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Mate tea with Lactobacillus probiotics: viability, chemical characterization and in vitro resistance to the gastrointestinal tract

Chá mate com probióticos Lactobacillus: viabilidade, caracterização química e resistência in vitro ao trato gastrointestinal

Té de mate con probióticos Lactobacillus: viabilidad, caracterización química y resistencia in vitro al tracto gastrointestinal

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Abstract

To meet the consumer demand for functional drinks with bioactive compounds, this study aimed to evaluate the addition of *Lactiplantibacillus plantarum* LP299V, *Lacticaseibacillus rhamnosus* GG and *Lactobacillus acidophilus* in mate tea. The viability of the probiotic bacteria, microbiological and physicochemical characteristics, the *in vitro* resistance to the gastrointestinal tract and the antioxidant capacity of the mate teas were evaluated during 28 days. Unlike *L. plantarum* and *L. rhamnosus, L. acidophilus* did not remain viable in the product. The physicochemical characteristics of the teas with *L. plantarum* and *L. rhamnosus* were maintained over time. Phenolic compounds of mate tea containing *L. plantarum* were identified by HPLC. There was stability of phenolic compounds and flavonoid content in teas over time. At the end of the gastrointestinal assay, there was 4.52 Log CFU/mL of *L. plantarum*, suggesting that consumption of 100 mL of tea may provide > 6.0 Log CFU/mL of the probiotic. Mate tea can carrier *L. rhamnosus* and *L. plantarum*. There is an increase in the search by consumers for health drinks and at the same time, there is a growing demand for alternatives carriers of probiotics, due to individuals with intolerance and allergy to dairy products, vegetarians and others. This study confirms the possibility of using mate tea, widely consumed in

Brazil, as a positive carrier of of *L. plantarum* and *L. rhamnosus*, making it an attractive alternative beverage to the dairy matrix.
Keywords: Mate tea; Probiotic; Gastrointestinal resistance; Antioxidants; HPLC analyses.

Resumo

Para atender a demanda do consumidor por bebidas funcionais com compostos bioativos, este estudo objetivou avaliar a adição de *Lactiplantibacillus plantarum* LP299V, *Lacticaseibacillus rhamnosus* GG e *Lactobacillus acidophilus* em chá mate. A viabilidade das bactérias probióticas, características microbiológicas e físico-químicas, a resistência *in vitro* ao trato gastrointestinal e a capacidade antioxidante dos chás mate foram avaliadas durante 28 dias. Ao contrário de *L. plantarum* e *L. rhamnosus*, *L. acidophilus* não permaneceu viável no produto. As características físico-químicas dos chás com *L. plantarum* e *L. rhamnosus* foram mantidas ao longo do tempo. Os compostos fenólicos do chá mate contendo *L. plantarum* foram identificados por HPLC. Houve estabilidade dos compostos fenólicos e do teor de flavonoides nos chás ao longo do tempo. Ao final do ensaio gastrointestinal, havia 4,52 Log UFC/ml de *L. plantarum*, sugerindo que o consumo de 100 ml de chá pode fornecer > 6,0 Log UFC/ml do probiótico. O chá mate pode transportar *L. rhamnosus* e *L. plantarum*. Há um aumento na busca por parte dos consumidores por bebidas saudáveis e, ao mesmo tempo, há uma demanda crescente por alternativas carreadoras de probióticos, devido a indivíduos com intolerância e alergia a laticínios, vegetarianos e outros. Este estudo confirma a possibilidade de utilização do chá mate, amplamente consumido no Brasil, como carreador positivo de *L. plantarum* e *L. rhamnosus*, tornando-se uma bebida alternativa atrativa à matriz láctea.
Palavras-chave: Chá mate; Probiótico; Resistência gastrointestinal; Antioxidantes; Análises de HPLC.

Resumen

Para satisfacer la demanda de los consumidores de bebidas funcionales con compuestos bioactivos, este estudio tuvo como objetivo evaluar la adición de *Lactiplantibacillus plantarum* LP299V, *Lacticaseibacillus rhamnosus* GG y *Lactobacillus acidophilus* en el té mate. La viabilidad de las bacterias probióticas, las características microbiológicas y fisicoquímicas, la resistencia *in vitro* al tracto gastrointestinal y la capacidad antioxidante de los tés de mate se evaluaron durante 28 días. A diferencia de *L. plantarum* y *L. rhamnosus*, *L. acidophilus* no permaneció viable en el producto. Las características fisicoquímicas de los tés con *L. plantarum* y *L. rhamnosus* se mantuvieron en el tiempo. Los compuestos fenólicos del té mate que contienen *L. plantarum* fueron identificados por HPLC. Hubo estabilidad de los compuestos fenólicos y el contenido de flavonoides en los tés a lo largo del tiempo. Al final del ensayo gastrointestinal, hubo 4,52 Log CFU/ml de *L. plantarum*, lo que sugiere que el consumo de 100 ml de té puede proporcionar > 6,0 Log CFU/ml del probiótico. Mate té puede portador *L. rhamnosus* y *L. plantarum*. Existe un aumento en la búsqueda por parte de los consumidores de bebidas saludables y, al mismo tiempo, existe una creciente demanda de alternativas portadoras de probióticos, debido a personas con intolerancia y alergia a los productos lácteos, vegetarianos y otros. Este estudio confirma la posibilidad de utilizar el té mate, ampliamente consumido en Brasil, como portador positivo de *L. plantarum* y *L. rhamnosus*, convirtiéndolo en una atractiva bebida alternativa a la matriz láctea.
Palabras clave: Té de mate; Probiótico; Resistencia gastrointestinal; Antioxidantes; Análisis HPLC.

1. Introduction

The importance that nutrition plays in maintaining health and preventing disease through the inclusion of functional foods in the diet becomes increasingly evident over the years (Banwo et al., 2021; Yahfoufi et al., 2018). In view of this, the production and consumption of functional foods and beverages gained prominence since they provide health and guarantee basic nutrition. Functional drinks are the most consumed within the functional food category, because in addition to being easy to store, they are good means of supplying nutrients and bioactive compounds such as vitamins, antioxidants, fatty acids, fibers, probiotics, and prebiotics (Bader-Ui-Ain et al., 2019).

The United Nations and the World Health Organization define probiotics as living microorganisms that provide benefits to the individual when ingested in adequate quantities (FAO/WHO, 2001). Probiotics must be live species of microorganisms safe for use, which guarantee health benefits; they can have different means of administration, target host species and target sites of action (Binda et al., 2020; Hill et al., 2014). Functional probiotic foods confer benefits to the consumer, as they provide protection from pathologies, pathogens, and maintain a balanced gut microbiota (Peng et al., 2020).

In the market for practical and healthy drinks, those based on yerba mate (Ilex paraguariensis A. St.-Hil.) are widely accepted and consumed by the public (Frizon et al., 2018). Ready-to-drink herbal teas can be found in versions such as natural

mate tea, lemon, peach and guarana flavors, as well as the dehydrated herb to be prepared in hot or cold water, with varied flavors.

The extract of yerba mate is made up of many non-volatile bioactive compounds that are responsible for beneficial effects on human health, such as vitamin A, vitamin C and the B complex, minerals and tannins, as well as flavonoids and phenolic acids that act to protect the body against the effects of free radicals, due to its antioxidant activity (Piovezan-Borges et al., 2016; Bracesco et al., 2011).

Characteristics of fermented yerba-based teas have been studied (Gonzalez-Gil et al., 2014; Lima et al., 2012), however, analyses that evaluate the ability to carry probiotic bacteria and maintain the concentrations of bioactive compounds in these products without fermentation are scarce in the literature. Thus, considering the importance of probiotic foods for health and the benefits of ingesting yerba mate, this study evaluated the enrichment of mate tea with probiotic bacteria as a viable alternative for the introduction of practical, tasty and healthy drinks to the human diet and performed a chemical characterization and *in vitro* resistance to the gastrointestinal tract.

2. Methodology

This work is based on a laboratory study. The addition of probiotic bacteria in mate tea, the viability of *L. rhamnosus* GG, *L. plantarum* LP299V and *L. acidophilus* in mate tea, the evaluation of the microbiological quality of the mate tea and the determination of the physicochemical characteristics as well as the evaluation of the resistance of *L. plantarum* to the simulated *in vitro* gastrointestinal tract was carried out at the Food Science and Technology Department of IF Sudeste MG, Rio Pomba campus. The other analysis were carried out at the Higher Institute of Engineering of Porto, Portugal.

Chemicals

For carrying out hydrogen peroxide elimination activity were used potassium phosphate dibasic trihydrate and potassium phosphate monobasic from Sigma-Aldrich (Sintra, Portugal) and hydrogen peroxide 35.5% w/w from Labchem (Zelienople, PA, USA).

The standards for HPLC analyses were purchased from Sigma-Aldrich (4-O-caffeoylquinic acid, ≥98%, 3,5-di-O-caffeoylquinic acid, ≥95%, 4,5-di-O-caffeoylquinic acid, ≥90%), Alfa Aesar (5-O-caffeoylquinic acid, ≥95%), Riedel-de Haën (caffeine, ≥99%) and Extrasynthèse (3-O-caffeoylquinic acid).

Addition of probiotic bacteria in mate tea

Commercial Mate tea Original (Leão®, 450 mL bottle) was obtained on the market in Rio Pomba, MG, Brazil, and sent to the Food Science and Technology Department, where it was stocked until the beginning of the experiment. A ready-to-use freeze-dried culture containing approximately 10^{10} CFU/g of *Lactiplantibacillus plantarum* LP299V (Nature's Bounty, United States) or *L. rhamnosus* GG (Culturelle®, United States) or *L. acidophilus* (Leiba, São Paulo, Brazil) was used. A capsule of each culture was added individually under aseptic conditions to every 100 mL sample of mate tea. The control treatment consisted of mate tea without probiotic culture.

The beverages were placed in an incubator for 24 hours at 37 °C and labeled pre-inoculum. Twenty-four hours later, 10 mL of the pre-inoculum was transferred to 100 mL of a new tea which was placed under the same conditions. It was labeled inoculum. After the fermentation of the inoculum, the teas were stored at 5 °C in a stove B.O.D. (Novatecnica NT 704, Piracicaba, São Paulo, Brazil) and submitted to the analyses during 28 days.

Viability of L. rhamnosus GG, L. plantarum LP299V and L. acidophilus in mate tea

After 24 hours of inoculum fermentation and after 0, 7, 14, 21 e 28 days of storage, a serial dilution of fermented tea in sterile saline peptone solution [0.85% of NaCl (Synth, Diadema, São Paulo, Brazil) and 0.1% of peptone (Acumedia, Michigan, EUA)] was performed until 10^{-5} of dilution was achieved. Aliquots of 1.0 mL of the respective dilution of the tea was inoculated in duplicate in Petri dishes (Cial, Paulina, São Paulo, Brazil) containing Man Rogosa and Sharpe agar, through the pour plate method, as the plates were incubated at 37 ± 1 ºC for 72 hours in anaerobic chamber (Richer and Vedamuthu, 2001). After this period, the *L. rhamnosus* GG, *L. plantarum* LP299V and *L. acidophilus* typical colonies were calculated.

Evaluation of the microbiological quality of the mate tea

Mate teas containing *L. rhamnosus* GG and *L. plantarum* LP299V were evaluated at 0 and 28 days of storage.

The presence or absence of *Salmonella* sp. was determined in 25 mL of the samples, homogenized in 225 mL of lactose broth using the methodology of Andrews et al. (2001).

Thermotolerant coliforms were determined by the Most Probable Number (MPN) technique according to Kornacki & Johnson (2001) at t=0 day and t=28 days of storage.

Determination of the physicochemical characteristics

The pH value (Tecnopon NT PHM, Piracicaba, São Paulo, Brasil) and titratable acidity (citric acid/100 g of product) of mate tea were determined according to AOAC (2016) procedures. Total soluble solids (ºBrix) was performed by refractometry according to Godshall (2016). These analyzes were done at 0, 7, 14, 21 e 28 days of storage of mate tea with *L. rhamnosus* GG and *L. plantarum* LP299V.

Evaluation of the resistance of L. plantarum to the simulated in vitro gastrointestinal tract when carried in mate tea

The methodology described by Bedani et al. (2013) was used, simulating the gastric, enteric I and enteric II phases, with enzymes of the gastrointestinal tract and applying at 0 and 28 days. Briefly, 10 mL of each serial dilution (10^{-3}, 10^{-4}, 10^{-5}) of mate tea with L. plantarum was transferred in triplicate to 3 sterile bottles (gastric phase), and the pH adjusted to 2.3 - 2.6 with 1 N 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. The same bottles at the end of the time 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 N 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 4 h of initiation of the in vitro test, 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.

Nine flasks containing the teas were incubated at 37 °C using a 150-rpm shaker for 2 h each sequentially, simulating the gastric, enteric I and enteric II phases, respectively. The survival of *L. plantarum* was evaluated shortly after the addition of the enzymes and after 2, 4 and 6 h, using the plating method with De Man, Rogosa and Sharpe agar (MRS, Merck, Darmstadt, Germany) added with 0.004% Bromocresol purple and calcium. The result of the plate count was expressed in Log CFU/mL and all averaging was done before taking logs.

Antioxidant activity of teas

The evaluation of the antioxidant activity of mate teas from the control treatments and containing *L. plantarum* LP299V over the 28 days was done by the ABTS [2,2'-azinobis (3-ethylbenzthiazoline sulfonic acid-6)] radical capture and Ferric Reducing Antioxidant Power (FRAP) microplate assays. The ABTS radical capture assay followed Gião et al. (2007) procedure, the ascorbic acid (AA) was used as standard. The ABTS scavenging activity was calculated by the following equation: ABTS scavenging activity = [(Abs of control - Abs of sample)] / (Abs of control) x 100. Results were expressed as µg AA equivalents per mL of sample (µg AA eq/ mL).

FRAP assay followed the methodology described by Paz et al. (2015). Ascorbic acid (AA) was the standard, and the absorbance was measured at 593 nm, at 37 ºC after 10 min. Results were expressed as µg AA equivalents per mL of sample (µg AA eq/ mL).

Hydrogen peroxide elimination activity

The evaluation of the hydrogen peroxide elimination activity in the control sample and in the mate tea containing *L. plantarum* LP299V was performed at 0, 7, 14, 21 e 28 days following the methodology of Ruch et al. (1989) with slight modifications (Delerue et al., 2021; Mancini et al., 2018). The elimination of hydrogen peroxide was achieved using the following equation: % Scavenging [H_2O_2] = [(A0 - A1) /A0] x 100, in which A0 is the absorbance of the negative control (phosphate buffer) and A1 is the absorbance of the samples (Gulçin, 2006).

Determination of phenolic content in mate teas

Determination of total phenolic content (TPC)

The quantification of the total phenolic content (TPC) in the control sample and in the sample containing *L. plantarum* LP299V was performed at 0 and 28 days, based on the Folin-Ciocalteu procedure (Paz *et al*., 2015) using gallic acid (GA) as standard. The absorbance was measured at 765 nm after 90 min of reaction and results were expressed as µg of GA equivalents per mL of sample (µg GAE/ mL).

Determination of total phenolic content by high performance liquid chromatography (HPLC)

Samples were analyzed in a Shimadzu system (Kyoto, Japan) equipped with a low-pressure quaternary gradient unit (model LC-20AD) with an inline degasser (model DGU-20A5R) and an auto-sampler (model SIL-20A HT). The system is equipped with a photodiode array detector (model SPD-M20A). Samples were analyzed (n=3) using a C18 Spherisorb ODS2 (25.0×0.46 cm; 5 µm particle size) column from Waters (Ireland). The solvent system consisted in formic acid 5% (A) and methanol (B), starting with 5% B and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42min, 55% B at 47 min, 75% B at 56 min, 100% B at 60 min, 100% B at 65 min, 5% at 66 min, end at 80 min (Grosso et al., 2015; Mancini et al., 2018). The solvent flow rate was 920 µL/min. External calibration curves were prepared for quantification purposes using six concentrations (n=3, each concentration) of caffeine (λ=272 nm; $y=5.02\times10^7-1.21\times10^4$, R^2=1.0000), and 3-*O*-caffeoylquinic (λ=320 nm; $y=7.29\times10^7-9.60\times10^5$, R^2=0.9963), 4-*O*-caffeoylquinic (λ=320 nm; $y=6.81\times10^7-2.64\times10^5$, R^2=0.9995), 5-*O*-caffeoylquinic acid (λ=320 nm; $y=8.11\times10^7-4.27\times10^4$, R^2=0.9998), 3,5-di-*O*-caffeoylquinic (λ=320 nm; $y=9.28\times10^7+1.23\times10^5$, R^2=0.9950) and 4,5-di-*O*-caffeoylquinic (λ=320 nm; $y=5.12\times10^7+7.72\times10^4$, R^2=0.9939) acids. All compounds were quantified with their corresponding standards, except 3,4-di-*O*-caffeoylquinic acid which was quantified as 3,5-di-*O*-caffeoylquinic acid.

Determination of the total flavonoid content (TFC) in mate teas

The total flavonoid content (TFC) in the control sample and in the sample containing *L. plantarum* LP299V, was quantified at 0 and 28 days by the spectrophotometric assay described by Barroso et al. (2011), using catechin (CA) as standard. The absorbance was measured at 510 nm and the TFC was expressed as µg of CA equivalents per mL of sample (µg CA/ mL).

Statistical analysis

In the physicochemical and viability analyses of probiotic cultures, completely randomized design (CRD) was used, with 3 replications and a 3x5 factorial design with 3 treatments (mate tea containing *L. plantarum*, mate tea containing *L. rhamnosus* and mate tea containing *L. acidophilus*) and 5 storage times (0, 7, 14, 21, 28 days).

For the analysis of the resistance of the probiotic culture to the simulated gastrointestinal tract, phenolic compounds and flavonoids, a completely randomized design (CRD) with 3 replications and a 2x2 factorial design with 2 treatments (mate tea containing *L. plantarum* and mate tea control) and 2 storage times (0 and 28 days) were used.

To perform the analysis of antioxidant activity, FRAP and ABTS analyses, a completely randomized design (CRD) was used with 3 repetitions and a 2x5 factorial design with 2 treatments (mate tea control sample and mate tea containing *L. plantarum*) and 5 storage times (0, 7, 14, 21 and 28 days).

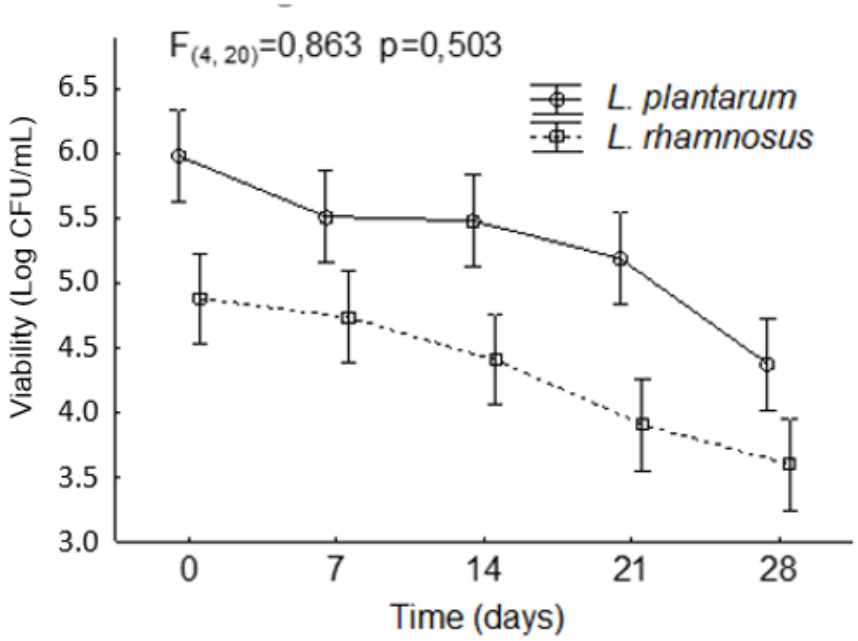
The averages of the different treatments (mate tea control sample, mate tea containing *L. plantarum*, mate tea containing *L. rhamnosus* and mate tea containing *L. acidophilus*) were analyzed by ANOVA Fatorial and by Tukey test, at the 5% significance level, using the statistical program Sisvar version 5.3 (Ferreira, 2010) and STATISTICA (TIBCO Software Inc., 2017).

3. Results and Discussion

Viability of L. rhamnosus GG, L. plantarum LP299V and L. acidophilus in the mate tea

The refrigerated storage period did not alter the viability of *L. rhamnosus* GG and *L. plantarum* LP299V ($p > 0.05$) (Figure 1). However, no viability of *L. acidophilus* was detected in mate tea. Jafarei & Ebrahimi (2011) demonstrated that the best conditions for the development of *L. acidophilus* correspond to temperatures between 37 to 41 ºC and pH in the range of 6 and 7. As mate teas showed lower pH values (Table 1), it is believed that this is the cause of the low growth of this microorganism, $<1.0 \times 10^1$ CFU/mL estimated. In addition, Galgano et al. (2015) reported that not all probiotic strains present good results in terms of survival when added in plant products, which can be seen in this study, so limiting its use in some types of food and processing.

Figure 1 - Viability of *L. plantarum* and *L. rhamnosus* in mate tea for 28 days.



Source: Authors.

Table 1 - Mean results of pH, acidity (% citric acid) and soluble solids of control mate tea and containing *L. rhamnosus* GG and *L. plantarum* LP 299V.

Treatments	pH	% acidity	Soluble solids
Control	4.04 a	1.19 a	8.46 a
L. rhamnosus GG	4.03 a	1.22 a	8.44 a
L. plantarum LP 299V	4.04 a	1.24 a	8.37 a

Means followed by the same letter in the column did not differ by Tukey's test ($p > 0.05$). Source: Authors.

The counts of *L. plantarum* LP299V during the 28 days of storage did not differ from the counts of *L. rhamnosus* GG ($p > 0.05$). According to FAO/WHO (2011) the product must contain at least 10^6 to 10^7 CFU/mL of the microorganism to be considered a probiotic.

At the end of the shelf life, the difference in probiotic counts was approximately one cycle Log ($p > 0.05$). Thus, the consumption of 100 mL of mate tea containing *L. plantarum* LP299V may provide an average of $10^{6.5}$ CFU while the intake of 100 mL of tea containing *L. rhamnosus* GG may provide an average of $10^{5.5}$ CFU. This way, mate tea proved to be a better vehicle of *L. plantarum* LP299V, based on FAO / WHO (2011) considerations. The viability of probiotic microorganisms can be affected by several factors such as temperature, pH, dissolved oxygen and storage time. In addition, mate tea can influence the resistance of different microorganisms due to its physicochemical characteristics, besides to the fact that each strain is unique and has its survival characteristic.

Microbiological quality of mate tea

The mate tea samples containing *L plantarum* LP299V, *L. rhamnosus* GG and the control sample, at t=0 day and t=28 days of storage at 5 ºC, presented coliform count at 45 ºC <1.0 x 10^{-1} MPN/mL and absence of *Salmonella sp.* in 25 mL of the product. Thus, the preparations are microbiologically safe for human consumption, as they are in accordance with the standards established by Brazilian legislation (Brazil, 2001).

Physicochemical characteristics of mate teas containing L. rhamnosus GG and L. plantarum LP299V

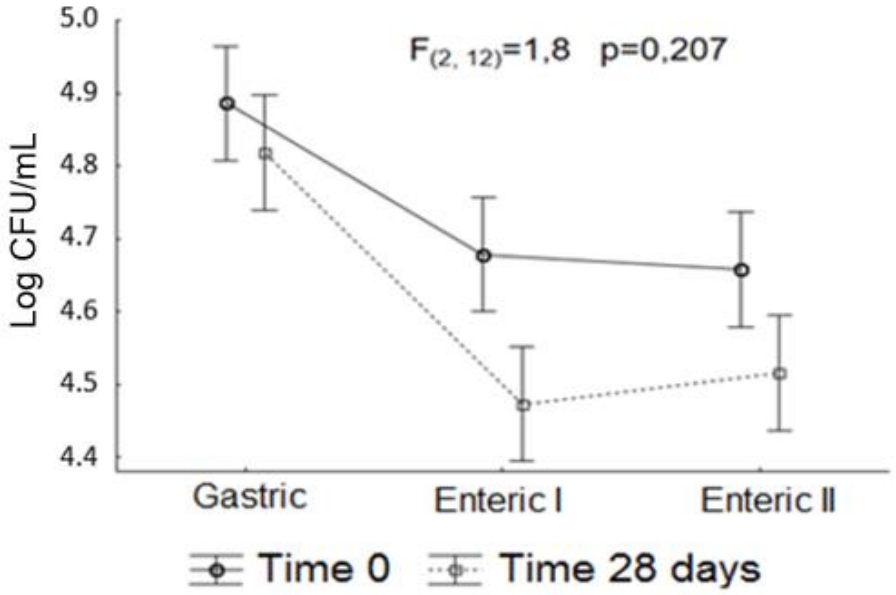
During the storage period, the pH, acidity, and soluble solids values of mate teas did not differ significantly ($p > 0.05$) and the control sample and samples containing probiotics did not differ either ($p > 0.05$) (Table 1). As results, probiotic cultures did not affect the physical and chemical characteristics of mate tea because they did not ferment the product, which is expected in the development of refrigerated drinks and food products.

Lima et al. (2012) observed pH values ranging from 3.5 to 6.65 and acidity from 0.10 to 1.2% during 28 days of storage of a fermented non-dairy drink, with the addition of the probiotic *L. acidophilus* and addition of mate herb extract, being these values compatible with the ones found in the present study.

Survival of L. plantarum LP299V to gastrointestinal conditions simulated in vitro when carried in mate tea

As *L. plantarum* LP299V showed better viability in mate tea, its survival was evaluated *in vitro* by testing its resistance to gastrointestinal conditions. There was no significant interaction ($p > 0.05$) between the different phases of the *in vitro* test and the storage time. By t=28 days of storage, the average count of *L. plantarum* LP299V in mate tea was 4.52 log CFU/mL (Figure 1). Through simulation of the enteric phase II, which corresponds to the large intestine, an average viability of 4.52 Log CFU/mL equivalent to 3.3 x 10^4 CFU/mL of L. *plantarum* LP299V was also found in tea (Figure 2), demonstrating the stability of the probiotic after passing through the gastrointestinal tract. Majeed et al. (2019) highlight that is indispensable that the probiotic remain alive and active during the shelf life of the product at high counts to promote health benefits. Therefore, mate tea is a promising matrix for delivering *L. plantarum* LP299V since through the consumption of 100 mL of the product enriched with the probiotic, approximately 6.52 Log CFU will reach the intestine to exercise the benefits. These results suggest that the probiotic maintains a constant count at the end of the gastrointestinal simulation even after 28 days of storage, being a desirable characteristic in the development of new products. According to Hussain et al. (2016), from 10^6 to 10^8 CFU/mL of the probiotic microorganisms cells must be able to reach the intestinal colon to guarantee the desired therapeutic effect. So, it is believed that mate tea containing *L. plantarum* LP299V can be considered potentially probiotic as long as 100 mL of the product is consumed.

Figure 2 - Viability of *L. plantarum* mate tea after different phases of the *in vitro* gastrointestinal resistance test at different times.



Source: Authors.

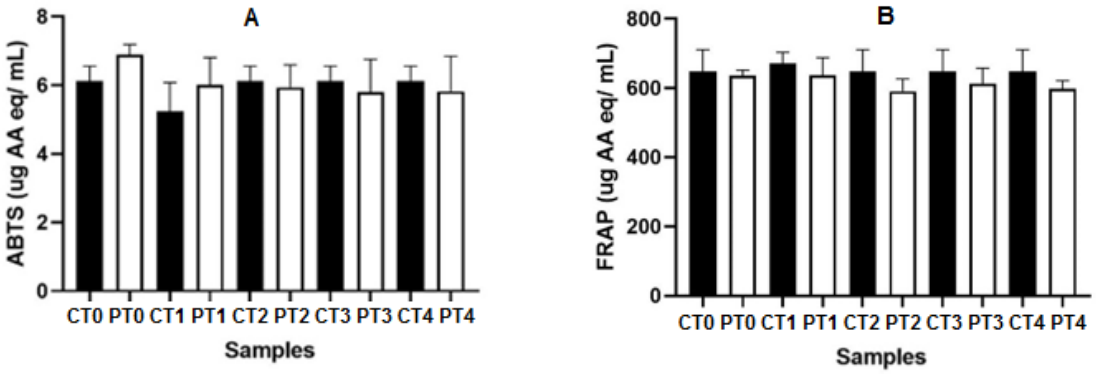
Antioxidant activity of mate teas

Antioxidant activity evaluated by the ABTS and FRAP assays

There was no significant difference ($p > 0.05$) in the antioxidant activity of mate teas by the ABTS and FRAP methods when the control samples and samples containing *L. plantarum* LP299V (Figure 3) were evaluated at different times.

The consumption of natural antioxidants found in plants and fruits, inhibits the formation of free radicals and ensures lower reports of oxidative stress related diseases (Dumanović et al., 2021; Droge, 2002). This protection against oxidative stress was displayed by mate tea and maintained with the addition of the probiotic. In this sense, the results of the present study are important for showing the stability of the antioxidant activity of mate teas during the storage period, which is one of the most important characteristics in the development of products that provide these benefits, besides the results show that the addition of the probiotic does not altered the antioxidant activity over 28 days of storage at 5.0 ºC.

Figure 3 - Average variation and standard deviation of the *in vitro* antioxidant activity of mate teas by the ABTS (A) and FRAP (B) methods over time. CT0 – CT4 (Control tea, CT0=0 day, CT1=7 days, CT2=14 days, CT3=21 days and CT4=28 days), PT0 -PT4 (Probiotic tea, PT0=0 day, PT1=7 days, PT2=14 days, PT3=21 days and PT4=28 days).

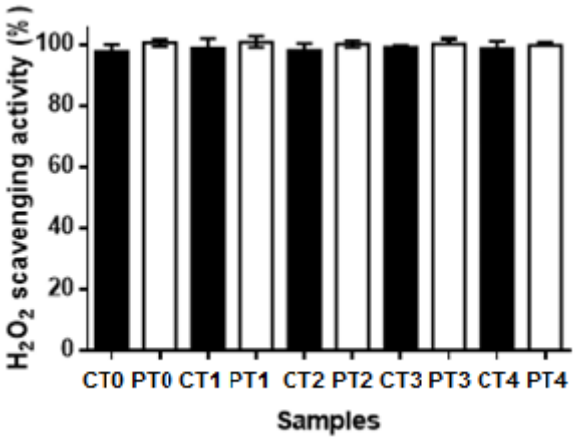


Source: Authors.

Hydrogen peroxide elimination

All mate teas, including control samples and those containing *L. plantarum* LP299V, showed 100% inhibition of hydrogen peroxide scavenging along the 28 days of storage (Figure 4). The addition of probiotic to mate tea did not alter the inhibiting power of this chemical compound. Hydrogen peroxide is a non-radical reactive oxygen species (ROS) that is responsible for several pathologies (Collin, 2019). Therefore, the consumption of mate tea from both samples is desirable for inhibiting this ROS and, consequently, delaying oxidation reactions, preventing damage to consumers' organisms.

Figure 4 - H_2O_2 scavenging in the control mate tea and in the tea containing *L. plantarum* LP 299V during the 28 days of storage. CT0 – CT4 (Control tea, CT0=0 day, CT1=7 days, CT2=14 days, CT3=21 days and CT4=28 days), PT0 -PT4 (Probiotic tea, PT0=0 day, PT1=7 days, PT2=14 days, PT3=21 days and PT4=28 days).

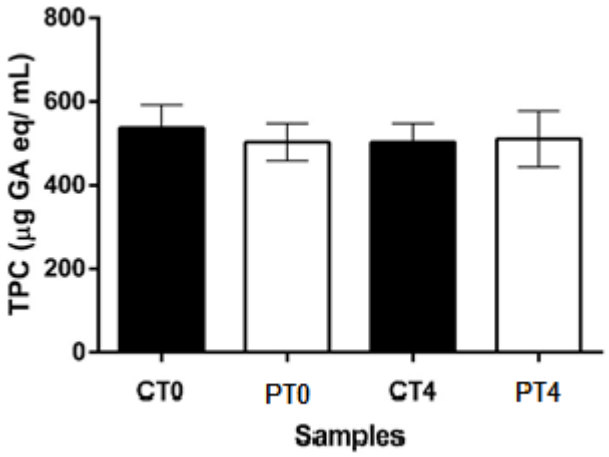


Source: Authors.

Phenolic composition

There was no difference (p> 0.05) in the content of phenolic compounds in the control mate tea and that inoculated with *L. plantarum*, as well as over time. The average TPC in teas at 0 and at 28 days were, respectively, 503.25 µg/mL (0.50 mg/mL) and 510.9 µg/mL (0.51 mg/mL) (p> 0.05) (Figure 5). The TPC in mate tea as well as its maintenance during storage reinforces the antioxidant potential of this product. The phenolic contents found in herbal teas have several biological properties, such as, anti-inflammatory, antioxidant and antiplatelet actions. Its antioxidant role in food is the result of its potential to inhibit the formation of ROS (Yan et al., 2020) and to maintain vitamin C (Koo & Suhaila, 2001).

Figure 5 - Total phenolic compounds (TPC, µg GA eq/ mL) in the control mate tea (CT) and in mate tea containing *L. plantarum* LP 299V (PT) at T=0 day (CT0 and PT0) and T=28 days (CT4 and PT4).

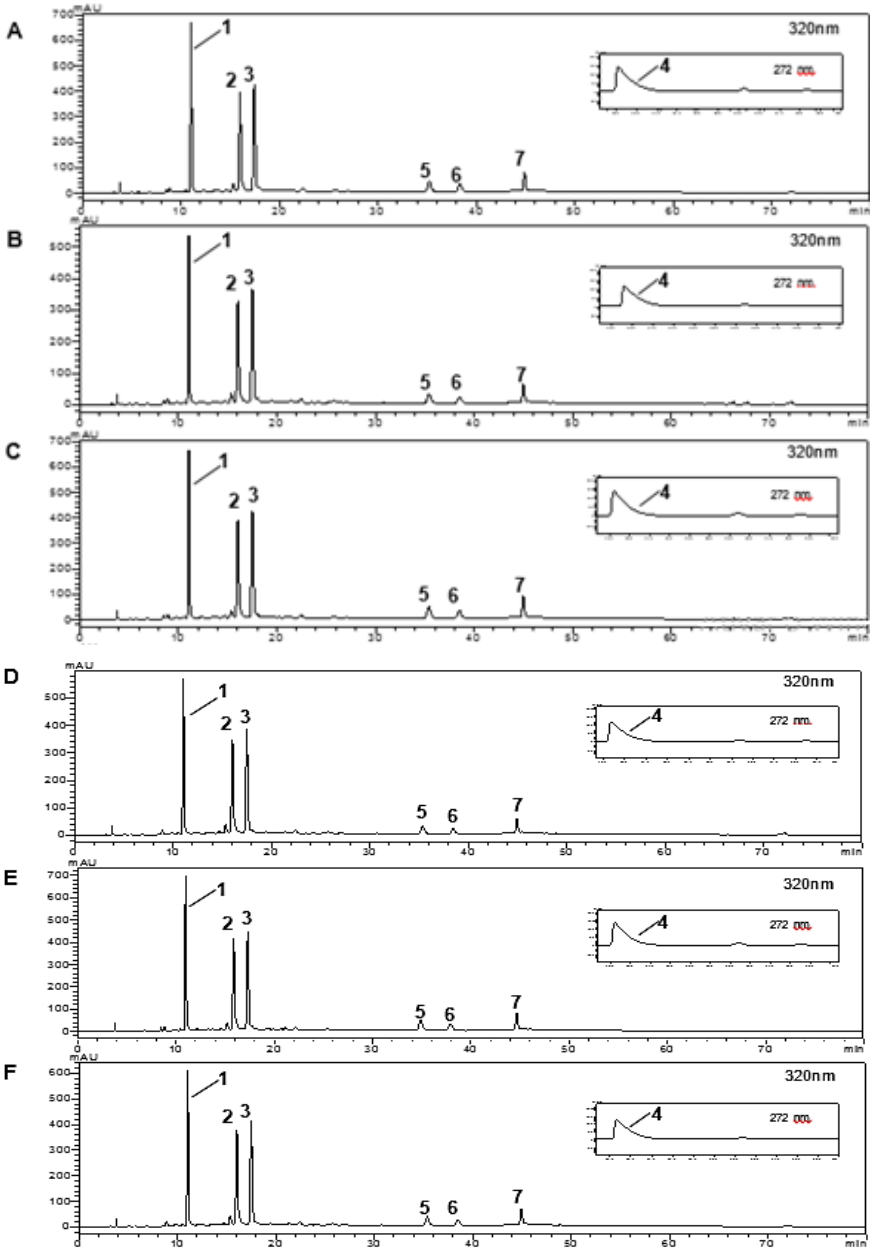


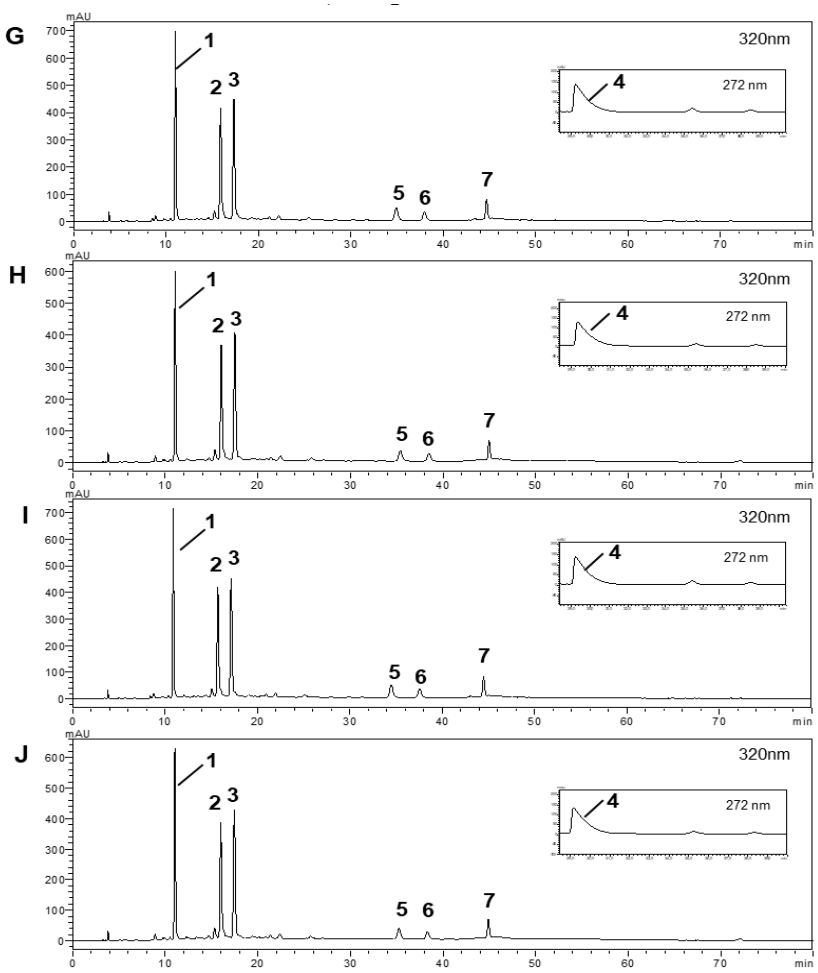
Source: Authors.

Six hydroxycinnamic acids (3-*O*-caffeoylquinic acid (**1**), 4-*O*-caffeoylquinic acid (**2**), 5-*O*-caffeoylquinic acid (**3**), 3,4-di-*O*-caffeoylquinic acid (**5**), 3,5-di-*O*-caffeoylquinic acid (**6**) and 4,5-di-*O*-caffeoylquinic acid (**7**)) were identified by HPLC-DAD, in addition to a methylxanthine (caffeine (**4**)) (Figure 6). This identification is in accordance with previously published works on the chemical composition of mate tea as well as in other species of the same genus (Yi et al., 2018; Peres et al., 2013; Burris et al., 2012). However, some flavonoids already identified in the species were not detected by HPLC-DAD, such as quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside (Wianowska et al., 2017).

According to Bravo et al. (2007), 28 different compounds were detected in the herb extract by HPLC. About 80% of the chromatographic peak was composed of isomers of caffeoylquinic acid, compounds that are also present in the mate teas of this study (Table 2).

Figure 6 - HPLC-DAD chromatograms of control mate tea (A, C, E, G, I) and tea containing *L. plantarum* (B, D, F, H, J). A - T0 control (T =0 day); B - PT0 with *L. plantarum* (T =0 day); C - T1 control (T=7 days); D - PT1 with *L. plantarum* (T=7 days); E - T2 control (T=14 days); F - PT2 with *L. plantarum* (T=14 days); G - T3 control (T=21 days); H - PT3 with *L. plantarum* (T=21 days); I - T4 control (T=28 days); J - PT4 with *L. plantarum* (T=28 days). Identification of the peaks: **1** - 3-*O*-caffeoylquinic acid; **2** - 4-*O*-caffeoylquinic acid; **3** - 5-*O*-caffeoylquinic acid; **4** - caffeine; **5** - 3,4-di-*O*-caffeoylquinic acid; **6** - 3,5-di-*O*-caffeoylquinic acid; **7** - 4,5-di-*O*-caffeoylquinic acid.





Source: Authors.

Table 2 - Concentrations (µg/mL) of hydroxycinnamic acids and methylxanthine in the control mate tea and containing *L. plantarum* LP 299V. Values presented as mean±SD of 3 determinations. CT0 – CT4 (Control at T=0 day, T1=7 days, T2=14 days, T3=21 days and T4= 28days), PT0 - PT4 (mate tea with *L. plantarum* at T=0 day, T1=7 days, T2=14 days, T3=21 days and T4= 28days).

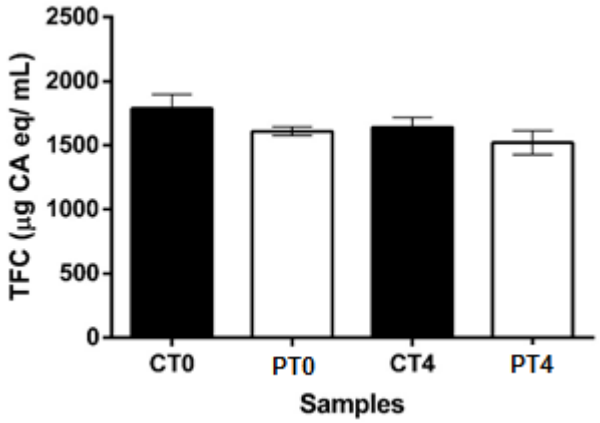
		CT0	T0	CT1	T1	CT2	T2	CT3	T3	CT4	T4
3-CQA	Average	79.96	63.79	79.46	68.09	79.24	74.16	78.42	72.08	77.91	75.45
	SD	0.04	0.36	0.10	0.07	1.11	0.36	0.19	0.04	0.07	0.32
4-CQA	Average	71.60	58.66	70.44	62.23	68.47	67.53	67.52	65.36	66.59	68.43
	SD	2.22	0.41	0.49	0.19	2.67	0.25	0.18	0.05	0.30	0.63
5-CQA	Average	76.80	66.26	77.29	69.51	78.06	75.83	78.36	73.47	77.84	76.88
	SD	0.22	0.19	0.53	0.11	0.18	0.22	0.22	0.03	0.21	0.76
Caffeine	Average	106.64	90.40	106.18	93.86	108.92	101.13	107.62	99.83	108.92	103.24
	SD	0.06	0.11	0.02	0.06	0.26	0.02	1.12	0.04	0.29	0.69
3,4-di-CQA	Average	14.87	10.30	15.88	9.99	15.10	11.58	15.04	11.15	15.50	11.65
	SD	0.03	0.14	0.09	0.16	0.07	0.03	0.01	0.23	0.09	0.17
3,5-di-CQA	Average	10.47	7.51	11.50	7.21	10.37	8.31	10.28	8.16	11.05	8.21
	SD	0.03	0.02	0.01	0.02	0.05	0.01	0.04	0.02	0.05	0.25
4,5-di-CQA	Average	16.46	12.60	17.95	12.16	16.28	13.96	16.10	13.50	17.11	13.85
	SD	0.13	0.03	0.02	0.23	0.12	0.20	0.07	0.30	0.03	0.14
Total		376.81	309.52	378.71	323.04	376.44	352.49	373.34	343.55	374.91	357.71

Source: Authors.

Total flavonoid content

Mate teas presented significant flavonoid contents in all control samples and samples containing the probiotic. At 0 and 28 days, an respective average of 1609.8 µg CA eq/ mL (1.60 mg CA eq/ mL) and 1522.0 µg CA eq/ mL (1.52 mg CA/ mL) were found (p> 0.05) (Figure 7). This fact shows the stability of these bioactive compounds in teas, recognized for their positive influence in kidney and brain function, as well as aiding digestion and heart function (Qadir, 2017). One class of components that most contributes to the body's antioxidant protection is flavonoids. Several studies are found in the literature regarding the quantification of these compounds; however, most of them assess the quantification in herbal tea extracts and not in their infusion or liquid form. Nakamura et al. (2013) found a significant presence of flavonoids in mate tea and Souza et al. (2008) identified flavonoids such as as myricetin and quercetin in mate tea, fennel, chamomile, green tea, and black tea.

Figure 7 - Total flavonoid content (TFC, µg CA eq/ mL) in the control mate tea (CT) and in the tea containing *L. plantarum* LP 299V (PT) at T=0 day (CT0 and PT0) and T=28 days (CT4, PT4).



Source: Authors.

4. Conclusion

This study showed that it is possible to add *L. plantarum* LP299V and *L. rhamnosus* GG with success in mate tea. The viability of these strains was ensured during the storage period of the tea, demonstrating that the matrix is promising for the maintenance of these microorganisms. At the end of the *in vitro* gastrointestinal simulation, 6.52 Log CFU/100mL of *L. plantarum* LP299V remained viable to the simulated large intestine conditions, suggesting the guarantee of the desired therapeutic effects. The addition of probiotic did not modify the antioxidant capacity of the teas, which was maintained along its 28 days of storage. Furthermore, the mate teas have totally inhibited hydrogen peroxide, showing preventive power for many pathologies caused by the presence of this ROS. It is believed that the presence and the stability of phenolic and flavonoid compounds enhances the antioxidant capacity of the teas. The compounds identified by HPLC-DAD in mate teas, i.e. hydroxycinnamic acids and methylxanthine, were found at the end of the experiment meaning that they were preserved. Thus, this study found that the widely consumed mate teas in Brazil has been able to carry *L. plantarum* LP299V and *L. rhamnosus* GG, becoming an innovative alternative for the beverage industries which are aimed at people whose target is healthy eating.

This study signals a potential future in the mate tea market with added functional ingredients such as probiotics. New studies should be conducted to evaluate techniques for incorporating these microorganisms into teas, such as microencapsulation.

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