RECyT
Year 27 / Nº 43 / 2025 /
DOI: https://doi.org/10.36995/j.recyt.2025.43.006

Chemical Stability, Viability, and *In Vitro* Gastrointestinal Resistance of the Probiotic *Bacillus* in Organic Mango Juice

Estabilidad Química, Viabilidad y Resistencia Gastrointestinal *In Vitro* del Probiótico *Bacillus* en Jugo de Mango Orgánico

Estabilidade Química, Viabilidade e Resistência Gastrointestinal *In Vitro* de *Bacillus* Probiótico em Suco de Manga Orgânico

Peron, Taciane[1, *]; dos Santos, Ramon Araújo[1, 4]; Lopes Martins, Maurílio[1]; de Castro Leite Júnior, Bruno R.[2]; Almeida do Nascimento Benevenuto, Wellingta C.[1]; de Souza Viana, Eliseth[3]; Furtado Martins, Eliane Maurício[1]

1- Federal Institute of Education, Science and Technology of Southeast Minas Gerais -IFSUDESTEMG, Rio Pomba, Minas Gerais, Brazil.
2- Federal University of Viçosa - UFV, Viçosa, Minas Gerais, Brazil.
3- Brazilian Agricultural Research Corporation - EMBRAPA, Cruz das Almas, Bahia, Brazil.
4- Federal Institute of Education, Science and Technology of Rio Grande do Norte - IFRN, Currais Novos, Rio Grande do Norte, Brazil.
* E-mail: taciane.peron@gmail.com

Received: 22/10/2024; Accepted: 17/03/2025

Abstract
Probiotic juices must ensure their survival after processing and in the gastrointestinal tract to achieve their benefits. This study was divided into two stages. Initially, the objective was to evaluate the pH and viability of *Bacillus clausii* and *Bacillus coagulans* BC30 in organic mango juices subjected to heat treatment and, subsequently, to simulated accelerated shelf-life conditions. In the second stage, the pH, acidity, total soluble solids, the viability of *B. clausii,* as well as its resistance to the *in vitro* gastrointestinal tract (GIT) digestion were evaluated. In the first stage, at all analyzed times, the juice with *B. clausii* presented a lower pH than the juice with *B. coagulans*. The *B. coagulans* count decreased during the accelerated shelf-life test, whereas the *B. clausii* count remained stable. In the second stage, the lowest count of *B. clausii* was observed in the juice subjected to simulated industrial heat treatment (96 °C/35 s). However, as regards the other treatments (non-heat-treated and 80°C/10 min), the probiotic count was > 10^6 CFU/mL. An excellent performance of *B. clausii* was found in the juice that was not heat-treated and stored at different temperatures (5°C and 25°C) after the GIT test, with counts of > 10^7 CFU/mL.

Keywords: tropical fruit juice, *Bacillus clausii,* shelf life, thermal treatments, resistance to i*n vitro* gastrointestinal digestion.

Resumen
Los jugos probióticos deben asegurar su supervivencia después del procesamiento y en el tracto gastrointestinal para lograr sus beneficios. Este estudio se dividió en dos etapas. Inicialmente, el objetivo fue evaluar el pH y la viabilidad de *Bacillus clausii* y *Bacillus coagulans* BC30 en jugos de mango orgánico sometidos a tratamiento térmico y, posteriormente a condiciones de vida útil acelerada. En la segunda etapa, se evaluó el pH, la acidez, los sólidos solubles totales, la viabilidad de *B. clausii* y, su resistencia a la digestión *in vitro* en el tracto gastrointestinal (TGI). En la primera etapa, en todos los tiempos analizados, el jugo con *B. clausii* presentó un pH menor que el jugo con *B. coagulans*. Durante la prueba de vida útil acelerada, el recuento de *B. coagulans* disminuyó, mientras que el recuento de *B. clausii* se mantuvo estable. En la segunda etapa, el recuento más bajo de *B. clausii* se observó en el jugo sometido a tratamiento térmico industrial simulado (96 °C/35 s). Sin embargo, en cuanto a los otros tratamientos (sin tratamiento térmico y 80 °C/10 min), el recuento fue > 10^6 UFC/mL. Se observó un excelente desempeño de *B. clausii* en el jugo que no fue tratado térmicamente y almacenado a diferentes temperaturas (5 °C y 25 °C) después de la prueba del tracto gastrointestinal *in vitro*, con recuentos > 10^7 UFC/mL.

Palabras clave: jugo de frutas tropicales, *Bacillus clausii*, duración, tratamientos térmicos, resistencia a la digestión gastrointestinal *in vitro.*

Resumo
Os sucos probióticos devem garantir sua sobrevivência após o processamento e no trato gastrointestinal para obter benefícios. Este estudo foi dividido em duas etapas. Inicialmente, objetivou-se avaliar o pH e a viabilidade de *Bacillus clausii* e *Bacillus coagulans* BC30, em sucos orgânicos de manga submetidos a tratamento térmico e

posteriormente expostos a condições de vida de prateleira acelerada. Na segunda etapa foram avaliados o pH, acidez, sólidos solúveis totais, viabilidade de *B. clausii* e sua resistência ao trato gastrointestinal *in vitro* (TGI). Na primeira etapa, em todos os tempos analisados, o suco com *B. clausii* apresentou pH menor que o suco com *B. coagulans*. A contagem de *B. coagulans* diminuiu ao longo do teste de vida de prateleira acelerada, enquanto a contagem de *B. clausii* permaneceu estável. Na segunda etapa, a menor contagem de *B. clausii* foi observada no suco submetido ao tratamento térmico industrial simulado (96 °C/35 s). Porém, para os outros tratamentos (não tratado termicamente e 80 °C/10 min), a contagem foi > 10^6 UFC/mL. Excelente resultado de *B. clausii* foi encontrado em suco não tratado termicamente e armazenado em diferentes temperaturas (5 e 25 °C), após ensaio *in vitro* do trato gastrointestinal, com contagens > 10^7 UFC/mL.

Palavras-chave: suco de frutas tropicais, *Bacillus clausii,* vida de prateleira, tratamentos térmicos, resistência à digestão gastrointestinal *in vitro*.

Introduction

Non-alcoholic ready-to-drink beverages have a considerable effect on the worldwide economy since they provide not only hydration to people, but also pleasure from consumption. In this context, to supply the demands from consumers, the industry began to develop formulae with the addition of functional ingredients and/or reduction or remotion of undesirable ingredients, with satisfying prices and flavors (1). Therefore, investing in the development of juices and beverages based on fruits enriched with healthy ingredients is important, since this factor has conditioned the consumer's purchase decision over the last few years.

The incorporation of probiotics in fruit juices to produce functional and nutraceutical food is notable for its potential to control lifestyle-related diseases, and increase the availability of food not only for lactose-intolerant populations, but also for vegetarians and those with certain dietary restriction (2,3).

Mango juice is rich in minerals and a natural source of vitamins, dietary fiber, phenolic, and photochemical compounds. It may also be an excellent alternative to the addition of probiotics. However, the development of these products involves shelf life-related studies, which are a challenge to science, but also, fundamentally, to the food industry. Thus, the accelerated shelf-life test is a quicker and more practical evaluation carried out at an industrial scale, aimed at simulating the product's storage under extreme temperature conditions. The incidence of both temperature and light is the main factor contributing to deterioration and is the most suitable option for this process (4).

Concerning this matter, the formulations must supply an adequate number of living probiotics (> 10^6 CFU/g or mL) and ensure their survival in the gastrointestinal environment to reach the desirable benefits (5). The spore-forming probiotic bacilli can be delivered in the human intestine through new products because the spores can bear high-temperature environments, acid conditions, and salinity. This makes them viable for the production of new functional food, thanks to the probiotic features of certain species and their great resistance to intrinsic and extrinsic stress conditions (6).

For that reason, the objective of this study was to assess the pH and evaluate the viability of *Bacillus clausii* and *Bacillus coagulans* BC30 probiotics in organic mango juice subjected to thermal treatment, which simulated the industrial process during the accelerated shelf-life test. Moreover, the evaluation of physical and chemical quality, the viability of *B. clausii,* and its resistance in the *in vitro* gastrointestinal tract when transported in organic mango juice was carried out. These evaluations were done using various thermal treatments during a 60-day storage period, under both ambient and refrigeration temperatures.

Materials and Methods

This research was carried out in two stages. In the first, there was simulation of thermal treatment used industrially to assess the accelerated shelf life of juices. In the second stage, a comparison between the simulated industrial thermal treatment and the proposed alternative for the product was made.

Addition of *Bacillus clausii* and *Bacillus coagulans* BC30 to Organic Mango Juices

Organic mango juices sold in glass bottles of 290 mL each were used, with the product consisting of organic mango pulp (52%), water, organic sugar, and citric acid.

In the first stage, after cleaning the packaging, the juices were homogenized and aseptically opened. Then, the juices were sampled in 100 mL to be transferred into sterile bottles, to which 5 mL of *B. clausii* (Enterogermina®, Italy) containing 4.0 x 10^9 spores was added to obtain 10^7 viable spores. The 100 mL juice sample from the bottles was homogenized, and the product was distributed in sterile threaded tubes, with 10 mL per tube.

To add *B. coagulans* BC30 (GanedenBC$^{30®}$), 10 g of probiotic powder containing 1.0 x 10^9 spores for 100 mL of juice was used to obtain 10^7 viable spores, which were then homogenized and also distributed in sterile threaded tubes of 10 mL each. In the juices obtained from the first stage, there was an application of 96 ± 3°C/35 seconds of thermal treatment was applied to simulate the time and temperature used by the industry.

In the second stage, *B. clausii* (Enterogermina®, Italy) was selected for continuity of the experiments and, as mentioned above, 10^9 variable spores were added to the juices. After homogenizing the juice in the bottle, the product was distributed in sterile threaded tubes of 10 mL each, which were divided and identified as untreated and heat-treated juices for two time-temperature binominal (96 ± 3°C/35 seconds and 80 ± 1 °C/10 minutes). At the end of the heat treatments, half of the samples from both treatments were stored at 25°C, and the other half were stored under refrigeration (5°C) for 60 days.

Thermal Treatments Used

Industrial Thermal Treatment Simulation

To simulate industrial thermal treatment, with temperature variation, a water bath was used to reach the temperature of 96°C along with the COD digestive block (SL20 - SOLAB, Piracicaba, São Paulo, Brazil) for cooling at 85°C and 70°C. Then, after adding probiotics to the juices, the latter underwent pasteurization, with the time-temperature binomial similar to those described by Kunitake *et al*. (7). The pasteurization was carried out at 96 ± 3 °C/35s, with subsequent cooling at 85 °C/2.5 min and 70 °C/2.5 min. After reaching 70°C, juices were cooled to 25°C in cold water (± 8 °C), simulating the time and temperature used in the industrial process.

At the end of the first stage, juice samples were stored in an oven at 37ºC to perform pH analysis and the assessment of probiotics used over the course of six days at 37ºC to accelerate chemical degradation and/or physical changes in the product, characterizing the accelerated shelf-life test. Non-thermally treated juices were made from the product added with *B. coagulans* or *B. clausii* that were not subjected to thermal treatment.

On the other hand, at the end of the second stage, the juice samples were stored at 25ºC and under refrigeration at 5ºC for 60 days. During this time, physical chemical analysis was conducted along with the assessment of *B. clausii* spore viability in thermally treated and non-treated juices.

Alternative thermal treatment to the industrial one

This treatment was only used in the second stage. After adding probiotics to the juices, these underwent thermal treatment for 10 minutes with subsequent cooling to 25°C in cold water, similar to the one performed by Faraoni *et al.* (8). To reach the temperature of 80°C, a digesting block (SL20 – SOLAB, Piracicaba, São Paulo, Brazil) was used. The time count for the thermal treatment began when the bottles reached 80°C, using a bottle to control the temperature with a thermometer. The average time to reach the temperature of 80ºC was 10 minutes and 10 seconds (± 2 minutes).

After the thermal treatment, physical-chemical analyses were performed. Moreover, the determination of the viability of *B. clausii* spores in the juices was carried out, with subsequent storage of the samples at 25°C and under 5°C refrigeration, over the lapse of 60 days. Non-thermally treated juices added with *B. clausii* were also analyzed and stored under the same conditions.

Physical Chemical Evaluation of Juices

The determination of the pH was determined using a digital pH meter (Tecnopon NT PHM, Piracicaba, São Paulo, Brazil) (9). On the first stage, the pH was determined over the course of the six days of storage at 37 °C. The analyses were performed on days 0 and 6 for the juices not subjected to thermal treatment, and on days 0, 2, 4, and 6 days for the thermally treated juices.

In the second stage, the analysis of pH, acidity and total soluble solids (ºBrix) was performed on days 0, 20, and 60 for both non-treated juice samples and those thermally treated (industrial thermal treatment and alternative one), stored at 25°C and under refrigeration at 5°C.

The acidity was assessed by potentiometric volumetry and converted to citric acid percentage. A mixture of 10 g of the sample, dissolved in 100 mL of distilled water, was titrated with a standard solution of NaOH 0.1 mol/L (Impex, Diadema, São Paulo, Brazil) until reaching the pH value of 8.2–8.4, which is equivalent to the turning point of phenolphthalein (10).

The total soluble solids were analyzed by refractometry using a digital refractometer (Atago®, Ribeirão Preto, São Paulo, Brazil) according to manufacturer's instructions, and methodology described by Godshall (11).

Viability of *B. clausii* and *B. coagulans* BC30 in Organic Mango Juices

To analyze probiotic viability, a serial dilution was performed using 1 mL of homogenized mango juice in 9 mL of peptone saline solution, containing 0.85% of NaCl (Synth, Diadema, São Paulo, Brazil), and 0.1% of peptone (Acumedia, Michigan, USA), thus obtaining a 10^{-1} dilution. Serial dilutions (10^{-1} a 10^{-6}) were plated using the pour plate technique, with the addition of 1 mL of each dilution, and about 20 mL TSA in Petri plates. The plates were kept at 37°C for 72 hours, and after the incubation, colony-forming units (CFU) were calculated.

The viability assessment of *B. coagulans* BC30 and *B. clausii* in first-stage juices was performed on days 0 and 6 for non-thermally treated juices and on days 0, 2, 4, and 6 for thermally treated ones.

In the second stage, the counting of *B. clausii* was determined on days 0, 30, and 60 of storage at 25°C and 5°C for juice samples that were not thermally treated and for those thermally treated (industrial thermal treatment and the alternative one, respectively, under 96 ± 3 °C/35 s and 80 ± 1 °C for 10 minutes).

Evaluation of *B. clausii* Resistance to Simulated *In Vitro* Gastrointestinal Digestion

The *in vitro* simulation of gastrointestinal conditions was carried out in non-thermally treated mango juices from the second stage. It was performed according to the methodology described by Bedani, Rossi, and Saad (12) on days 0 (after addition of *B. clausii* to the juice), 30, and 60 of storage at 25°C and under refrigeration at 5°C. Initially, the gastric phase was simulated by preparing a 10^{-1} dilution of the sample in 0.85% saline solution, using 10 mL aliquots transferred to 100 mL dilution bottles. The pH was adjusted to 2.0 – 2.5 with 1 mol/L HCl (Impex, Diadema, São Paulo, Brazil), 3 g/L pepsin (isolated from porcine gastric mucosa, Sigma-Aldrich), and 0.9 mg/L of lipase (G Amano lipase, isolated from *Penicillium Camemberti*, Sigma-Aldrich), respectively. Then, all samples were incubated at 37°C for 2 at 150 rpm in a shaking incubator (Tecnal TE – 424, Piracicaba, São Paulo, Brazil).

After the incubation period, enteric stage I (corresponding to the small intestine) was simulated, in which the pH was adjusted to 4.5 – 5.5, using a pH 12.0 sodium phosphate solution. This solution was prepared from a mixture containing 150 mL NaOH 1 mol/L (Vetec, Duque de Caxias, Rio de Janeiro, Brazil), 14 g $NaH_2PO_4.2H_2O$ (Vetec, Duque de Caxias, Rio de Janeiro, Brazil) and distilled water until it reached 1000 mL, along with bile (bovine bile, Sigma-Aldrich) and pancreatin (porcine pancreas isolated pancreatin, Sigma-Aldrich), at concentrations of 10.0 g/L and 1.0 g/L, respectively. The bottles were incubated again for 2 hours, under the conditions previously described.

Afterward, enteric stage II (corresponding to the large intestine) was simulated, adjusting the pH to 6.8 – 7.2, using the same alkaline solution containing bile (10.0 g/L) and pancreatin (1.0 g/L). Subsequently, a third incubation period was conducted under the same conditions, resulting in an assay duration of six hours. At the end of each incubation cycle (2, 4, and 6 hours), which corresponded to the simulation of gastric and enteric stages I and II, a deep plating on TS was performed to assess probiotic viability. The Petri plates were incubated at 37 ± 1ºC for 72 hours, and the results of the assessment were measured in log CFU/mL.

Statistical analyses

The procedures were carried out in three independent repetitions, and the results obtained from them were expressed as the mean ± standard deviation. The unidirectional analysis of variance (ANOVA) was performed to compare the effects of the different treatments. The Tukey test was used to assess significant differences between the treatments at a 5% significance level ($p < 0.05$). The statistical analyses were carried out using the software Statistica (Statistical Analysis System - SAS Institute, Cary, NC, USA; version 9.2).

Results and Discussion

ph And Viability of *B. Clausii* and *B. Coagulans* in Organic Mango Juice after Thermal Treatment at 96 °C/35 s throughout 6 Days of Accelerated Shelf Life at 37°C

There was a difference in pH between juices containing *B. clausii* and *B. coagulans* ($p < 0.05$) for each analyzed time during the accelerated shelf-life test. However, no significant pH difference was observed ($p > 0.05$) in juices that underwent the same treatment during the storage time (Fig. 1). The juices containing probiotics and not thermally treated also did not present pH variation ($p > 0.05$) between day 0 (*B. clausii* 3.93 ± 0.12; *B. coagulans* 4.49 ± 0.03) and day 6 (*B. clausii* 3.92 ± 0.08; *B. coagulans* 4.50 ± 0.18).

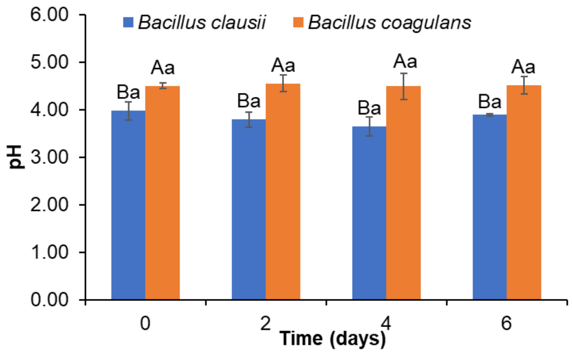

Fig 1. pH of organic mango juice after heat treatment at 96 °C/35 s during six days of accelerated shelf life at 37°C. Different uppercase letters indicate a significant difference between samples at the same time, while different lowercase letters indicate a difference between times for the same sample, using Tukey's test at a 5% probability level ($p < 0.05$).

These results demonstrate probiotic mango juices' pH stability throughout their accelerated shelf life, under extreme storage conditions, which indicates that the addition of probiotic bacilli is viable. Furthermore, this technique is believed to enable a simulation study under storage conditions similar to those that these products would undergo once available in the market.

Poshadri *et al.* (6) highlight that the spores can bear high-temperature environments, acid conditions and salinity, and that makes their usage promising for the production of new foods with stability throughout their shelf life, such as the juice analyzed in the present essay.

A difference in viability ($p < 0.05$) between juices containing *B. clausii* and *B. coagulans* was observed only after thermal treatment on day 0 (Fig. 2). There was a reduction ($p < 0.05$) of viability of *B. clausii* in juices after thermal treatment, but the counting kept stable ($p < 0.05$), reaching the end of accelerated shelf life at 4.29 log CFU/mL. However, as regards the juices containing *B. coagulans*, it was observed that there was a reduction in the count ($p < 0.05$) until day 4 of storage (Fig. 2), leading to a count of 3.60 log CFU/mL on day 6.

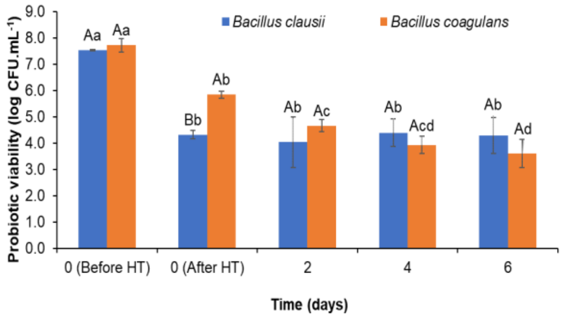

Fig 2. Viability of *Bacillus clausii* and *Bacillus coagulans* in organic mango juice after heat treatment at 96 °C/35 s during 6 days of accelerated shelf life at 37 °C. Different uppercase letters indicate a significant difference between samples at the same time, while different lowercase letters indicate a difference between times for the same sample, using Tukey's test at a probability level of 5% ($p < 0.05$).

The study conducted by the present research group on *B. coagulans* demonstrates sustenance or little alteration of this probiotic's viability after the storage period, as observed in the present study (13).

In non-thermally treated juices, a reduction in the viability ($p < 0.05$) of both probiotic microorganisms was observed over time. The magnitude of the difference for *B. clausii* was small: a reduction of 0.26 log CFU/mL (7.54 ± 0.03 to 7.28 ± 0.04). On the contrary, as for *B. coagulans*, the difference over time was of 1.15 log CFU/mL (7.72 ± 0.26 to 6.57 ± 0.35). Adebayo-Tayo, Olomitutu, and Adebami (2), when assessing probiotic mango juice with *Pediococcus pentosaceus* and *Pediococcus acidilactici* stored at 4ºC and 25 °C for 4 weeks, observed a reduction in the viability during the storage time, which indicates that the major benefits of probiotic juice can be obtained in the first week of storage.

However, Palencia-Argel *et al.* (14) highlight that the viability of probiotic microorganisms is generally affected by storage temperature, suggesting that refrigeration can extend these microorganisms' survival in beverages and fruit juices, while exposure to higher temperatures can cause adverse effects, such as those observed in this study.

In the international market, where probiotic juices are widely commercialized, it's possible to observe that some brands recommend storage under beverage refrigeration. For example, "Garden of Flavor, Surja Organic e Pressed Juicery" provides some juices added with *B. coagulans,* and "GoodBelly Probiotics", which commercializes juices with *Lactobacillus plantarum* 299V. In this way, it is believed that refrigerated storage can contribute to these microorganisms' continuation, and further studies on tropical mango juice are needed.

Physical Chemical Features of Mango Juice Containing *B. Clausii* after Simulated Industrial Thermal Treatment or the Alternative Method

There was no pH difference ($p > 0.05$) among mango juices containing *B. clausii* from different treatments during the 60 days of storage both for samples stored at ambient temperature and those kept under refrigeration (Fig. 3A). pH stability is a desirable result, since it can interfere on the total number of viable probiotic cells in fruit matrices. According to Cosme, Inês, and Vilela (15), the low pH of fruit juices is considered one of the main disadvantages for the counting and activity of probiotic bacteria.

As for the acidity figures, it was verified that the non-thermally treated juice kept at 5ºC and at 25ºC differed ($p < 0.05$) from those thermally treated at day 0 (Fig. 3B). The development of acidity is common when juices are subjected to thermal treatment due to the organic acid formation, and this may have occurred in the present study. On the other hand, the storage temperature did not influence ($p > 0.05$) juice acidity (Fig. 3B).

No alteration was observed ($p > 0.05$) in the total soluble solids of juices from different treatments stored at different temperatures (Fig. 3C), indicating that the probiotic bacteria did not cause physical and chemical alteration in the juices.

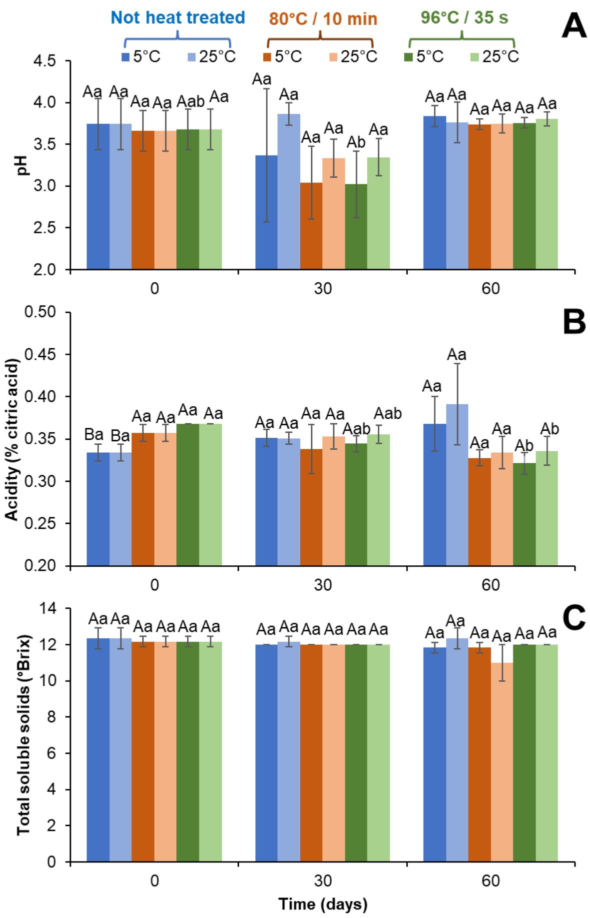

Fig 3. pH (A), acidity (B), and total soluble solids (C) of organic mango juice containing *B. clausii* subjected to different heat treatments (80 °C/10 min and 96 °C/35 seconds) during a 60-day storage period at 5 °C and 25 °C. Different uppercase letters indicate a significant difference between samples at the same time, while different lowercase letters indicate a difference between times for the same sample, using Tukey's test at a probability level of 5% ($p < 0.05$).

Viability and *In Vitro* Gastrointestinal Resistance *of B. Clausii* in Mango Juice after Simulated Industrial Thermal Treatment or the Alternative Method

The viability of *B. clausii* was not influenced ($p > 0.05$) by storage temperature (Fig. 4). However, the simulated industrial thermal treatment caused a reduction ($p < 0.05$) in *B. clausii* viability. These results reinforce the findings of Palencia-Argel *et al.* (14), who highlighted that the variables related to processing methods, especially thermal treatments, have an impact on probiotic counting.

The juices subjected to simulated industrial thermal treatment, with pasteurization at 96 ± 3°C/35 seconds, and then cooled at approximately 70°C for about 5 minutes, showed a considerable reduction in viability when compared to those non-thermally treated ($p < 0.05$), with figures higher than 2.0 log CFU/mL (Fig. 4).

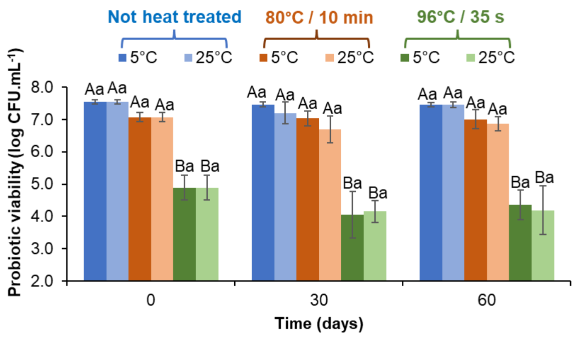

Fig 4. Viability of *B. clausii* in organic mango juice subjected to different heat treatments (80 °C/10 min and 96 °C/35 seconds) during a 60-day storage period at 5 °C and 25 °C. Different uppercase letters indicate a significant difference between samples at the same time, while different lowercase letters indicate a difference between times for the same sample, using Tukey's test at a probability level of 5% ($p < 0.05$).

Almada-Érix *et al.* (16), when assessing pasteurization at 95ºC for 30 seconds in orange juice with probiotic bacilli, also found a reduction at a range of 0.43–2.5 log CFU/mL. In the orange juice, pasteurization caused a larger viability reduction of *B. coagulans* BC30 in comparison to *B. subtilis* Bn1, showing the distinct resistance of microorganisms to thermal processing. Nonetheless, the authors highlight that the loss of viability observed in probiotic bacilli is still smaller compared to other non-spore-forming bacteria.

The usual portion of fruit juice consumption, according to Normative Instruction 75/2020, is 200 mL (17), which enables a proper supply of > 10^6 CFU/mL of probiotics from the present study, even after a viability reduction following thermal treatments.

As for the *in vitro* simulation of gastrointestinal conditions of mango juice containing *B. clausii*, non-thermally treated and stored at 5ºC and 25 °C showed excellent probiotic performance once viability was greater than 7.0 log CFU/mL throughout stages tested during 60 days (Fig. 5). The final count of *B. clausii* on enteric stage II, the final portion of the gastrointestinal tract was > 7.08 CFU/mL (Fig. 5). This was enough to increase defense in the host organism, suggesting that organic mango juice stored at ambient temperature is a promising matrix.

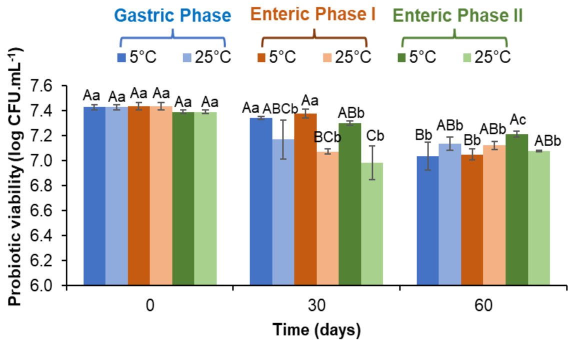

Fig 5. Viability of *B. clausii* after *in vitro* gastrointestinal digestion test in non-heat-treated mango juice during a 60-day storage period at 5 °C and 25 °C. Different uppercase letters indicate a significant difference between samples at the same time, while different lowercase letters indicate a difference between times for the same sample using Tukey's test at a probability level of 5% ($p < 0.05$).

Some studies indicate that mango is a promising matrix of non-lactic probiotic formulations, such as Moreira *et al.* (18), when developing Mango Ubá and Juçara juice, with addition of *Lactobacillus rhamnosus* GG; Oliveira *et al.* (19) with mixed mango juice and carrot as vehicle of probiotic lactobacilli; and Mousanejadi *et al.* (20) with the addition of *L. casei* UK 318 in mango juice.

In the literature, no studies were found about consumers' perception and behavior towards the administration form of probiotics. Nevertheless, considering the high acceptability and consumption of juices by the population, it is believed that having it as a matrix is also a positive way to make ingestion easier. In this case, it is worth highlighting that groups with major vulnerabilities, such as seniors and children, can also show greater difficulty and resistance to the ingestion of capsules and other forms of administration with an unpleasant taste. This is another relevant factor for choosing mango juice, which has a pleasant taste, as a vehicle for these microorganisms.

B. clausii is widely used for the treatment and prevention of diarrhea (21, 22, 23*)*, a clinical picture that requires attention to hydration. Thus, combining this probiotic with a product that can also help with this matter is a very promising alternative to the market.

Conclusion

The simulation of thermal industrial treatment in juices, after the addition of probiotic bacilli, promoted a viability reduction during the accelerated shelf life, this being more evident for *B. coagulans* BC30. The consumption of 100 mL of organic mango juice containing *B. clausii*, and that was thermally treated, provides more than 6.0 log CFU/mL of probiotic at the end of accelerated shelf life. The accelerated shelf-life test carried out in the first stage proved to be viable in simulating the storage conditions to which juices are subjected before consumption.

Mango juices added with *B. clausii*, submitted to different thermal treatments and stored for 60 days in ambient temperature and under refrigeration, presented good stability regarding pH and total soluble solids. The viability of *B. clausii* in juices was not altered when these were stored at ambient or refrigeration temperature. Although the industrial thermal treatment promoted a viability reduction of *B. clausii* compared to the non-thermally treated juice.

The *in vitro* simulation of the gastrointestinal conditions of organic mango juice containing non-thermally treated *B. clausii* and stored at 5 *in vitro* and 25°C has evidenced an excellent performance as regards the probiotic. Concerning the viability, it was greater than 7.0 log CFU/mL in the final portion of the simulated gastrointestinal tract for 60 days. The counting suggests that the organic mango juice stored at ambient temperature is a promising matrix for *B. clausii*.

Acknowledgements

The authors are grateful to the Federal Institute of Southeast Minas Gerais for their financial support, and to the GanedenBC30®/Kerry for the donation of *B. coagulans* BC30.

References

Acosta-Rodríguez-Bueno, C. P.; Abreu, A. T. A.; Guarner, F.; Guno, M. J. V.; Pehlivanoğlu, E.; Perez III, M. *Bacillus clausii for Gastrointestinal Disorders: A Narrative Literature Review*, Advances in Therapy, v. 39, p. 4854-4874, 2022. Available in: https://doi.org/10.1007/s12325-022-02285-0

Adebayo-Tayo, B. C.; Olomitutu, F. O.; Adebami, G. E. *Production and evaluation of probioticated mango juice using Pediococcus pentosaceus and Pediococcus acidilactici during storage at different temperature*. Journal of Agriculture and Food Research, v. 6, p. 100202, 2021. Available in: https://doi.org/10.1016/j.jafr.2021.100202

Almada-Érix, C. N.; Almada, C. N.; Pedrosa, G. T. S.; Lollo, P. C.; Magnani, M.; Sant'ana, A. S. *Development of a semi-dynamic in vitro model and its testing using probiotic Bacillus coagulans GBI-30, 6086 in orange juice and yogurt*. Journal of Microbiological Methods, v. 183, p. 106187, 2021. Available in: https://doi.org/10.1016/j.mimet.2021.106187

Bedani, R.; Rossi, E.A.; Saad, S. M. I. *Impact of inulin and okara on Lactobacillus acidophilus La-5 and Bifidobacterium animalis Bb-12 viability in a fermented soy product and probiotic survival under in vitro simulated gastrointestinal conditions*. Food Microbiology, v. 34, p. 382-389, 2013. Available in: https://doi.org/10.1016/j.fm.2013.01.012

Brasil. *Requisitos técnicos para a declaração da rotulagem nutricional dos alimentos embalados*. Instrução Normativa No 75 de 8 de outubro de 2020 da Agência Nacional de Vigilância Sanitária. Diário Oficial da União (DOU) de 09 de outubro de 2020.

Calligaris, S.; Manzocco, L.; Anese, M.; Nicoli, M. C.; *Accelerated shelf life testing*. In: GALANAKIS, C. M. (ed.). Food Quality and Shelf Life, New Delhi: Academic Press, 2019. Chapter 12, p. 359-392. Available in: https://doi.org/10.1016/B978-0-12-817190-5.00012-4

Castro, J. A.; Kesavelu, D.; Lahiri, K. R.; Chaijitraruch, N.; Chongsrisawat, V.; Jog, P. P. *Recommendations for the adjuvant use of the poly-antibiotic–resistant probiotic Bacillus clausii (O/C, SIN, N/R, T) in acute, chronic, and antibiotic-associated diarrhea in children: consensus from Asian experts.* Tropical Diseases, Travel Medicine and Vaccines, v. 6, p. 1-15, 2020. Available in: https://doi.org/10.1186/s40794-020-00120-4

Clark, D.H. *Fruits and Fruit Products*. In: JATIMER Jr. G.W. (ed.). Official Methods of Analysis of the Association of Official Analytical Chemists international, Rockville: AOAC International: 2016. p. 1-39.

Cosme, F.; Inês, A.; Vilela, A. *Consumer's acceptability and health consciousness of probiotic and prebiotic of non-dairy products*. Food Research International, v. 151, p. 110842, 2022. Available in: https://doi.org/10.1016/j.foodres.2021.110842

Ellis, C. *Vegetable Products, Processed*. In: JATIMER Jr. G.W. (ed.). Official Methods of Analysis of the Association of Official

Analytical Chemists international, Rockville: AOAC International: 2016. p. 1-14.

Faraoni, A.S.; Ramos, A.M.; Stringheta, P.C.; Laureano, J. *Efeito dos métodos de conservação, tipos de embalagem e tempo de estocagem na coloração de polpa de manga "Ubá" produzida em sistema orgânico*. Revista Ceres, v. 55, p. 504-511, 2008. Available in: https://www.redalyc.org/articulo.oa?id=305226820001

Ghelardi, E.; Mazzantini, D.; Celandroni, F.; Calvigioni, M.; Panattoni, A.; Lupetti, A.; Bois De Fer, B.; Perez, M. *Analysis of the microbial content of probiotic products commercialized worldwide and survivability in conditions mimicking the human gut environment*. Frontiers in Microbiology, v. 14, p. 1127321, 2023. Available in: https://doi.org/10.3389/fmicb.2023.1127321

Godshall, M.A. *Sugars and Sugar Products*. In: JATIMER Jr. G.W. (ed.). Official Methods of Analysis of the Association of Official Analytical Chemists International, Rockville: AOAC International: 2016. p. 1-53.

iiKunitake, M.; Ditchfield, C.; Silva, C.; Petrus, R. *Effect of pasteurization temperature on stability of an acidified sugarcane juice beverage*. Ciência e Agrotecnologia. v. 38, p. 554-561, 2014. Available in: https://doi.org/10.1590/S1413-70542014000600004

Moreira, R. M.; Martins, M. L.; Leite Júnior, B. R. C.; Martins, E. M. F.; Ramos, A. M.; Cristianini, M.; Campo, A. N. R.; Stringheta, P. C.; Silva, V. R. O.; Canuto, J. W.; Oliveira, D. C.; Pereira, D. C. S. *Development of a juçara and Uba mango juice mixture with added Lactobacillus rhamnosus GG processed by high pressure*. LWT - Food Science and Technology, v. 77, p. 259-268, 2017. Available in: https://doi.org/10.1016/j.lwt.2016.11.049

Mousanejadi, N.; Barzegar, H.; Behbahani, B. A.; Jooyandeh, H. *Production and evaluation of a functional fruit beverage consisting of mango juice and probiotic bacteria.* Journal of Food Measurement and Characterization, v. 17, p. 3240-3253, 2023. Available in: https://doi.org/10.1007/s11694-023-01862-3

Oliveira, P. M.; Leite Júnior, B. R. C.; Martins, E. M. F.; Martins, M. L.; Vieira, E. N. R.; Barros, F. A. R. *Mango and carrot mixed juice: a new matrix for the vehicle of probiotic lactobacilli.* Journal of Food Science and Technology, v. 58, p. 98-109, 2021. Available in: https://doi.org/10.1007/s13197-020-04518-y

Palencia-Argel, M.; Rodríguez-Villamil, H.; Bernal-Castro, C.; Díaz-Moreno, C.; Fuenmayor, C. A. *Probiotics in anthocyanin-rich fruit beverages: research and development for novel synbiotic products*. Critical Reviews in Food Science and Nutrition, v. 64, p. 110-126, 2022. Available in: https://doi.org/10.1080/10408398.2022.2104806

Pirozzi, C.; Opallo, N.; Coretti, L.; Lama, A.; Annunziata, C.; Comella, F. *Alkalihalobacillus clausii (formerly Bacillus clausii) spores lessen antibiotic-induced intestinal injury and reshape gut microbiota composition in mice*. Biomedicine & Pharmacotherapy, v. 163, p. 114860, 2023. Available in: https://doi.org/10.1016/j.biopha.2023.114860

Poshadri, A.; Deshpande, H. W.; Khodke, U. M.; Katke, S. D.; *Bacillus coagulans and its Spore as Potential Probiotics in the Production of Novel Shelf- Stable Foods*. Current Research in Nutrition & Food Science, v. 10, p. 858-870, 2022. Available in: https://dx.doi.org/10.12944/CRNFSJ.10.3.4

Soares, M. B.; Martinez, R. C. R.; Pereira, E. P. R.; Balthazar, C. F.; Cruz, A. G.; Ranadheera, C. S.; Sant'Ana, A. S. *The resistance of Bacillus, Bifidobacterium, and Lactobacillus strains with claimed probiotic properties in different food matrices exposed to simulated gastrointestinal tract conditions*. Food research international, v. 125, p. 108542, 2019. Available in: https://doi.org/10.1016/j.foodres.2019.108542

Tireki, S. *A review on packed non-alcoholic beverages: Ingredients, production, trends and future opportunities for functional product development*. Trends in Food Science & Technology, v. 112, p. 442-454, 2021. Available in: https://doi.org/10.1016/j.tifs.2021.03.058

Vivek, K.; Mishra, S.; Pradhan, R. C.; Nagarajan, M.; Kumar, P. K.; Singh, S. S.; Manvi, D.; Gowda, N. N. *A comprehensive review on microencapsulation of probiotics: technology, carriers and current trends*. Applied Food Research, v. 3, p. 100248, 2023. Available in: https://doi.org/10.1016/j.afres.2022.100248