flask containing supplemented whey (10 g L^{-1} peptone, 10 g L^{-1} yeast extract, 0.04 g L^{-1} $MnSO_4$, and 0.2 g L^{-1} $MgSO_4$), previously sterilized by autoclaving (Phoenix Luferco, Araraquara, Brazil) at 121°C for 20 min, without rotation at 37°C for a period of 13~15 h. The volume of the medium was 20% of the volume of the flask used. The pre-cultured *L. plantarum* UFSJP2 strain was centrifuged (MPW Med. Instruments, Warsaw, Poland) at a rotation of 1,370 g for 30 min and washed with 0.85% saline. The cells remaining after the washes were resuspended in sterile distilled water, and an aliquot was measured in a spectrophotometer (Nova Instruments, Piracicaba, Brazil) at 600 nm to perform a controlled inoculum with an optical density (OD) of 0.2.

2.4 Deproteinization of whey

The whey's pH was adjusted to 4.5 and autoclaved at 120°C for 20 min. Next, the serum was filtered with its pH adjusted to 6.3 and autoclaved again at 120°C for 20 min.

2.5 Optimization of biomass production of *L. plantarum* UFSJP2

2.5.1 Selection of the significant variables by a fractional factorial design

A fractional factorial design (FFD) (24^{-1}) was performed to evaluate the effect of independent variables on the response biomass production by *L. plantarum* UFSJP2 strain. Individual supplements found within the whey were isolated and utilized as described: yeast extract (0–10 g L^{-1}), $MgSO_4$ (0–0.4 g L^{-1}), $MnSO_4$ (0–0.08 g L^{-1}), and peptone (0–10 g L^{-1}). A total of eight combinations of the supplements and four repetitions of the central point were tested. A first-order model was used to describe the individual supplements' effects on biomass production in response (Equation 1).

$$Answer = \beta_0 + \Sigma \beta_i X_i \quad (1)$$

Where:

β_0: the constant;

β_i: the coefficient of the linear term;

X_i: the independent variable.

2.5.2 Rotatable central composite design

A rotatable central composite design (RCCD) was used to determine the conditions that maximize biomass production by the *L. plantarum* P2 (CFU mL^{-1}) and to fit second-order models to describe them. The main factors influencing the responses, previously analyzed according to FFD, were

Table 1. CCRD outcomes following FFD analysis, detailing adjustments in yeast extract, peptone, and manganese sulfate concentrations after an 18-h culture.

Rum	YE	Peptone	Mn	Experimental CFU mL-1	Predicted CFU mL-1
1	7.02 (-1)	2.82 (-1)	0.064 (-1)	1.20E+10	1.55756E+10
2	12.97 (+1)	2.82 (-1)	0.064 (-1)	2.10E+10	2.36364E+10
3	7.02 (-1)	8.17 (+1)	0.064(-1)	1.30E+10	1.52032E+10
4	12.97 (+1)	8.17 (+1)	0.064 (-1)	2.11E+10	2.23140E+10
5	7.02 (-1)	2.82 (-1)	0.135 (+1)	1.30E+10	1.34707E+10
6	12.97 (+1)	2.82 (-1)	0.135 (+1)	1.90E+10	1.84816E+10
7	7.02 (-1)	8.17 (+1)	0.135 (+1)	2.60E+10	2.50483E+10
8	12.97 (+1)	8.17 (+1)	0.135 (+1)	3.10E+10	2.91092E+10
9	5 (-1.68)	5.5 (0)	0.100 (0)	1.19E+10	9.56215E+09
10	15 (+1.68)	5.5 (0)	0.100 (0)	1.98E+10	1.97553E+10
11	10 (0)	1 (-1.68)	0.100 (0)	2.02E+10	1.73469E+10
12	10 (0)	10 (+1.68)	0.100 (0)	2.55E+10	2.59705E+10
13	10 (0)	5.5 (0)	0.040 (-1.68)	2.85E+10	2.35867E+10
14	10 (0)	5.5 (0)	0.160 (+1.68)	2.50E+10	2.75307E+10
15	10 (0)	5.5 (0)	0.100 (0)	3.01E+10	2.50848E+10
16	10 (0)	5.5 (0)	0.100 (0)	2.91E+10	2.50848E+10
17	10 (0)	5.5 (0)	0.100 (0)	2.00E+10	2.50848E+10
18	10 (0)	5.5 (0)	0.100 (0)	2.45E+10	2.50848E+10
19	10 (0)	5.5 (0)	0.100 (0)	2.65E+10	2.50848E+10
20	10 (0)	5.5 (0)	0.100 (0)	1.99E+10	2.50848E+10

YE: yeast exact; Mn: manganese sulfate.

selected and used in an RCCD. The variables evaluated were yeast extract (5–15 g L^{-1}), peptone (1–10 g L^{-1}), and $MnSO_4$ (0.04–0.16 g L^{-1}). There were 15 combinations between the factors and five repetitions of the central point, as shown in Table 1. A second-order model was used to describe the relationship between these supplements and biomass production according to Equation 2.

$$Answer = \beta_0 + \Sigma \beta_i X_i + \Sigma \beta_{ij} X_i X_j + \Sigma \beta_{ii} X_i^2 \quad (2)$$

Where:

β_0: the constant β_i;

β_{ii}: the terms of the linear and quadratic coefficients;

β_{ij}: the interaction term;

X_i: the independent variable.

2.5.3 Validation of models

The best conditions determined in the previous steps will be used for new benchtop experimentation, performing production in 25 mL of culture medium. The predictive capacity of the established models will be evaluated using bias factors (F_B) and accuracy factors (F_A) as calculated in Equations 3 and 4, respectively, described by Baranyi et al. (1999).

Validation was performed for two culture media: conditions 1 and 2. Condition 1 (10.55 g L^{-1} of yeast extract, 10 g L^{-1} of peptone, and 0.16 g L^{-1} manganese sulfate) concerns the optimal condition given by the model. Condition 2 (7.5 g L^{-1} of yeast extract, 2.5 g L^{-1} of peptone, and 0.08 g L^{-1} manganese sulfate) is an economically more viable condition that maintains the UFC mL^{-1} log scale with lower spending on supplementing the medium.

$$F_B = 10^{(\Sigma \frac{log\,(\frac{P}{O})}{n})} \quad (3)$$

$$F_A = 10^{(\Sigma \frac{|log\,(\frac{P}{O})|}{n})} \quad (4)$$

Where:

P: the predicted values;

O: the observed values;

n: the number of experimental data.

The F_B indicates the relative mean deviation between predicted and observed values, providing the model's confidence. An F_B value of 1 indicates that the predicted and observed values fully agree. The F_A shows how the experimental values are arranged around the predicted values, providing the model's accuracy. Acceptable F_A values are close to 1. These factors are appropriate for comparing two or more models.

2.6 Scanning electron microscopy of lyophilized cells

Lyophilized powders of *L. plantarum* UFSJP2 strain growth in MRS and optimized medium were fixed to a sample slide and were transferred to stubs, and then platinum plated on Leica equipment on SCD500 (LEICA, Wetzlar, Germany). The morphology of platinum-coated samples was examined using a Helios NanoLab 650 scanning electron microscope (FEI, Oregon, USA).

2.7 Antioxidant activity

2.7.1 Scavenging 2,2-diphenyl-1-picrylhydrazyl radicals

For this study, the medium used was MRS. The *L. plantarum* UFSJP2 strain culture was incubated at 37°C for 24 h. Antioxidant activity was performed in two groups: (a) intact cells and (b) lysed cells. For the intact cells, 10^{10} cells per mL were guaranteed through centrifugation at 1,400 g for 15 min, washing three times in autoclaved distilled water, and resuspending in sterile distilled water. The lysed cells of the grown probiotic culture were obtained through centrifugation at 1,400 g for 15 min. Lysis of these strains was carried out using the enzyme lysozyme at a concentration of 1 mg mL^{-1}, incubated at 37°C for 1 h, followed by sonication at 90 mA for 1 min, divided into five pulses with an interval of 1 min, between each pulse, in an ice bath. The cells for the lysis process were in autoclaved distilled water. After lysis, centrifugation was performed at 1,500 g for 10 min to remove cell debris and possible unlysed cells, with only the supernatant resulting from this process being used to determine the activity of antioxidants.

Notably, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity was determined according to the method described by Li et al. (2012) with some modifications. An aliquot of 1 mL of each group mentioned above was added to 2 mL of DPPH radical in a 0.05 mM ethanolic solution. The mixture was shaken vigorously using a vortex and incubated at room temperature in the dark for 30 min. The resulting absorption of the solution was measured in triplicate at 517 nm. For samples that contained intact cells, centrifugation was performed at 1,500 g for 10 min before dosing at 517 nm to remove these cells.

The DPPH radical capture capacity was defined based on Equation 5. In addition to the two test groups mentioned above, the antioxidant activity of two groups was performed for negative controls, namely, autoclaved distilled water and autoclaved distilled water with lysozyme at 1 mg mL^{-1}. Negative controls' antioxidant activity was subtracted from their tests' antioxidant activity. The absorbance of the test control refers to the ethanolic DPPH solution (0.05 mM) (Equation 5). The test was performed in triplicate on three different days:

$$Antioxidant\ activity\ (\%) = \left[1 - \left(\frac{Sample\ abs}{Control\ abs}\right)\right] \times 100 \quad (5)$$

2.7.2 Resistance of intact cells to hydrogen peroxide

The method of Buchmeier et al. (1997) was used with some modifications. A pre-inoculum of the *L. plantarum* UFSJP2 strain was performed; it was inoculated at 1% (v/v) in MRS broth and MRS broth containing 0.4, 0.7, or 1.0 mM hydrogen peroxide (30% by weight of solution) and incubated at 37°C for 8 h. Cell growth was measured spectrophotometrically at 600 nm. The results were presented in OD. Spectrophotometric measurements were performed in triplicates on three different days.

2.8 Sorbet production and cell viability

Graviola was purchased in the local market, processed to produce sorbet, and pasteurized. To produce 250 mL of graviola (*A. muricata*) sorbet, 65 g of silver banana, 196 g of graviola pulp, and 10 g of xylitol were used. Using a multiprocessor, all ingredients were mixed for 3 min at 4°C. The mixture, at this point, had a creamy and uniform consistency. Subsequently, the mixture was stored in a freezer at an average temperature of -20°C for 30 min. After this period, the mixture was removed from the freezer and homogenized again, now in a domestic mixer, for 3 min to incorporate air into the mass, thus leaving the product with an airy characteristic. This last step of the procedure was repeated five more times until it acquired a consistency close to creamy ice cream. After that, 10^9 CFU mL^{-1} of *L. plantarum* UFSJP2 was incorporated into the graviola sorbet.

Cell viability was assessed by counting the cells on MRS agar. The plates were incubated in anaerobic conditions for 48 h at 37°C. The strain was pre-incubated for 16 h at 37°C and then inoculated into 45 mL of MRS broth for 24 h under the same incubation conditions. After the cells were washed once with 0.85% saline, 0.9 mL of this suspension was added to 4.1 mL of sorbet so that the final concentration of bacteria was around 10^9 cells mL^{-1}. About 5 mL of sorbet containing the bacteria was frozen at -20°C. Counts of the number of live cells were performed at the time of inoculation in the sorbet (T0) and after 30 days (T1). A 1 mL aliquot was taken from the samples, diluted, and plated on MRS agar. The plates were incubated anaerobically for 48 h at 37°C. The experiments were performed in triplicate and on three different days. The control was the pasteurized sorbet without the addition of bacteria.

2.9 Statistical analysis

A student's t-test ($\alpha = 0.05$) was used to verify the significance and the amount of data, compared to the DFF and the coefficients of the fitted models, in relation to the RCCD. Fisher's test (ANOVA of linear regression) was used to determine the statistical significance of the factors on the *L. plantarum* UFSJP2 biomass in the RCCD. The coefficient of determination, R2, the statistical significance of the regression, and the model's fit were used to assess the quality of the adjusted regressions. Statistical and graphical analysis were performed on the Minitab® 18 software (Minitab Inc.). For the antioxidant activity, the analysis was conducted utilizing a normality test for all data. Unpaired t-test and one-way ANOVA were used to analyze two or more groups, respectively. Statistical significance was considered when $p < 0.05$.

3 RESULTS

After the bioprocess was carried out in the 12 evaluated conditions of the FFD, plating was performed to assess the cell concentration (CFU mL^{-1}) of the serial dilutions ($10^{-6}/10^{-7}/10^{-8}$). Table 2 shows the CFU mL^{-1} values.

The results were analyzed by means of variance analysis, with significance indicated by a $p < 0.05$ (Suppl. Table 1). The variables that significantly influenced the biomass (CFU mL^{-1}) of the *L. plantarum* UFSJP2 strain were indicated by the p-value analysis. When observing Suppl. Table 1, the first data found is in relation to the model that was significant with $p < 0.05$. Analyzing in detail, it can be observed that for all variables except

Table 2. CFU mL^{-1} derived from post-bioprocess in the 12 conditions evaluated through fractional factorial design.

Rum	YE (g L^{-1})	Peptone (g L^{-1})	Mg (g L^{-1})	Mn (g L^{-1})	CFU mL^{-1}
1	0 (-1)	0 (-1)	0 (-1)	0 (-1)	4.70E+09
2	10 (+1)	0 (-1)	0 (-1)	0.08 (+1)	3.20E+10
3	0 (-1)	10 (+1)	0 (-1)	0.08 (+1)	3.88E+10
4	10 (+1)	10 (+1)	0 (-1)	0 (-1)	2.43E+10
5	0 (-1)	0 (-1)	0.4 (+1)	0.08 (+1)	4.60E+09
6	10 (+1)	0 (-1)	0.4 (+1)	0 (-1)	4.00E+10
7	0 (-1)	10 (+1)	0.4 (+1)	0 (-1)	1.51E+10
8	10 (+1)	10 (+1)	0.4 (+1)	0.08 (+1)	4.20E+10
9	5 (0)	5 (0)	0.2 (0)	0.04 (0)	9.40E+09
10	5 (0)	5 (0)	0.2 (0)	0.04 (0)	1.04E+10
11	5 (0)	5 (0)	0.2 (0)	0.04 (0)	1.21E+10
12	5 (0)	5 (0)	0.2 (0)	0.04 (0)	1.18E+10

YE: yeast extract; Mg: magnesium sulfate; Mn: manganese sulfate.

magnesium sulfate, the p-value was lower than 0.05, showing the influence of these components on biomass production. Magnesium sulfate did not influence the increase in biomass production and was therefore disregarded for the following experiment stages. For the second-order interactions, influence on production was observed in the interaction between all the factors combined since they presented a $p < 0.05$.

The coefficient of determination, also called R^2, measures the adjustment of a generalized linear statistical model with linear regression concerning the observed values. The coefficient of determination, denoted as R^2, was calculated to be 99.84%. This suggests that 99.84% of the variation in the dependent variable can be accounted for by the regressors present in the model.

The coefficients of the regression of the FFD are presented in Suppl. Table 2. These coefficients express the intensity and the signal of the effect of the factors on the response in biomass production. From this, it is observed that the yeast extract has the most expressive positive effect on biomass production, calculated through CFU mL^{-1}. The peptone, manganese sulfate, and magnesium sulfate also showed a positive but lower interference in biomass production; we emphasize that the interference of magnesium sulfate was not statistically significant, as shown in Suppl. Table 1. With the increase in the yeast extract, peptone, and manganese sulfate concentration, it is evident that the biomass production of *L. plantarum* UFSJP2, calculated by CFU mL^{-1}, also increases.

From the results obtained in the FFD, a central composite rotatable design (CCRD) was planned with the following decision-making: (1) upward shift of the concentration range of yeast extract; (2) maintenance of the peptone concentration range; (3) upward shift of the concentration range of manganese sulfate. We decided to maintain the range of peptone concentration due to the non-significant interaction of yeast peptone extract and the high cost of this raw material in the industrial scope.

The CCRD results were obtained after 18 h culture with experimental units, according to Table 1. A variance analysis was performed, and the variables that significantly influenced the *L. plantarum* UFSJP2 biomass (CFU mL^{-1}) and the interference of each medium component and the second-order interactions were analyzed (Suppl. Table 3). The results showed that only the yeast extract, peptone, and manganese sulfate significantly influenced the UFSJP2 biomass production (CFU mL^{-1}), according to Figure 1. Second-order interactions do not influence *L. plantarum* UFSJP2 biomass production (CFU mL^{-1}). In addition, the coefficients of the CCRD regression are presented in Suppl. Table 4.

The maximum adjusted *L. plantarum* UFSJP2 biomass production (CFU mL^{-1}) could be achieved at the following concentrations: 10.55 g L^{-1} of yeast extract, 10 g L^{-1} of peptone, and 0.16 g L^{-1} manganese sulfate (condition 1). With this optimal condition after the bioprocess development, it was possible to produce 3.7×10^{10} CFU mL^{-1}. Thus, with the supplementation of the whey, the biomass production was approximately 787% about the whey, which provides a CFU mL^{-1} of 4.7×10^9 (Table 2), demonstrating the importance of the process for increasing yield and decreasing costs.

In addition to the optimum point, another experimental condition was chosen to validate the models, selecting a condition with a larger biomass production cost-to-benefit ratio. Thus, concentrations of 7.5 g L^{-1} of yeast extract, 2.5 g L^{-1} of peptone, and 0.08 g L^{-1} of manganese sulfate were chosen (condition 2). The validation of these two points was then performed.

The conditions used for the validation experiment were based on the estimated values of each factor for biomass production, calculated by CFU mL^{-1} (condition 1 and condition 2). The values chosen corresponded to the desirability function (d) of 1. The experiments were conducted in triplicate, with results expressed in CFU mL^{-1}, and the values obtained for each fermentative parameter were used to calculate the bias factors and accuracy, which were found to be close to 1. For condition 1, the value was 1.027, and for condition 2, the value was 0.904, indicating that the function expressed by the CCRD has good accuracy of results.

A scanning electron micrograph of GP lyophilized cells of *L. plantarum* UFSJP2 growth in MRS and optimized medium

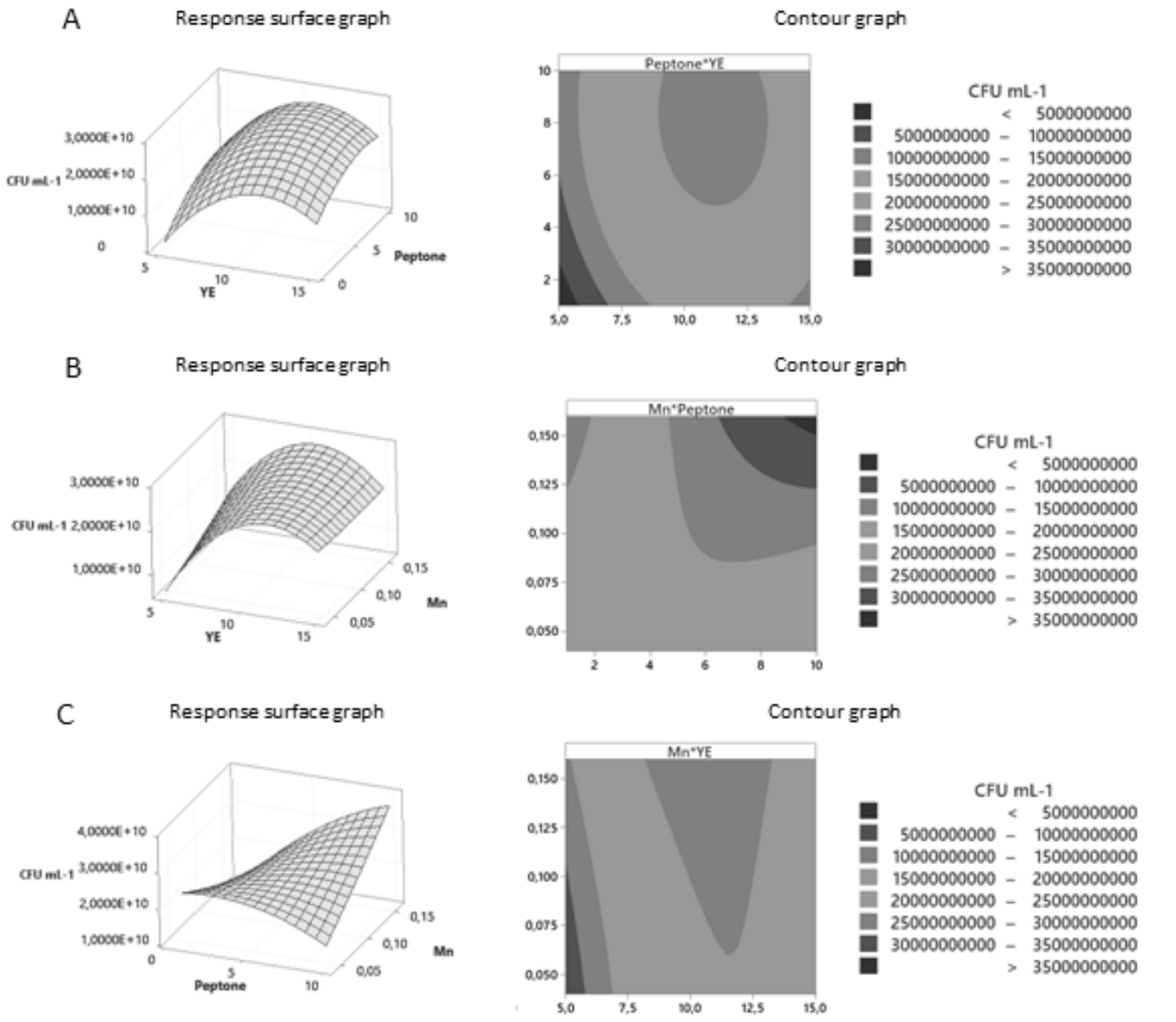

Figure 1. Response surface graph and contour graph for (A) yeast extract and peptone, (B)yeast extract and manganese sulfate, and (C) peptone and manganese sulfate.

was demonstrated in Figure 2. The freeze-dried powder demonstrated a lamellar structure and no aggregate or dispersed particles (Figures 2A and 2C), such as no bareness, smooth surface, and almost no porosity (Figure 2D). On the other hand, the freeze-dried powders from *L. plantarum* UFSJP2 growth in optimized medium demonstrated a granular shape and good particle dispersion (Figures 2B and 2D). Arrows indicated porous, long filamentous cellular structures and organic material from the cheese whey (Figure 2E).

The percentage of DPPH antioxidant or free-radical scavenging activity was performed with intact and lysed cells (Figure 3A). It was possible to observe that the *L. plantarum* UFSJP2 strain presented an antioxidant activity of 79 and 46%, respectively, for intact and lysed cells, with a statistically significant difference.

The resistance of *L. plantarum* UFSJP2 intact cells to hydrogen peroxide was demonstrated in Figure 3B. The intact cells showed tolerance to hydrogen peroxide at concentrations of 0.4–1 mM for 8 h and were concentration-dependent. At the highest concentration of hydrogen peroxide (1.0 mM for 8 h), the *L. plantarum* UFSJP2 intact cells showed an absorbance of 1.886, transforming it into a percentage of *L. plantarum* UFSJP2 survival under hydrogen peroxide. We found an approximate value of 77.5% compared to the negative control (0 mM for 8 h).

The survival of the *L. plantarum* UFSJP2 strain in graviola sorbet over time was evaluated (Table 3). After 30 days, the ΔLog CFU mL^{-1} in the number of cells was 0.3.

4 DISCUSSION

Lactobacillus sp. are demanding microorganisms, and culture media such as MRS are necessary for their growth (Ficoseco et al., 2018; Maldonado et al., 2018), but they are not

economically suitable for industrial applications (Manzoor et al., 2017). An alternative strategy is the use of some industrial by-products, such as cheese whey, cassava effluents, and biodiesel by-products (Amorim et al., 2018; Fu et al., 2014). Applying one of these industrial wastes can lead to high productivity and lower production costs. Cheese whey is a liquid by-product that remains after the cheesemaking process. It is associated with the dairy industry's environmental contaminants due to its negative effect on rivers or streams (Carvalho et al., 2013). The whey nutrient contents are lactose (70–72%), whey proteins (8–10%), and mineral salts (12–15%) (Panesar et al., 2007). Although whey contains these nutrients, it cannot support the growth of lactic acid bacteria. For this reason, we performed the FFD and CCRD to optimize the whey, supplementing it with nitrogen sources such as yeast extract, peptone, and mineral salts such as manganese sulfate and magnesium sulfate.

Table 3. The survival of the *L. plantarum* UFSJP2 strain in graviola sorbet over time.

Time	CFU mL-1	DSV	Log CFU mL-1	Δ Log CFU mL-1
T0	8.3 x 109	5.4 x 109	9.91	0.15
T1	5.8 x 109	4.1 x 109	9.76	

T0: Time zero; T1: 30 days.

Previous studies demonstrated that the concentration of the protein and reducing sugar in the deproteinized whey were 3.4 and 0.6 g L^{-1}, respectively (Colares et al., 2021), demonstrating the necessity to improve it with more nutrients. The optimization of the biomass production of *L. plantarum* UFSJP2 provided fundamental information on the components needed to obtain high biomass through a cheap medium, making industrial-scale production viable. The primary nitrogen source was yeast extract, which served as a source of carbon, nitrogen, and vitamins needed to meet the growth needs of microorganisms (Colares et al., 2021; Hayek & Ibrahim, 2013). Supplementation with yeast extract (2%) caused an increase in the growth of *Lacticaseibacillus casei* NCIM 5,752 more efficiently (Nanjaiah et al., 2024).

Another important finding is that peptone and yeast extract can be alternated, and no high concentrations are required to provide high biomass yields. Studies have already shown that a medium containing numerous nitrogen sources hinders the intensive propagation of *L. plantarum* NCIMB 8014 (Zacharof & Lovitt, 2013). The authors also demonstrated that, as in the present work, yeast extract was chosen as the primary source of nitrogen, just as the absence or reduction of peptone can improve bacterial growth rate.

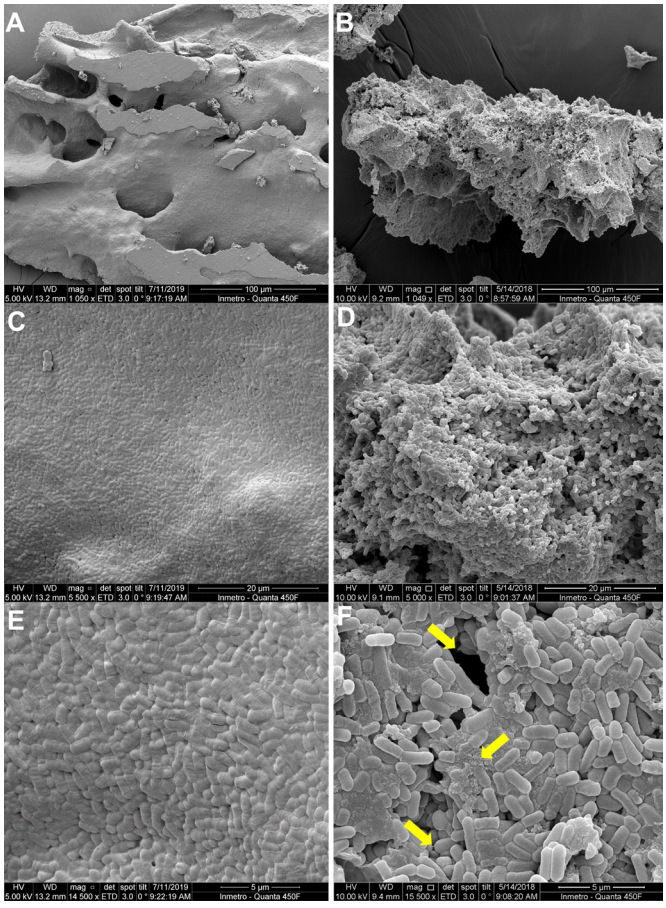

Figure 2. Scanning electron micrographs of lyophilized cells of *Lactiplantibacillus plantarum* UFSJP2 growth (A) in MRS and (B) optimized medium.

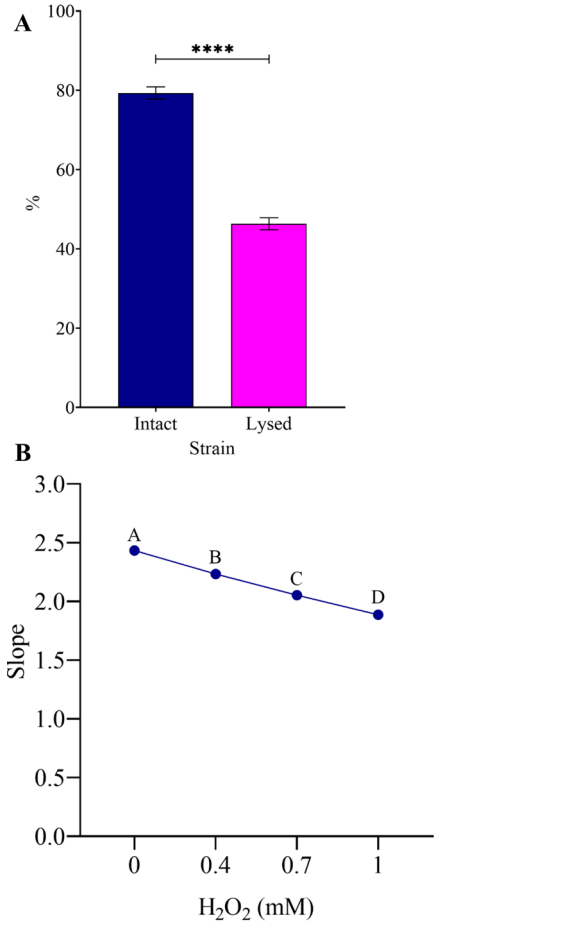

Figure 3. (A) Antioxidant activity of intact and lysed cells of *L. plantarum* and (B) the resistance of *L. plantarum* across varying concentrations of H2O2.

When we analyzed the variation in the concentration of divalent metal ions Mg and Mn during the FFD study, only Mn showed a significant increase in the *L. plantarum* UFSJP2 biomass, being used in an optimal concentration between 0.08 and 0.16 g L^{-1}. Studies have verified the importance of metal ions and highlighted their impact on lactobacilli growth (Colares et al., 2021; Hayek & Ibrahim, 2013). Divalent metal ions contributed to the growth of *Lactococcus lactis*, influencing the β oxidation system and the activity of *La. lactis* thioesterase (Li & Ma, 2014).

We achieved a high level of growth of *L. plantarum* UFSJP2 in this work, > 10 log CFU mL^{-1}, after the successful optimization of the bioprocess. After validating the models, we demonstrated an increase of 787% in biomass production compared to the non-optimized process, which was higher than that of other studies in the literature. The *Lac. casei* NCIM 5,752 produced 26.45% more biomass than the conventional MRS media (Nanjaiah et al., 2024). The optimized culture medium for different probiotics, such as *Lactobacillus acidophilus* CRL2074, *Lact. amylovorus* CRL2116, *Lact. mucosae* CRL2069, and *Lact. rhamnosus* CRL2084 found increases in CFU mL^{-1} of 259, 250, 372, and 148%, respectively, compared to the non-optimized RSM (Ficoseco et al., 2018). In contrast to this study, the optimal composition in paneer whey medium for *Lac. casei* NCIM 5,752 was 16.22 g L^{-1} of yeast extract, 19.31 g L^{-1} of dextrose, and 2.12 g L^{-1} of dipotassium hydrogen phosphate (Nanjaiah et al., 2024).

The disequilibrium between oxidant and antioxidant actions is responsible for oxidative stress. Despite the efficient antioxidant defense system in human health, ROS accumulation can exceed its intrinsic antioxidant capacity. To avoid damage to cells or tissue, external antioxidants, such as LAB strains, have been used to decrease oxidative stress (Kim et al., 2022). Assays have been conducted to determine the free-radical scavenging activity of probiotics and confirm the decrease in levels of DPPH (Kim et al., 2022; Rwubuzizi et al., 2023). The percentage of DPPH antioxidants in this study was similar to that observed for the study of Wang et al. (2009), with 80% in the presence of *L. plantarum* and better than for the study of *L. plantarum* C88, which presented 53.05% of DPPH antioxidants for intact cells (Li et al., 2012). The production of cell surface compounds, proteins, or polysaccharides is related to the antioxidant activity of some LAB strains (Hoffmann et al., 2021). Removing these cell surface compounds by lysed cells resulted in a significant decrease of the DPPH free-radical scavenging capacity, as observed in the decreased antioxidant activity to 44.31% at 10^{10} CFU mL^{-1} for *L. plantarum* C88 (Li et al., 2012).

Many strains of *Lacticaseibacillus* have been found to resist hydrogen peroxide to varying extents. *Limosilactobacillus fermentum* can survive with about 90% of viable cells after 4 h of incubation at 1.0 mM hydrogen peroxide (Wang et al., 2009). However, data in the literature demonstrate that the effect is species-specific. In the presence of 1.0 mM hydrogen peroxide, *Lac. casei* KCTC 3260 and *Lact. rhamnosus* GG were viable after 8 h of incubation, whereas the *Lac. casei* 01 strain did not demonstrate resistance to hydrogen peroxide (Lee et al., 2005).

To diversify the application of probiotics into non-dairy food, we evaluated the viability of the *L. plantarum* UFSJP2 strain in graviola sorbet. The preliminary organoleptic properties were not altered during the storage of graviola sorbet infused with the *L. plantarum* UFSJP2 strain, indicating the potential acceptance of this food matrix for probiotic delivery. The survival of probiotic cultures and their impact on the quality parameters of food products is dependent on many factors, such as the processing steps, food matrix, probiotic strain, form of probiotic addition, storage conditions, addition of prebiotic components, storage at low temperatures, and protection from the light, which play an important role (Marinho et al., 2019; Tripathi & Giri, 2014). The Jussara pulp sorbet with linseed mucilage and *Lac. casei* 01 demonstrated viability for 30 days without significant changes (Machado et al., 2021). In frozen Brazilian berries with *Streptomyces harbinensis* Ca12, the viability after 180 days demonstrated a reduction of only 1 log CFU mL^{-1} (Colares et al., 2021). The high levels of phenolic compounds in graviola are associated with the viability of probiotics by protecting them against oxidative stress, as demonstrated in in vitro studies and as shown in the study on Juçara and banana sorbet (Marinho et al., 2019). Moreover, phytochemistry analysis indicated that graviola is rich in flavonols triglycosides, and polyphenol compounds (Errayes et al., 2020; Pineda-Ramírez et al., 2020). The aggregated spherical structure observed in SEM may have a certain protective effect on the *L. plantarum* UFSJP2 grown in whey as observed for other LAB strains (Wang et al., 2024). The presence of organic material in the aggregate powder as a prebiotic contributed to the availability of the probiotic on the graviola sorbet, as demonstrated in other studies (Wang & Zhong, 2023).

5 CONCLUSION

The use of FFD and CCRD to study the components of supplementation of the whey for the *L. plantarum* UFSJP2-optimized bioprocess presented an approximately 787% higher yield when compared to whey without supplementation and biomass production of 1×10^{10} UFC mL^{-1}, making the bioprocess economically viable and sustainable, contributing to the bioeconomy. The probiotic strain demonstrated antioxidant activity. The graviola sorbet has been demonstrated to support viability for the *L. plantarum* UFSJP2 strain and could be an essential vehicle for a potential probiotic strain with antioxidant properties that benefits human health.

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