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ORIGINAL ARTICLE

Viability and resistance of *Lacticaseibacillus rhamnosus* GG to passion fruit beverages with whey protein isolate

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Abstract

This study characterized the beverage of passion fruit and whey protein added with *Lacticaseibacillus rhamnosus* GG (LGG). The acidity, pH, soluble solids, protein, phenolic compounds, and color of the sample did not differ during the 28 days at 5°C. The LGG counts in the beverage remained >7.70 log CFU/mL during the evaluation period. However, the LGG count was significantly reduced after the *in vitro* simulated gastrointestinal tract (GIT) evaluation (reduction of 5 logarithmic cycles after processing). On the other hand, the LGG resistance to GIT increased during the shelf-life of the beverage (LGG count of ~ 4.0 log CFU/mL after 28 days). In addition, the LGG count after GIT increased by approximately 2.0 logarithmic cycles when microencapsulation and fermentation technologies were used in beverage processing. Thus, the product has the potential to be commercialized, due to high viability of LGG use to increase the resistance of LGG to GIT.

Keywords: Functional food; Probiotic; Tropical fruit; *Passiflora edulis*; Microencapsulation; Fermentation.

Highlights

- *L. rhamnosus* GG did not affect the quality of the unfermented beverage
- Probiotic resistance to *in vitro* simulated GIT was increased during product storage
- Microencapsulation and fermentation increased probiotic resistance to *in vitro* GIT

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1 Introduction

Innovations in product development have increased the demand for foods with beneficial health properties, including disease prevention. Passion fruit, due to its characteristic flavor, is a fruit that draws attention when incorporated into beverages, as well as being rich in vitamin C, iron, calcium, phosphorus, and fiber (Casierra-Posada & Jarma-Orozco, 2016).

On the other hand, among the most consumed supplements based on protein is Whey Protein Isolate (WPI), mainly due to its content of essential amino acids and easy digestibility and absorption, resulting in rapid increase in lean mass and post-exercise recovery, also being able to generate economic returns for the industries that use it, due to its potential for the production of different bioproducts (Hayes & Cribb, 2008; Karim & Aider, 2022). WPI contains 80 to 95% protein, and it is considered one of the purest commercial forms of whey proteins, which have potential as a functional ingredient (Sharma, 2019). Therefore, there is a strong interest in the consumption of beverages containing WPI due to the implications for health outcomes (Wagoner & Foegeding, 2017).

In addition to the use of these proteins in fruit drinks, studies are also carried out with the addition of probiotic bacteria, which are live microorganisms that, when administered in adequate quantities, confer health benefit to the host (Hill et al., 2014). However, Zendeboodi et al. (2020) defined probiotic, in terms of healthy or beneficial microorganisms, as viable or inviable microbial cell (vegetative or spore; intact or ruptured) that is potentially healthful to the host.

The most important characteristics of probiotics are their ability to resist against gastric juice, bile salts and digestive enzymes, as well as having the ability to adhere to the intestinal mucosa, live with the endogenous intestinal microbiota and produce substances that inhibit the growth of undesirable bacteria, since these characteristics are being specific to each strain (Food and Agriculture Organization of the United Nations, 2002).

Probiotics and the foods that contain them are used to contribute to general well-being and are consumed for health promotion, especially digestive, immunological, and respiratory disorders (Rauch & Lynch, 2012; Park et al., 2018; Soldi et al., 2018; Wu et al., 2019). However, there are many challenges in incorporating these microorganisms in foods, especially regarding viability during processing, product storage and survival in the human Gastrointestinal Tract (GIT) (Martins et al., 2022). These adversities represent a challenge from the technological point of view, thus, aiming to overcome these limitations, different techniques, such as the use of fermentation, adaptation to stress, the incorporation of micronutrients and microencapsulation are necessary (Ramos et al., 2016; Prates et al., 2020). Among the probiotics, *Lacticaseibacillus rhamnosus* stands out for its viability and stability when used in fruits and dairy products (Moreira et al., 2017; Campos et al., 2019; Mitra & Ghosh, 2020).

Due to the strong interest in the consumption of beverages containing WPI (Wagoner & Foegeding, 2017) and natural fruits, in order to obtain combinations of functional ingredients that meet the needs of the consumer, this study aimed to, as a novelty, evaluate whether the fermentation of beverages containing WPI and passion fruit pulp or if the addition of microencapsulated *L. rhamnosus* GG is technologically advantageous in relation to the delivery of the free probiotic in the product. Besides, this work aimed to characterize the unfermented beverage, as well as determining the survival to *in vitro* simulated GIT when this probiotic bacterium was carried by the unfermented and fermented beverages, and in which the microorganism was added microencapsulated.

2 Material and methods

2.1 Design of the experiment

In the first stage, the unfermented beverage added by LGG containing passion fruit pulp and WPI was developed and characterized. In the second stage, the fermented beverage or the beverage containing

microencapsulated LGG was elaborated, aiming to verify if these methods could influence the LGG resistance to *in vitro* simulated GIT (Figure 1).

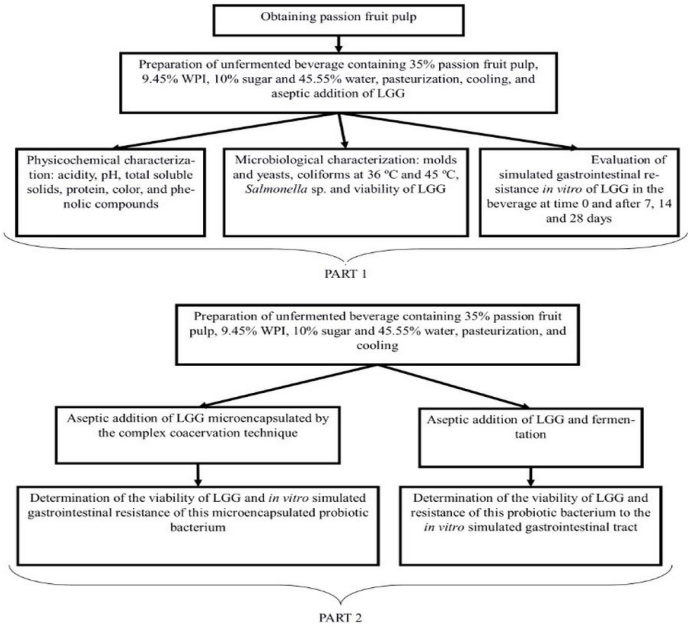

Figure 1. Study design for production and characterization of beverages containing passion fruit pulp and WPI.

2.2 Development and characterization of the unfermented beverage

2.2.1 Obtaining passion fruit pulp

Passion fruits were purchased in the commercial area of Rio Pomba, in the state of Minas Gerais (MG)/Brazil, when they reached the maturity stage and afterwards selected to separate fruits with deteriorated parts or in poor phytosanitary conditions. Then, the selected fruits were washed in running water to remove dirt and sanitized in water containing 100 mg/L of active chlorine, for 20 minutes to reduce the contaminating microbiota. Subsequently, the fruits were cut, and the pulp was removed with the aid of a stainless-steel spoon. The pulp was obtained using a low-speed blender (Philco PH 700) for ~ 1 min to prevent crushing the seeds, which were removed by filtration in two layers of cheesecloth. Then, the pulp was stored at -18 °C until use.

2.2.2 Preparation of unfermented beverage

For the beverage production, 35% of passion fruit pulp, 10% of sugar (sucrose), and 9.45% of WPI with 81.8% of protein without flavor (Proregi, Indústria, Comércio e Importação Ltda, Rio Pomba, Minas Gerais, Brazil) were used. After weighing the ingredients, 45.55% of water was added to complete the final mass of 200 g. The pulp, WPI, sugar and water were homogenized manually, and the prepared beverage was transferred to 200 g bottles and pasteurized at 65 °C for 30 min in a water bath. Subsequently, the product was cooled at 5 °C and the content of a capsule of ready-to-use lyophilized LGG probiotic culture (Culturelle®) containing 1.5 x 10^{10} cells was added to 200 g of beverage under aseptic conditions within a laminar flow (Thermo Fisher Scientific). Then, the beverage was homogenized manually and stored at 5 °C.

2.2.3 Determination of the physical-chemical quality of the unfermented beverage

The physical-chemical quality of the beverage was evaluated at time 0 (immediately after obtaining it) and at times 14 and 28 days of storage at 5 °C. Physico-chemical analyzes of titratable acidity (g lactic acid/100 mL

of product), pH, soluble solids and protein were performed according to Association of Official Analytical Chemists (2016). The surface color analysis of the beverages was evaluated using a Konica Minolta CR10 (Tecnal, BR) colorimeter. The color was determined by direct reading of the reflectance of the coordinates L*, a* and b* using the CIELAB L* scale adopted as standard by the International Commission on Illumination. To determine phenolic compounds, the Folin-Ciocalteu reagent (Sigma-Aldrich, Saint Louis, Missouri, USA) was used, according to the methodology described by Singleton et al. (1999). The results were expressed in mg of Gallic Acid Equivalents (GAE) per 100 g of sample (mg GAE/100 g).

2.2.4 Determination of the microbiological quality of the unfermented beverage

The microbiological quality of the beverage was determined on day 0 and day 28. The contaminating microbiota of the beverage was evaluated by the standard count of molds and yeasts (Beuchat & Cousin, 2001), of coliforms at 36 °C and 45 °C (Kornacki & Johnson, 2001) and by the evaluation of *Salmonella* spp. (Andrews et al., 2001). The viability of LGG in the beverage samples was determined immediately after manufacture, time zero (T0), and on days 7, 14, 21 and 28 of product storage at 5 °C. The viability of LGG in the samples was determined according to Richter & Vedamuthu (2001) in the de Man Rogosa Sharpe (MRS) culture medium (MRS, Neogen, USA).

2.2.5 Resistance to in vitro simulated GIT of LGG carried in unfermented beverage

The methodology described by Bedani et al. (2013) was used to simulate the resistance to the gastric, enteric I and enteric II phases of LGG carried in the unfermented beverage. This analysis was carried out after beverage elaboration (time 0), and after 7, 14 and 28 days of product storage kept at 5 °C. Initially to simulate the Gastric Phase (GP), 10 mL of each serial dilution of the beverage was transferred in triplicate to sterile bottle, and the pH adjusted to 2.3 - 2.6 with 1 M HCl (Impex, Diadema, SP, Brazil). Pepsin (from swine stomach mucosa, Sigma-Aldrich, St Louis, MO, USA) and lipase (from *Penicillium camemberti*, Sigma-Aldrich) were added to the 10 mL samples of the respective dilutions to achieve a concentration of 3 and 0.9 mg/L, respectively. Then, the bottles containing the samples were incubated at 37 °C using a 150 rpm shaker for 2 h. After this period, the same bottles were used for the next step. To simulate intestinal conditions (enteric I), the pH of the samples was increased to 5.4 - 5.7 using an alkaline solution containing 150 mL 1 M NaOH and 14 g $NaH_2PO_4.2H_2O$/L (Synth) in distilled water. Bovine bile (Sigma-Aldrich) and pancreatin (from swine pancreas, Sigma-Aldrich) were added to reach a concentration of 1 g/L and 10 g/L, respectively. After 2 h of samples incubation at 37 °C, the large intestine (enteral phase II) was simulated, where the pH was adjusted to 6.8 - 7.2 using the same 150 mL alkaline solution. Bovine bile and pancreatin (from pancreas, Sigma-Aldrich) were added to maintain the concentration. Then, the sample was incubated for 2 h at 37 °C, totaling 4 h for Enteric Phase (EP). The survival of *L. rhamnosus* GG was evaluated before GP and after GP and EP, using the plating method with MRS agar added according to Richter & Vedamuthu (2001). The result of the plate count was expressed in Log CFU/mL.

2.3 Development of the fermented beverage and the beverage containing LGG microencapsulated

2.3.1 Fermented beverage production

To produce the fermented beverage, after formulation, the product was pasteurized and cooled to 5 °C, as described in item 2.2.2. Subsequently, in 200 g of the same, 10^{10} cells of lyophilized LGG (Culturelle®) were added and incubated (Nova Ética 403 - 5D, Vargem Grande do Sul, São Paulo, Brazil) for 24 hours at 37 °C, characterizing the adaptation phase of the microorganism. After the incubation period, 10 mL of the pre-inoculum was inoculated into bottles containing 200 g of the beverage, which were incubated at 37 °C for 72 hours

for the fermentation of the product. After fermentation, the bottles were stored at 5 °C (Novatecnica NT 704, Piracicaba, São Paulo, Brazil).

2.3.2 Microencapsulation of LGG and addition to the unfermented beverage

2.3.2.1 Preparation of probiotic culture

The lyophilized culture of LGG was two times activated in 50 mL of MRS broth (Neogen, USA) at 37 °C (Nova Ética 403 – 5D, Vargem Grande do Sul, São Paulo, Brazil) for 24 h. Subsequently, it was transferred to 250 mL of MRS broth and incubated under the same conditions. Then, it was centrifuged for 10 min at 10,000 g in a refrigerated centrifuge at 4 °C (Thermo Scientific™, Heraeus™ Biofuge™ Stratos™, Germany). The culture medium supernatant was discarded and the LGG cell pellet was resuspended in saline solution for washing for two consecutive times. After the washing steps, 1.5 g of cells were obtained, which were stored at 5 °C (Novatecnica NT 704, Piracicaba, São Paulo, Brazil).

2.3.2.2 Production of microcapsules by complex coacervation

The production of microcapsules by complex coacervation was performed according to the methodology described by Alvim & Grosso (2010) and Silva et al. (2018), with modifications. Initially, the previously prepared LGG probiotic culture (1.5 g of cells) was added to 100 mL of sterile water containing 5 g of type B gelatin (Himedia Laboratories Pvt. Ltda, Mumbai, India) and kept under magnetic agitation (Lucadema, LUCA01/09, Brazil) at 40 °C for 25 min. Subsequently, 100 mL of gum arabic solution (5% at 40 °C) was slowly added to the mixture containing gelatin and probiotic cells, using a 1:1 ratio of biopolymers (gelatin: gum arabic). Then, 100 mL of sterilized water at 40 °C was carefully added and the pH was adjusted to 4.0 (Tecnopon NT PHM, Piracicaba, São Paulo, Brazil) with 1 M HCl (Labsynth produtos para laboratórios, Diadema, São Paulo, Brazil) under stirring at 40 °C. The resulting system was cooled to 10 °C with stirring and using an ice bath. The resulting material was stored at 7 °C for 2 h, to promote the settling of the microcapsules (coacervates), thus facilitating the removal of the aqueous phase. Soon after, the microcapsules were removed by filtration using sterile filter paper (Melitta^{R}) and stored for, approximately, 2 h at 7 °C until the preparation of the beverage. In addition, after preparation, microscopic images of the microcapsules containing probiotic were obtained. For this, a drop of the 10-fold diluted sample was placed on a microscope slide, carefully covered with a glass coverslip, and images were visualized and photographed (Canon, Poxer Shot A620) after optical microscopically magnification with an objective lens of 4x and 100x (Anatomic Opton®, Model TIM-18, Brazil) (Paula et al., 2019).

To produce the unfermented beverage, after formulation, it was pasteurized and cooled to 5 °C, as described in item 2.2.2. Subsequently, microcapsules were added until, approximately, 8 log CFU/mL of LGG was obtained into the beverage.

2.3.3 Determination of viability and resistance to in vitro simulated GIT of LGG

The viability of the probiotic bacterium in the fermented beverage and in the beverage containing LGG microencapsulated was determined at time zero (immediately after processing) as described in item 2.2.4. The evaluation of resistance to the GIT of LGG in these beverages was conducted using an *in vitro* model at zero time (immediately after processing) as described in item 2.2.5.

2.4 Statistical analysis

In the experiments of physical-chemical characterization (acidity, pH, total soluble solids, protein, and color) and of the viability of LGG in the unfermented beverage, a completely randomized design was used.

To evaluate the resistance to *in vitro* simulated GIT of LGG in unfermented beverage, a 3x4 factorial design was used, with three phases (before GP, after GP and after EP) and four storage times (0, 7, 14 and 28 days). A 3x3 factorial design was also used, with three phases (before GP, after GP and after EP) and three treatments (unfermented beverage, fermented beverage and beverage containing LGG after microencapsulation) to evaluate the resistance to *in vitro* simulated GIT of LGG in beverages at time 0.

The results obtained were submitted to an Analysis of Variance (ANOVA) and Tukey's test for comparisons between the means. All experiments were carried out in three repetitions and the statistical procedures were carried out considering a 5% level of probability and using STATISTICA 13.0 software (Tibco Software Inc, 2017).

3 Results and discussion

3.1 Physical-chemical quality of the unfermented beverage

There was no difference ($p > 0.05$) in the values of acidity, pH, Total Soluble Solids (TSS) and protein in the unfermented beverage during the 28 days of storage at 5 °C. After the beverage production was observed acid values of 2.16 ± 0.16% lactic acid, pH of 3.99 ± 0.14, TSS of 22.9 ± 0.53 °brix, and protein of 7.79 ± 0.19%. After 28 days of storage, these values were 2.23 ± 0.10% of lactic acid; 3.85 ± 0.13; 22.3 ± 0.78 °brix; 7.96 ± 0.15%, respectively. Therefore, storage under refrigeration was efficient to avoid a probiotic fermentative action. This was expected since this microorganism is not psychotropic.

For the color parameters (L*, a* and b* coordinates), there was also no difference in the unfermented beverage during the storage time ($p > 0.05$), with L* values of 40.73 ± 0.63, a* of 4.45 ± 0.52, b* of 28.51 ± 1.15 after production and after 28 days these values were 41.36 ± 1.99 for L*, 4.73 ± 0.53 for a* and 29.88 ± 2.15 for b*, respectively. Therefore, the addition of LGG also did not change the color of the unfermented beverage, as it had already been verified in other works (Campos et al., 2019; Montanari et al., 2020). In general, the unfermented beverage tended to be yellow due to the addition of the passion fruit pulp (evidenced by the high b* values).

The mean concentration of phenolic compounds in the unfermented beverage was 129.6 mg GAE/100 g after processing and remained constant throughout the 28 days of product storage at 5 °C ($p > 0.05$), which can improve the ability of probiotics to adhere and survive during exposure to GIT.

3.2 Microbiological quality of unfermented beverage

The beverage had < 1.0 x 10^1 CFU/mL of molds and yeasts, < 3.0 MPN/mL of coliforms and absence of *Salmonella* in the unfermented beverage on Day 0 and Day 28, which demonstrated the hygienic conditions of the manipulators and the efficiency of the heat treatment.

The average counts of LGG in the beverage remained above 7.70 log CFU/mL throughout the 28 days of refrigerated storage (Figure 2). The results obtained are in line with international standards for probiotic products, since these foods must have a minimum count of >10^6 CFU/mL (Hussain et al., 2016; Martins et al., 2022). In addition, according to the literature, counts between 10^6 and 10^8 of viable probiotic cells must reach the intestinal colon to exert the therapeutic effect (Martins et al., 2013; Hussain et al., 2016).

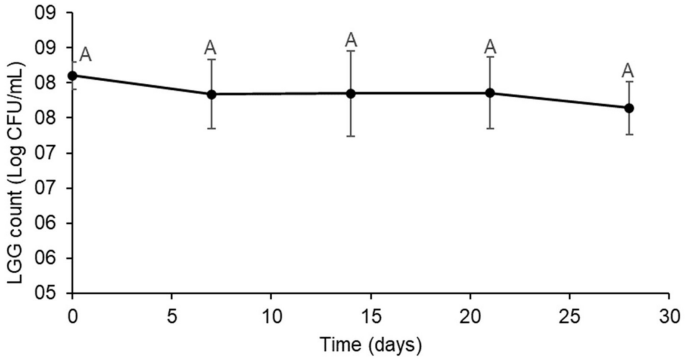

Figure 2. Viability of LGG in the unfermented beverage over time. Different capital letters indicate a significant difference evaluated by the Tukey's test ($p < 0.05$) for unfermented beverage evaluated over time.

Thus, the beverage developed using passion fruit pulp and WPI can be considered a promising vehicle for probiotic bacteria (Figure 2). The viability of the probiotic in food products is dependent on the strain and is affected by associated microorganisms, culture conditions, food composition, processing, final pH, dissolved oxygen, and storage temperature. Regarding the survival of *Lactobacillus*, the most important limiting factors are low pH, low temperatures, and the presence of organic acids (Martins et al., 2022). In the present study, it was found that these factors did not affect the LGG viability.

However, Buriti et al. (2007) found that the viability of *Lactobacillus acidophilus* decreased by up to 4.7 log CFU/g on the 21st day in mousses containing passion fruit. Thus, the addition of fruits to probiotic products should be carefully planned since there may be inhibition of the strains.

3.3 *In vitro* resistance of LGG to the GIT when carried in the unfermented beverage

Regarding the *in vitro* assay of LGG resistance to GIT when carried in the unfermented beverage, there was no reduction in the count of this microorganism after the GP, regardless of the evaluated time ($p > 0.05$). However, after the EP, a reduction in the count was observed at all evaluated times ($p < 0.05$) (maximum reduction of ~ 5 logarithmic cycles) (Figure 3).

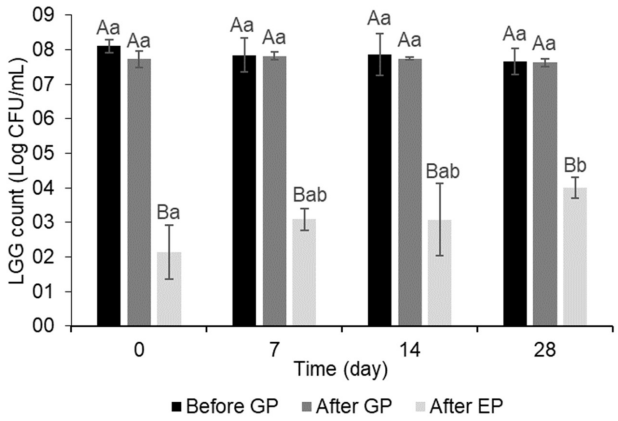

Figure 3. *In vitro* resistance of LGG to the GIT when carried in the unfermented beverage during 28 days of storage at 5 ºC. GP: Gastric Phase and EP: Enteric Phase. Different capital letters indicate a significant difference evaluated by the Tukey's test ($p < 0.05$) for unfermented beverage evaluated at different phase and same time. Different lowercase letters indicate a significant difference evaluated by the Tukey's test ($p < 0.05$) for unfermented beverage evaluated at different time and same phase.

In addition, the LGG count remained constant after the GP during the shelf-life of the beverage ($p > 0.05$). On the other hand, there was an increase in the LGG count after the EP during the storage period ($p < 0.05$),

with a count of 4 log CFU/mL after EP when the test was carried out after 28 days of product storage (Figure 3). This demonstrated an increase in LGG resistance to the GIT. This increase may be correlated with several factors. Bacterial cells naturally have many defense mechanisms to increase survival in hostile environments (Fiocco et al., 2020). Natural adaptation to the intrinsic characteristics of fruits can help lactic acid bacteria to survive during processing and storage, as well as being exposure to stressful conditions imposed during the GIT (Garcia et al., 2016). As an adaptive response, they express specific genes that allow them to survive and multiply under stress conditions (Hlaing et al., 2018; Prates et al., 2020), which were imposed on LGG in the developed beverage and during product storage.

There are some limitations that can prevent the production of non-dairy probiotic beverages at the industrial level, such as survival of probiotics during storage (Perricone et al., 2015). Madureira et al. (2011) considered that at least 10^6 to 10^7 of viable probiotic cells must reach the colon for the food to have a therapeutic effect. Therefore, based on results obtained, the ingestion of 100 mL of the beverage after 28 days of storage can provide to consumers an amount of LGG cells (10^6 CFU) sufficient to exert a possible therapeutic effect.

3.4 Viability and in vitro resistance of LGG to the GIT when carried in the fermented beverage and in the beverage added of this microencapsulated bacterium

After the production of beverages, the LGG viability in the fermented beverage (8.08 log CFU/mL) was similar to the viability in the beverage with the microencapsulated microorganism (7.32 log CFU/mL) ($p > 0.05$). In addition, these counts showed no difference compared to the unfermented beverage ($p > 0.05$) (Figure 4).

After the gastric phase, there was a reduction ($p < 0.05$) in LGG survival only in the treatment that used the microencapsulation technique (Figure 4). Similarly, Bosnea et al. (2014) observed a reduction of ~ 4.0 log CFU/g for *Lactobacillus paraplantarum* in microcapsules produced from WPI and gum arabic by complex coacervation. Silva et al. (2018), when encapsulating *Bifidobacterium* Bb-12 using gelatin and gum arabic by complex coacervation, also observed a reduction of ~ 4.16 log CFU/g for free cells and of ~ 6.56 log CFU/g for microcapsules.

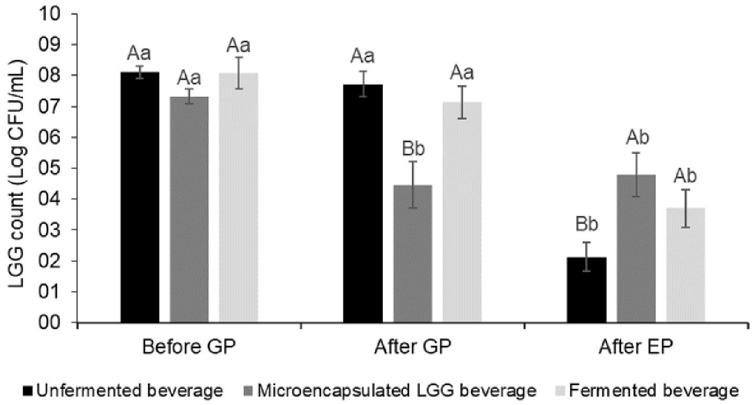

Figure 4. *In vitro* resistance of LGG to the GIT when carried in the unfermented beverage and the beverages submitted to the microencapsulation and fermentation processes immediately after processing. GP: Gastric Phase and EP: Enteric Phase. Different capital letters indicate a significant difference evaluated by the Tukey's test ($p < 0.05$) for different beverages at same phase. Different lowercase letters indicate a significant difference evaluated by the Tukey's test ($p < 0.05$) for different phases in the same beverage.

In the complex coacervation method, the microcapsules formed may be of the matrix type where the encapsulated material is distributed throughout the particle volume, including on the surface (Silva et al., 2018, 2019). In this case, the LGG cells are in the central region and on the surface of the microcapsules

(Figure 5). Thus, part of the cells may be exposed to acidic conditions and, as a result, they may have suffered the action of acid stress of the gastric phase, which limited their recovery. Etchepare et al. (2020), when encapsulating *L. acidophilus* in particles coated with up to three multilayer levels, found that the greater the number of layers, the lower the exposure of probiotic cells and, with that, obtained greater cell viability during storage, heat treatments and gastrointestinal conditions. Silva et al. (2019) found that crosslinked microcapsules with transglutaminase offered greater protection to cells, especially against gastric conditions, releasing cells only in the duodenum and maintaining high viability until the end of the process, demonstrating the relevance of the use of crosslinking.

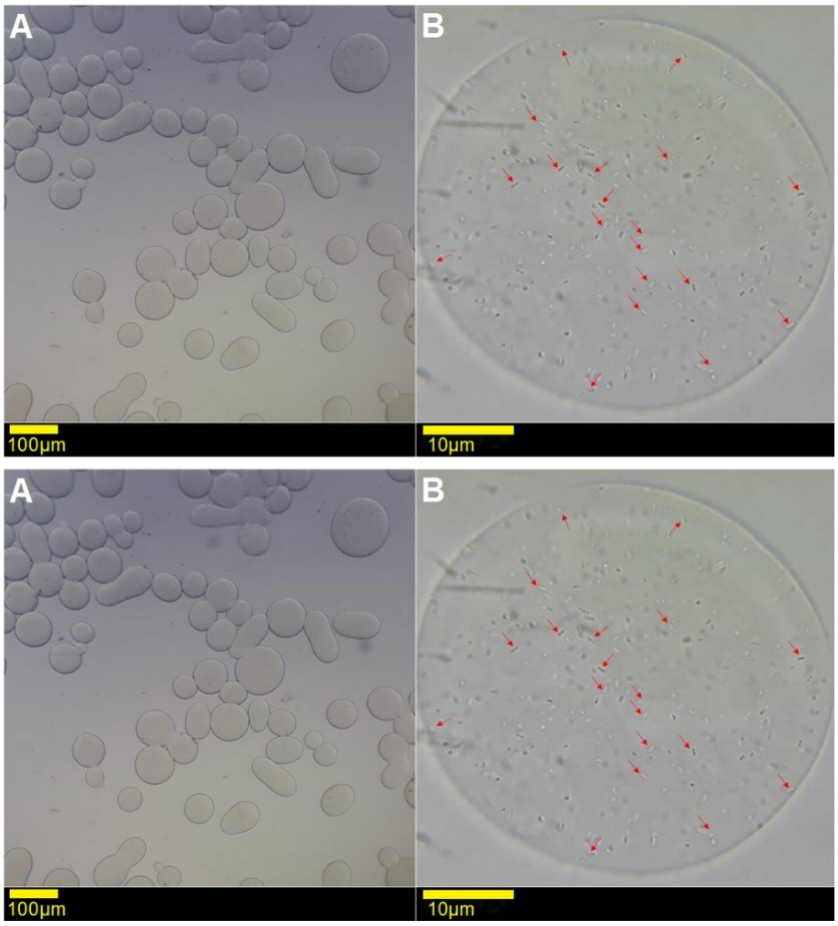

Figure 5. Microscopic observation (4x (A) and 100x (B) magnification) of the microcapsules containing *Lactobacillius rhamnosus* GG. The images were obtained after the microcapsules production. Red arrows indicate the cells of LGG were detected in the central region and on the surface of microcapsules. The scale bars are 100 μm (A) 10 μm (B) in length.

However, in the unfermented beverage and the one that passed by fermentation, the number of survivors in the gastric phase was greater than 7.0 log CFU/mL (Figure 4). Probiotic cells can express specific genes that allow them to survive and multiply under stress conditions (Hlaing et al., 2018), which were imposed on LGG in the developed beverage. In addition, WPI may have provided protection for probiotic cells by increasing the tolerance to gastric juice in fermented and unfermented beverages. However, in the beverage with microencapsulated cells due to the formation of a barrier imposed by the gelatin and gum arabic biopolymers, the cells were not subjected to this protection. Vargas et al. (2015) in a study with pure cultures of *Streptococcus thermophilus* ST-M5 and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 found that the use of WPI significantly increased cell viability under gastric acid conditions and bile salts.

The count of LGG at the end of enteric phase increased by approximately 2.0 log cycles when microencapsulation and fermentation technologies were used compared to unfermented drink ($p < 0.05$) (Figure 4). Due to the higher pH conditions (pH > 5,0) in the enteric phase, the gelatin and arabic gum biopolymers that formed the microcapsule presented predominantly negative electrical charges, favoring the repulsion between them and, with this, consequent rupture, and release of probiotic cells (Paula et al., 2019). Therefore, the microcapsules may have provided protection and release of the cells, thus explaining the higher counts in relation to the cells present in the unfermented beverage. Similar results were obtained by Silva et al. (2018), when these authors observed in the final phase of the *in vitro* gastrointestinal simulation the microencapsulated cell count of 7.77 log CFU/g whereas in the gastric phase the count obtained was 5.89 log CFU/g demonstrating that, despite the initial cell reductions, microcapsules were effective in protecting probiotics.

Regarding the fermented beverage, the technology used may have induced a response to the stress suffered by the microorganism in the product. According to Pereira et al. (2017), fermentation has some advantages, including the growth of more adapted microbial cells, which can contribute to higher survival rates. Campos et al. (2019), when evaluating the viability of LGG and its resistance *in vitro* and *in vivo* to the GIT when transported by a mixed fermented juice of pineapple and juçara palm, found that the formulated juice was an excellent matrix carrier for probiotic cells.

According to the results of this study, it was verified that the ingestion of 100 mL of the fermented beverage or containing microencapsulated probiotic cells can provide consumers with 10^6 log CFU immediately after its elaboration (Figure 4) in contrast to the unfermented beverage which such result is obtained only after 28 days of storage (Figure 3). Thus, the use of microencapsulation and fermentation technologies may have protected the cells or induced a response to the stress suffered by the microorganism in the products, respectively. Therefore, they are promising for use in the development of beverages added with probiotic bacteria, but there is a need of performing sensory studies in a further research. Lima et al. (2022) highlighted that the importance of generating rapid/easy consumer perception of a new product is an urgent need, and Torres et al. (2020) suggested identify consumer needs and expectations, which may assist in developing a new brand, *i.e.*, more competitive products.

4 Conclusions

The addition of LGG to the unfermented beverage did not cause physical-chemical changes in the product during storage. The beverage also had satisfactory microbiological quality and good viability of LGG. However, it can be potentially used as probiotic solely after 28 days of storage and the ingestion of at least 100 mL since the *in vitro* resistance of LGG to GTI is satisfactory in this amount.

The count of LGG at the end of the enteric phase in beverages subjected to microencapsulation and fermentation technologies was superior in approximately 2.0 logarithmic cycles compared to the unfermented beverage, which indicates that they are promising for use in the development of new probiotic beverages.

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