Food Research International 116 (2019) 1247–1257

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

In vitro and *in vivo* resistance of *Lactobacillus rhamnosus* GG carried by a mixed pineapple (*Ananas comosus* L. Merril) and jussara (*Euterpe edulis* Martius) juice to the gastrointestinal tract

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ARTICLE INFO

Keywords:
Jussara
Probiotic
Gastrointestinal resistance
Pre-neoplastic lesions

ABSTRACT

This study evaluated the viability of *Lactobacillus rhamnosus* GG (LGG) and its *in vitro* and *in vivo* resistance to the gastrointestinal tract (GIT) when carried by a mixed fermented pineapple and jussara juice. The effects of product ingestion on the biochemical characteristics of the blood and on the development of aberrant crypt foci (ACF) in Wistar rats were also determined. The LGG viability in probiotic juice was higher than 7.2 log CFU/mL throughout 28 days at 8 °C. The mean count of the probiotic microorganism in the fecal samples of the rats was 5.6 log CFU/g, identical to the count at the end of the *in vitro* trial (enteric phase II), indicating that the mixed pineapple and jussara juice was an excellent vehicle for LGG. No difference ($p > .05$) was verified to ACF among the groups. However, the results for the probiotic culture viability and its *in vitro* and *in vivo* resistance to the gastrointestinal tract suggest that this juice is an excellent carrier matrix for LGG and contributed to a reduction in the levels of the LDL (low density lipoprotein) fraction of the blood cholesterol, thus being an aid in the control of coronary diseases.

1. Introduction

The high incidence of chronic non-transmittable diseases (CNTD) has been modifying the habits of the population, who are now looking for healthier life styles. In recent decades, the concept of diet has become linked to that of health, increasing the consumption of natural foods (Saad, Cruz, & Faria, 2011). This change in behavior of the consumer market has directed research to develop products with functional appeal such as those containing antioxidants and probiotic cultures (Silva, Barreira, & Oliveira, 2016). In this context, antioxidants are substances capable of acting in the inhibition and/or elimination of oxidizing compounds, whereas probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014).

The carrying of probiotic cultures by fruit based beverages is promising, since it associates the functionality of these microorganisms with the bioactive compounds inherent to the matrix (Moreira et al., 2017). In addition it offers a new food product option to consumers who, for cultural or health reasons, do not consume dairy-based probiotic foods (Martins, Ramos, Martins, & Leite Júnior, 2016).

Cancer prevention, specifically that of colorectal cancer (CRC), is one of the numerous health benefits attributed to the antioxidants and probiotics (Gomides et al., 2015; Jobin, 2012; Klewicka, Nowak, Zduńczyk, Juśkiewicz, & Cukrowska, 2012; Klewicka, Zduńczyk, Juśkiewicz, & Klewicki, 2015). Like all cancer types, CRC is characterized as an aggressive pathology with an invasive and disseminative character (Pande et al., 2008), aberrant crypt foci (ACF) or pre-neoplastic lesions being the precursory lesions (Bird, 1987). The induction of colorectal carcinogenesis in animal models by administering the drug 1,2-dimethylhydrazine (DMH) is a tool considered to be simple, practical and economic as a bioassay to evaluate the preventative character of dietetic agents (Newell & Heddle, 2004).

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https://doi.org/10.1016/j.foodres.2018.10.012
Received 5 November 2017; Received in revised form 22 September 2018; Accepted 2 October 2018
Available online 04 October 2018
0963-9969/

As a function of the benefits attributed to the consumption of probiotics, the elaboration of a juice containing jussara pulp as the carrier of *Lactobacillus rhamnosus* GG (LGG) results in a nutritive product with excellent bioactive potential, not just because of the presence of the probiotic, but also due to the elevated anthocyanin content present in this exotic fruit from the Brazilian Atlantic Forest (Moreira et al., 2017; Schulz et al., 2015).

Thus, the main objective of this study was to evaluate the resistance of LGG in a mixed fermented pineapple and jussara juice when submitted to *in vitro* and *in vivo* simulated gastrointestinal conditions, and investigate the effects of product ingestion on the biochemical characteristics of the blood and in the development of aberrant crypt foci in Wistar rats.

2. Material and methods

2.1. Preparation of the pineapple and jussara pulps

Three batches of pineapple fruits (*Ananas comosus* L. Merril) were acquired from the market in the city of Rio Pomba, MG, Brazil in an adequate state of ripeness. The fruits were washed under running water, sanitized in a 200 mg/L chlorinated solution, rinsed in potable water containing 10 mg/L of active chlorine, peeled, cut into pieces and the pulp obtained using an industrial blender (model R16720, Walita).

Three batches of frozen jussara (*Euterpe edulis* Martius) pulp (20 Kg) were obtained from a producer in the city of Rio Pomba, MG, Brazil and packaged in polyethylene containers each with a capacity of 500 g. The pineapple and jussara pulps were frozen a − 18 °C.

2.2. Elaboration of the pineapple and jussara juice fermented by LGG

After thawing of fruit pulps for 6 h at 5 °C, the juice was formulated containing 50% pineapple pulp, 45% jussara pulp and 5.0% sucrose. After the formulation, the mixed juice was subjected to the pasteurization process at 88 °C / 2 min (Moreira et al., 2017). Then, the product was cooled to 37 °C and 10^{10} cells of LGG (Culturelle®) added to 200 mL of the cooled juice. This bacterium was chosen due to reports in the literature as a probiotic with high resistance to the gastrointestinal tract (GIT) besides technological robustness (Goldin et al., 1992). This formulation was denominated the pre-inoculum and was incubated for 24 h at 37 °C in an incubator (Nova Ética 403 – 5D, Vargem Grande do Sul, São Paulo, Brazil), characterizing the adaptive phase of the microorganism.

After the incubation period, 10 mL aliquots of the pre-inoculum were added to flasks containing 200 mL of the juice and incubated at 37 °C for 72 h to ferment the product in order to increase the stability of phenolic compounds as anthocyanins due to pH reduction (He, Li, Lv, & He, 2015), besides the maintenance of the color of the juice. Thereafter, the flasks were subsequently stored at 8 °C (Novatecnica NT 704, Piracicaba, São Paulo, Brazil) for up to 28 days.

2.3. Evaluation of the physicochemical characteristics of the pulps and juices

The pH value, titratable acidity (g citric acid/ 100 mL of product), total soluble solids (TSS), lipid and protein contents of the pulps and juices were determined according to AOAC (2016). The moisture content and the fixed mineral residue of the pulps were also determined according to AOAC (2016). The carbohydrate content of the pulps was determined by difference (100 – (% moisture + % ash + % lipids + % protein)) according to James, Rotimi, and Bamaiyi (2010). The color was analyzed according to the parameters described by Moreira et al. (2017).

2.4. Determination of the total phenolic compounds, anthocyanins and antioxidant capacity of the pulps and juices

The phenolic compounds of the samples were determined using the Folin-Ciocalteau method (Singleton, Orthofer, & Lamuela-Raventós, 1999) and the results expressed in mg of gallic acid equivalents (GAE) per 100 g of sample (mg GAE/100 g). The anthocyanin contents of the pulp and juice samples were determined according to Lees and Francis (1972) and the results expressed in mg anthocyanin per 100 g of product. Aliquots of the pulps and juices were tested for their antioxidant capacity according to the ABTS [2,2′-azinobis (3-ethylbenzthiazoline sulfonic acid-6)] radical capture method described by Re et al. (1999) and the results expressed in μM Trolox/g of product.

2.5. Evaluation of the viability of LGG

The viability of LGG was determined immediately after elaborating the juice (time 0 day), after the fermentation period (time 3 days) and after 7, 14, 21 and 28 days of storage at 8 °C. This probiotic bacterium was quantified according to Richer and Vedamuthu (2001) in deMan, Rogosa and Sharpe agar (MRS, Merck, Darmstadt, Germany), once it develops well and the colonies have a unique morphology when cultured on MRS agar (Goldin et al., 1992). As a negative control, lactic acid bacteria were counted on MRS agar into non-LGG inoculated juice.

2.6. Simulation of in vitro gastrointestinal conditions

The methodology described by Bedani, Rossi, and Saad (2013) was used, simulating the gastric, enteric I and enteric II phases, and applying soon after elaboration of the juice (time 0 day), after fermentation of the juice (time 3 days) and after 7, 14, 21 and 28 days of storage of the fermented juice maintained at 8 °C.

2.7. Evaluation of the microbiological quality of the pulps and juices

Petrifilm™ plates (3 M™ EC 6404, Saint Paul, USA) were used to analyze for total coliforms with differentiation of *Escherichia coli*, according to the manufacturer's instructions. The presence or absence of *Salmonella* sp. was determined in 25 g of the samples, homogenized in 225 mL of lactose broth (MicroMed/Isofar, Duque de Caxias, Rio de Janeiro, Brazil) using the methodology of Andrews, Flower, Silliker, and Bailey (2001). The pulps were evaluated before processing of the juices, immediately after preparation (time 0 day) and after 4 weeks of storage at 8 °C.

2.8. Evaluation of the effect of ingesting the probiotic juice on the development of pre-neoplastic lesions in Wistar rats

2.8.1. Animals and treatments

Forty male Wistar rats with a mean weight of 105 g and 8 weeks old were randomly divided into four groups (G1, G2, G3 and G4), each group with 10 animals. The formula proposed by Mera, Thompson, and Prasad (1998) was used to calculate the number of animals in each experimental group, the baseline values being extracted from the study of Klewicka et al. (2012). The groups denominated as G1 and G2 represented the negative and positive controls, respectively, being fed exclusively on a commercial animal feed (Purina®). Groups G3 and G4, in addition to the animal feed, were force-fed 1.0 mL/day of the developed juice for a period of 10 weeks. To simulate the stress of force-feeding, the animals in G2 were force-fed 1.0 mL/day of distilled water. The experiment lasted 10 weeks and during this period the animals were maintained in individual cages under 12 h light/dark cycles at a mean temperature of 23 °C and received feed and water *ad libitum*. The weights of the animals were monitored at the start and end of the experiment.

2.8.2. Chemical induction of pre-neoplastic lesions

On the second and third weeks of the experiment the animals in groups G2 and G3 were submitted to a total of four intraperitoneal injections of 40 mg/Kg of body weight of 1,2-dimethylhydrazine (DMH) (Sigma-Aldrich, Saint Louis, Missouri, USA), with two applications per week on non-consecutive days, the drug being prepared according to Newell and Heddle (2004). On the seventh week counted as from the last application of DMH, the animals were euthanized in the Experimental Nutrition Laboratory (DNS/UFV) in an atmosphere which conferred peace to the animals. The entire protocol conformed with the Principals of Ethics in Animal Experimentation adopted by the Brazilian National Council for the Control of Animal Experimentation, in agreement with the approval provided on August 20th 2015 by the Ethics Commission for the Use of Animals of the Federal University of Viçosa (CEUA/UFV), Brazil (process number 46/2015).

2.8.3. Biochemical evaluation of the blood

The blood samples of the 40 animals were collected immediately after euthanasia in disposable tubes containing separation gel, and centrifuged at 3500 rpm for 10 min. The samples were maintained under refrigeration until analyzed. The determinations of the biochemical parameters of glucose, urea, creatinine, total cholesterol and fractions: high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL), triglycerides, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were carried out in the Biosystems apparatus – A15 (Barcelona, Spain) using Labtest® kits.

2.8.4. Weighing of right kidney and liver and determination of the hepatosomal index

The right kidney and liver of each animal were weighed (Shimadzu BL3200H, São Paulo, SP, Brazil). The hepatosomal index was determined from the ratio between the weight of the liver and the body weight of the animal (Sadekarpawar & Parikh, 2013).

2.8.5. Identification and quantification of LGG in the rat feces

A feces pool was collected for each experimental group after 14, 28, 49 and 70 days as from the start of the experiment, and placed in sterile flasks. The samples were diluted in saline solution and deep plated in *Lactobacillus* Anaerobic MRS Agar with Vancomycin and Bromocresol Green (LAMVAB), modified by the addition of a sterile 20 mg/mL aqueous solution of fluconazole (Pharmanostra, Rio de Janeiro, RJ, Brazil) which had the objective of inhibiting the development of yeasts, followed by incubation at 37 °C for 72 h.

The lactic bacteria were counted after the incubation period and the differential count of LGG based on the morphology of typical colonies of the strain under study, considering the creamy, regular and slightly yellow colonies in the modified LAMVAB agar. The morphotinal and biochemical characteristics were also considered, since LGG is characterized as short, narrow Gram-positive rods, grouped in palisades, catalase negative and differentiated from the other sub-species of L. *rhamnosus* for not fermenting lactose, maltose or sucrose (Goldin et al., 1992; Peña et al., 2004).

The number of CFU/g of feces was calculated as a function of the number of typical colonies counted, the dilution and the percent of colonies confirmed based on a minimum of 20 colonies tested per feces sample. The pure LGG culture (Culturelle®, ATCC 53103) was used as the positive control for identification of the strain under study and L. *rhamnosus* Lc 705 as negative control. The total DNA of the isolates and of the controls was also extracted according to the manufacturer's instructions (BIONEER - AccuPrepGenomic DNA Extraction Kit Cat.# K-3032) and the strain LGG confirmed using the polymerization chain reaction (PCR) technique as described by Brandt and Alatossava (2003).

The PCR reaction consisted of 10 mM Tris–HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0,1% Triton X-100, 0.2 mM deoxynucleotide triphosphates (dNTPs), 25 μL of 10 mM each primer, 0.2 μL of 5 U Taq DNA polymerase (GBT-Biosciences, Curitiba, PR, Brazil), and 100 ng of DNA in a final volume of 25 μL. Primers based on region of DNA encoding an inserted lysogenic phage, specific for L. *rhamnosus* GG (1F - 5' CAATC TGAATGAACAGTTGTC and 2R - 5' TATCTTGACCAAACTTGACG) were previously described by Brandt and Alatossava (2003) and synthesized by GBT-Biosciences (Curitiba, PR, Brazil).

The amplifications were carried out in a thermal cycler (Amplitherm, ThermalCyclers, modelo TX 96). The DNA was initially denatured at 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s. Finally, products were extended for 10 min at 72 °C. PCR products were separated on 1.5% agarose gel in Tris-borate/EDTA electrophoresis buffer (TBE) (89 mM Tris–HCl, 2.5 mM EDTA pH 8.0, 89 mM borate) and stained with GelRed (Biotium Corporate Headquarters, Start Bioscience, Fremont, Canada). The gels were photographed under UV light (Loccus Biotecnologia, L-PIX Imagem EX, Cotia, SP, Brasil).

In parallel, the viability of the LGG in the juice administered to the animals was determined by plating in MRS agar according to Richter & Vedamuthu (2001), for comparative purposes.

2.8.6. Determination of the fecal pH value

The fecal pH value of each experimental group was determined at approximately fifteen day intervals as from the start of the experiment, using a digital pH meter, the sample being diluted by mixing 1 g of feces in 10 mL distilled water (Asvarujanon, Satoshi, & Hara, 2005).

2.8.7. Determination of the short chain fatty acids in the feces

After euthanasia the feces in the caeca were collected in microtubes and stored at −80 °C for the short chain fatty acid (acetic, propionic and butyric) analysis. The feces were extracted with 12% orthophosphoric acid and the extracts analyzed by high performance liquid chromatography (HPLC) following the methodology of Zhao, Nyman, and Jönsson (2006) using the chromatograph with a C18 (Supelco) column, coupled to an ultraviolet detector (THERMO model ACCELLA PDA), taking readings at a wavelength of 210 nm.

2.8.8. Quantification and characterization of aberrant crypt foci

After euthanasia, the colons of the animals were removed and opened using a longitudinal cut along the mesenteric band. They were then washed using a 0.01 M phosphate buffer solution, placed in paraffin plates with the mucus uppermost and submitted to histological fixing for 24 h in 10% formol (Vetec, Duque de Caxias, Rio de Janeiro, Brazil). The histological preparations were measured and divided into three equal quadrants identified as proximal, medial and distal in relation to the caecum. The counting and characterization of the aberrant crypt foci (ACF) was carried out by two observers (double blind) according to the Bird technique (Bird, 1987) using an optical microscope (Alltion, Wuzhou, China) with an X10 lens. The ACF were characterized by observation of the luminal opening dilations, the epithelial thickness and the color intensity as compared to adjacent cells, being classified as ACF ≤ 3 and ACF > 3, based on the multiplicity of aberrant crypts per focus.

2.9. Statistical analyses

The experiments were carried out in three repetitions, the physicochemical, microbiological and LGG viability analyses being carried out in duplicate and the *in vitro* assay under gastrointestinal conditions in triplicate.

A (2×5) factorial entirely random design (ERD) was used to carry out the physicochemical analyses (pH, acidity, color and TSS) at 5 points in time for the juices with and without the probiotic culture.

The analyses for antioxidant capacity, and the phenolic compound and anthocyanin contents were evaluated using a (2×3) factorial ERD at 3 points in time for the juice with and without the probiotic culture.

The LGG viability and resistance experiment under simulated *in*

vitro gastrointestinal conditions was also carried out by ERD, the treatments being evaluated with time. A variance analysis of the regression was carried out with determination of the significance of the parameters by the F test at 5% of probability.

The *in vivo* experiment was carried out using a (2 × 3) factorial design (2 groups and 3 intestinal cuts) with 5 animals per treatment. An analysis of variance of the experiment was carried out, considering 5% of probability in the F test.

The results of the other analyses were evaluated by means of a variance analysis which was checked prior for its normality (Shapiro-Wilk test) and uniformity (Levene's Test) and the means compared by the Tukey test at 5% of probability. All statistical procedures were carried out considering a 5% level of probability and using the statistical software STATISTICA 7.0 (StatiSoft, Inc., Tulsa, Okla., USA).

3. Results and discussion

3.1. General characteristics of the pineapple and jussara pulps

The pineapple and jussara pulps conformed to the physicochemical and microbiological standards established by the Brazilian legislation (Brazil, 2000; Brazil, 2001). In addition, these pulps are nutritious and technologically viable for the manufacture of juice mixture (Table 1). The physicochemical composition and the presence of bioactive compounds in the pineapple pulp, combined with the aroma of this fruit showed that it has good features for the development of jussara and pineapple juice mixture.

3.2. pH values, acidity and TSS contents of the juices

The values for pH and acidity of the control and fermented juices differed ($p < .05$) and the time was significant for the treatment containing the probiotic culture. Fermentation of the juice caused a reduction in the pH value and increase in the titratable acidity between 0 and 3 days ($p < .05$). After fermentation pH and acidity values remained stable (Table 2). No differences ($p > .05$) were observed for the TSS between treatments or with time ($p > .05$) during the 28 days of product storage (Table 2).

Table 1
Mean results for the physicochemical and microbiological characterizations of the pineapple and jussara pulps.

Characteristic	Pineapple	Jussara
pH value	3.50 ± 0.02a	4.90 ± 0.03b
Total acidity expressed as citric acid (g/100 g)	0.82 ± 0.004b	0.13 ± 0.002a
Soluble solids (°Brix)	11.5 ± 0.06b	2.6 ± 0.06a
Moisture content (g/100 g)	89.8 ± 1.4a	92.0 ± 2.4b
Total solids (g/100 g)	10.2 ± 1.4a	8.0 ± 1.4b
Lipids (g/100 of dry matter)	0.98 ± 0.44a	33.36 ± 1.55b
Protein (g/100 g of dry matter)	4.51 ± 0.78a	7.87 ± 2.25b
Carbohydrate (g/100 g of dry matter)	91.57 ± 1.4b	54.75 ± 20.93a
Fixed mineral residue (g/100 g of dry matter)	2.94 ± 0.46a	4.00 ± 1.16b
L*	58.5 ± 0.02b	46.4 ± 0.08a
a*	−0.3 ± 0.06a	0.6 ± 0.06b
b*	2.4 ± 0.12b	1.1 ± 0.06a
Total phenolic compounds (mg GAE/100 g)	370.2 ± 2.22a	4918.5 ± 705.2b
Anthocyanin (mg/100 g)	–	1570.6 ± 18.5
Antioxidant capacity (μM Trolox/g)	347.1 ± 12.3a	933.0 ± 75.4b
Total coliforms (log CFU/g)	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$
Escherichia coli (log CFU/g)	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$
Salmonella sp. in 25 g	Absent	Absent

(−): Not determined.

Table 2
Results for pH value, acidity and TSS of the control pineapple plus jussara juice and of that fermented by L. *rhamnosus* GG.

Treatments	Time (days)	Physicochemical characteristics		
		pH	Acidity	Total soluble solids (°Brix)
Control juice	0	4.2 ± 0.13 Aa	0.31 ± 0.04 Ba	12.8 ± 0.6 Aa
	3	4.1 ± 0.17 Aa	0.31 ± 0.04 Ba	12.7 ± 0.4 Aa
	7	4.1 ± 0.25 Aa	0.32 ± 0.03 Ba	12.7 ± 0.5 Aa
	14	4.2 ± 0.10 Aa	0.33 ± 0.02 Ba	12.9 ± 0.5 Aa
	21	4.0 ± 0.12 Aa	0.34 ± 0.02 Ba	12.9 ± 0.7 Aa
	28	3.9 ± 0.19 Aa	0.33 ± 0.04 Ba	12.9 ± 0.3 Aa
Probiotic juice	0	4.1 ± 0.18 Aa	0.32 ± 0.04 Bb	12.6 ± 0.6 Aa
	3	3.2 ± 0.39 Bb	0.92 ± 0.18 Aa	12.4 ± 0.6 Aa
	7	3.1 ± 0.43 Bb	0.94 ± 0.12Aa	12.5 ± 0.6 Aa
	14	3.2 ± 0.21 Bb	0.94 ± 0.10 Aa	12.5 ± 0.7 Aa
	21	3.1 ± 0.25 Bb	0.99 ± 0.14 Aa	12.8 ± 0.7 Aa
	28	3.0 ± 0.29 Bb	1.00 ± 0.07 Aa	12.6 ± 0.5 Aa

Capital letters reflect comparisons between different treatments for the same time. Small letters reflect comparisons between different times for the same treatment. Means followed by the same letter in the same column do not differ from each other according to the Tukey test at 5% of probability.

3.3. Color of the mixed juices

There were no differences ($p > .05$) in luminosity (L*) between the treatments with time, providing evidence that the addition of LGG did not darken the juice. On the other hand, the coordinates a* and b* differed ($p < .05$) between the control juice and that containing LGG, indicating that the addition of the probiotic bacterium interfered with these color parameters (Table 3). There was no interaction with time ($p > .05$) within the same treatment.

Color is an extremely important characteristic in the evaluation of the sensory quality of food on the market (Stich, 2016; Wu & Sun, 2013). The color of the juice was based on the pigmentation of the jussara fruits, which, when ripe, tend to a purple/black color due to the elevated anthocyanin content (Schulz et al., 2015). Fermentation of the probiotic juice resulted in a fall in the pH values, and consequently the conversion of the anthocyanins to their most stable form, with a higher value for a* (Table 3) and tendency to red ($p < .05$).

The probiotic juice also presented a higher value for b* than the control treatment ($p < .05$), characterizing a greater tendency for yellow (Table 3). Moreira et al. (2017) obtained a similar result at the end of 30 days of refrigerated storage for a high-pressure processed mixed jussara and Ubá mango juice with added LGG.

3.4. Total phenolic compounds, total anthocyanins and antioxidant capacity

The control and fermented juices differed ($p < .05$) with respect to the total phenolic compound contents (Table 3) but the time was not significant for a given treatment ($p > .05$). This suggests that the lower pH of the probiotic juice was responsible for the difference in values of the phenolic compounds of the treatments, since the co-pigmentation of these compounds is favored by acidification of the medium (Trouillas et al., 2016).

With respect to the total anthocyanin contents and antioxidant capacities there was no difference ($p > .05$) between the treatments or throughout the storage period (Table 3), showing the stability of these compounds. Moreira et al. (2017) found similar results for antioxidant capacity when studying a mixed jussara and Ubá mango juice with added LGG.

Inada et al. (2015) considered the jussara fruits to be sources of vitamins A and E, and pineapple a source of vitamin C (Sun, Zhang, Soler, & Marie-Alphonsine, 2016; Wijeratnam, 2016). However, one

Table 3
Mean results obtained for the coordinates L*, a* and b* in the color determination and for the total phenolic compounds, total anthocyanins and antioxidant capacity of the mixed juices.

Treatments	Coordinates			Total phenolic compounds (mg GAE/100 mL)	Total anthocyanins (mg/100 mL)	Antioxidant capacity (μM Trolox/g)
	L*	a*	b*			
Control juice	18.6 ± 0.4a	2.55 ± 0.4b	0.57 ± 0.1b	2510.1 ± 55.0b	437.2 ± 227.6a	338.0 ± 36.6a
Probiotic juice	18.5 ± 0.3a	4.07 ± 0.6a	0.77 ± 0.2a	1978.3 ± 51.5a	438.5 ± 202.8a	339.0 ± 38.0a

Means followed by the same letter in the same column do not differ from each other according to the Tukey test at 5% of probability.

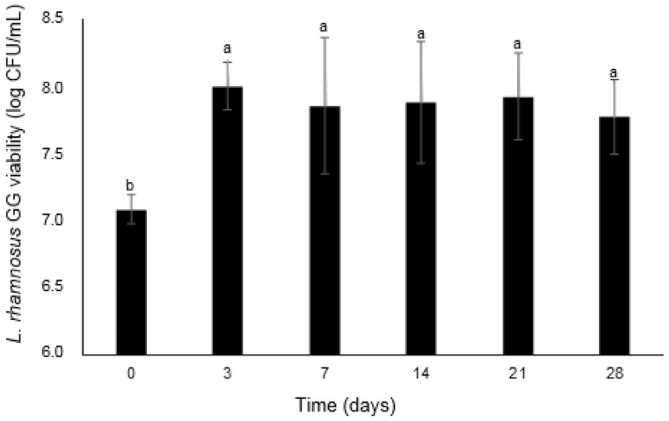

Fig. 1. Viability of L. *rhamnosus* GG in the fermented pineapple plus jussara juice stored for 28 days at 8 °C. Means followed by the same letter do not differ from each other according to the Scott-Knott test at 5% of probability.

should consider that the mixture of the two fruits contributed to the high antioxidant capacity of the mixed juices elaborated in the present study.

3.5. Viability of the LGG in the juices

The LGG presented counts above 7.2 log CFU/mL throughout the 28 days of storage of the juice at 8 °C (Fig. 1) and in the non-LGG inoculated juice (negative control), the count of lactic acid bacteria were < 1.0 log CFU/mL. Although consensus does not exist concerning the minimum amount of probiotic microorganisms necessary to provide beneficial effects for the organism (Martins et al., 2016), some authors consider that one needs > 10^6 CFU/g or mL of food (Hussain, Patil, Yadav, Singh, & Singh, 2016), whereas others suggest between 10^6 and 10^7 CFU/g (Madureira, Amorim, Gomes, Pintado, & Macata, 2011), or even 10^9 to 10^{10} CFU/g (Naidu, Adam, & Govender, 2012). The ingestion of 100 mL/day of the jussara plus pineapple juice fermented with LGG provides the consumer with a minimum of 9.2 log CFU/day, and hence the juice developed is a promising vehicle for LGG. Fermentation favored multiplication of the microorganism, presenting higher counts after this stage (Fig. 1), and maintenance of the LGG count in the product from the 3rd to the 28th day of refrigerated storage was verified (Fig. 1).

Fermentation causes desirable changes in the product, such as aggregation of characteristic aromas, increase in the bioavailability of minerals (Taylor, 2016) and the suppression of contaminating flora. This process also leads to microbiological safety due to lowering of the pH value as well as increasing the amount of short chain fatty acids, among other benefits (Klewicka et al., 2015; Klewicka, Zduńczyk, & Juśkiewicz, 2009).

The use of fruits and vegetables as vehicles for probiotic microorganisms represents a challenge (Shori, 2016). Moreira et al. (2017) verified that the jussara and Ubá mango juice mixture with added L. *rhamnosus* GG can be considered as a food with functional appeal, since this probiotic bacterium proved to remain viable during storage of the juice. Pereira et al. (2017) also verified that the probiotic cupuaçu (*Theobroma grandiflorum*) beverage containing L. *casei* NRRL B-442 was well accepted as an alternative functional food.

However, various factors can influence the viability of the probiotic microorganism. They might be inherent to the food matrix such as fat and protein contents, sugar composition, pH and the presence of antimicrobial substances, besides those linked to the process (oxygen level, presence of preservatives, storage time and temperature). In addition, the correct choice of probiotic strain is indispensable for the success of the formulation. Microorganism *versus* matrix interaction must aid resistance of the probiotic during its passage through the GIT (Burns, Lafferriere, Vinderola, & Reinheimer, 2014; Espírito Santo, Perego, Converti, & Oliveira, 2011).

3.6. In vitro survival of LGG under the simulated gastrointestinal conditions

One essential requirement for a food to be considered probiotic is that the bacterial strain remain viable in elevated concentration as from its inoculation into the food up to the end of the shelf life, and be capable of surviving the conditions of the GIT, tolerating the acid conditions and the presence of bile salts (Madureira et al., 2011; Peres et al., 2014). *In vitro* survival trials under simulated GIT conditions are frequently used to verify the potential of a probiotic strain and also its suitability for the carrier matrix (FAO/WHO, 2001a; Bedani, Vieira, Rossi, & Saad, 2014; Perricone, Bevilacqua, Altieri, Sinigaglia, & Corbo, 2015).

On evaluating the behavior of the probiotic in the different phases, it was shown that for all times, the viability of the LGG in the juice before exposure to the gastric phase did not differ ($p > .05$) from the viability after passage through this phase for 2 h of incubation at pH 2.0–2.5. However, the viable cell counts of the bacterium after passage through enteric phases I and II were lower (< 0.05) for all times (Fig. 2).

It was shown that in enteric phases I and II, which simulate the small and large intestines, respectively, the LGG started out with minimal counts of 3.57 log CFU/mL and 4.18 log CFU/mL (time 0 day) and subsequently increased to 6.99 log CFU/mL and 6.73 log CFU/mL, respectively, after the three day fermentation period. There was a subsequent reduction in the counts to 5.66 log CFU/mL and 5.01 log CFU/mL, respectively, after the first week of storage at 8 °C. This suggests adaptation of the strain to the sub-lethal acid pH values of the juice, such that the product reached the end of its shelf life with 5.99 log CFU/mL of viable cells after passage through enteric phase II of the GIT simulated *in vitro* (Fig. 2).

Elizaquível et al. (2011) also showed that the inoculation of probiotic strains into orange juice with a pH value of 3.8, promoted an increase in resistance to more acid pH values, favoring survival of the bacteria to gastrointestinal stress. On the other hand, Oliveira et al. (2017) obtained in jabuticaba (*Plinia cauliflora*) juice LGG counts below 1.0 log CFU/mL at the end of *in vitro* simulation of GIT. The maximum viability of this bacterium was 6.05 log CFU/mL during 28 days of refrigerated storage. Thus, the carrier matrix exerted an action on the probiotic during its passage through the GIT. However, in the present study, fermentation allowed for adaptation of the microorganism to the matrix, which may also have favored the viable cell count.

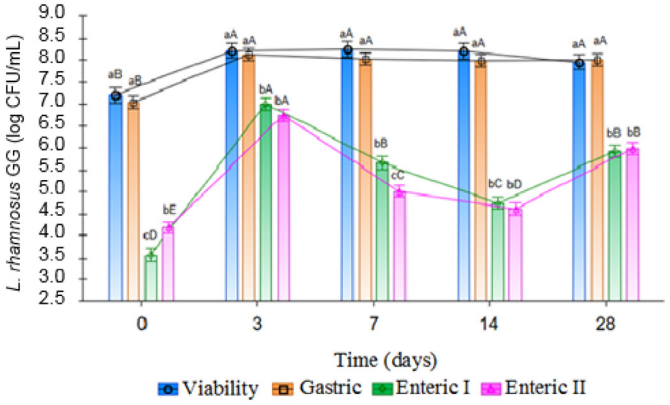

Fig. 2. Survival of L. *rhamnosus* GG in the *in vitro* GIT trial in pineapple plus jussara juice stored for 28 days of refrigerated storage. The bars show the viability of the strain in the juice not exposed to gastric stress, followed by the gastric, enteric I and enteric II phases of the GIT. The capital letters reflect comparisons between the same phases of the *in vitro* trial at distinct sampling times. Small letters refer to comparisons between different phases of the *in vitro* trial within the same sampling time. Means followed by the same letter do not differ between themselves according to the Tukey test at 5% of probability.

Madureira et al. (2011) considered that a minimum of 10^6 to 10^7 of live probiotic cells should reach the intestinal colon in order for a food to exert a therapeutic effect. Considering the viable cell count obtained after *in vitro* simulation of enteric phase II, and considering a minimal portion of 100 mL, one can allege that 7.6 log CFU of LGG would be available to promote benefits for the organism, making the food apt to be considered probiotic.

From the industrial point of view, the certification of probiotic viability is essential, due to the adverse conditions of the food matrix that affects the viability and the gastrointestinal resistance of these microorganisms. Thus, this work contributes to show the excellent viability and gastrointestinal resistance of *Lactobacillus rhamnosus* GG when carried in tropical fruit juice. Therefore, these results strengthen the processing and commercialization of this product ensuring the transmission of the probiotic to consumers.

3.7. Evaluation of the microbiological quality of the juices

The juices were conformed to Brazilian legislation (Brazil, 2000; Brazil, 2001), since the total coliform and *E. coli* counts were below 1.0×10^1 CFU/mL and all the samples showed the absence of *Salmonella* sp. in 25 mL.

3.8. Body mass of the rats with induced carcinogenesis

There was no difference ($p > .05$) in body mass gain between the groups of animals at the end of the 10 week experimental period (Table 4). Liboredo et al. (2013), on evaluating the protective effect of *Lactobacillus delbrueckii* UFVH2b20; *Bifidobacterium animalis* variety *lactis* Bb12; *L. delbrueckii* UFVH2b20 associated with *B. animalis* variety *Lactis* Bb12 and *Saccharomyces boulardii* in Swiss rats with pre-neoplastic lesions induced by DMH, also observed no difference in body mass gain at the end of 14 weeks of experimentation.

3.9. Evaluation of the blood parameters and the effect of the probiotic juice on the kidneys, liver and hepatosomal index of the Wistar rats

Despite the differences found ($p < .05$) in the biochemical parameters evaluated (urea, total cholesterol, LDL and AST) between the animal groups (Table 5), none of them were above the reference limits for Wistar rats (Melo, Dória, Serafini, & Araújo, 2012). Only the hepatic marker ALT was above the reference limit, extrapolating the normality standard for male Wistar rats (Table 5).

A difference was found ($p < .05$) in the values for urea between groups G1 and G4 (Table 5), suggesting that the juice promoted better nutrition for the animals. For total cholesterol there was a significant difference between groups G1 and G3 ($p < .05$), whereas for the fraction LDL, G1 differed from groups G3 and G4 ($p < .05$), both of which received the probiotic juice (Table 5). This result suggests a cardio-protective role for the juice under study. There are diverse factors that lead to coronary artery diseases, but an elevated cholesterol level is considered the main modifiable factor, and regulation of the serum LDL levels the main form of control, consequently avoiding infarction and death by heart disease (Baigent et al., 2010).

Huang, Chang, Kao, and Lin (2015) showed that antioxidant activity due to the presence of cianidin-3-glycoside promoted a reduction in total cholesterol and the LDL fraction in hamsters fed a fat-rich diet. It should be emphasized that cianidin-3-glycoside is the second largest anthocyanin present in jussara.

The hypocholesterolemic effect has also been attributed to probiotic microorganisms, and is one of the probable mechanisms for the inactivation of the bile salts function (Kumar et al., 2012). Thus, the results obtained indicate that the pineapple with jussara juice fermented by LGG could act in the reduction of cholesterolemia due to its antioxidant potential and action promoted by the probiotic.

With respect to the hepatic function markers, there was a difference ($p < .05$) between groups G3 and G4 for the enzyme AST (Table 5). Based on the values reported by Melo et al. (2012) for Wistar rats, this enzyme remained within the limits of normality, which differ considerably from the limits for humans (Table 5). However, the levels of the enzyme ALT were higher than the reference values for all the groups.

The administration of the drug DMH as an inducer of carcinogenesis leads to hepatocyte damage. DMH is metabolically activated in the liver by a series of reactions, giving rise to the intermediate metabolites azoxymethane and methylzoxymethanol, and finally the ion methyldiazonium, the metabolite responsible for alterations in the DNA molecules of various animal species in various organs including the liver (Rajeshkumar & Kuttan, 2003). The bioactivation process of DMH induces hepatotoxicity (Hedge, Mathews, Shivashankara, Prabhu, & Baliga, 2013). There were no indications that the mixed juice caused hepatocyte damage, which was desirable. This was proven once the

Table 4
Mean values for the body mass gain of the groups evaluated during the experimental period.

	Treatments			
	Group 1	Group 2	Group 3	Group 4
Initial mass	105.9 ± 19.9a	104 ± 20.8a	105.1 ± 24.0a	105.2 ± 22.4a
Final mass	368.4 ± 39.9b	345.1 ± 33.3b	345.6 ± 36.0b	367.5 ± 35.6b
Mass gain	262.5 ± 25.8c	241.1 ± 23.6c	240.5 ± 40.6c	262.3 ± 36.5c

Group 1 = Negative control (basal feed); Group 2 = Positive control (basal feed + DMH); Group 3 = Pineapple plus jussara juice fermented by L. *rhamnosus* GG + DMH; Group 4 = Pineapple plus jussara juice fermented by L. *rhamnosus* GG. Values expressed as the mean ± standard deviation, with $n = 10$. Means followed by the same letter in the same line do not differ from each other according to the Tukey test at 5% of probability.

Table 5
Mean values obtained for the biochemical analyses of the blood from the groups evaluated at the end of the experimental period.

Biochemical parameters	Treatments				Reference values	
	Group 1	Group 2	Group 3	Group 4	*Wistar* rats (males)*	Humans (males)**
Glucose (mg/dL)	130.7 ± 10.5a	124.1 ± 8.0a	125.7 ± 12.7a	129.4 ± 18.4a	79–144	60–99
Urea (mg/dL)	39.2 ± 7.3a	32.1 ± 4.0ab	32.8 ± 4.5ab	29.7 ± 7.2b	30–42	15–40
Creatinine (mg/dL)	0.25 ± 0.1a	0.25 ± 0.1a	0.25 ± 0.04a	0.25 ± 0.1a	0.44–0.64	0.6–1.1
Total cholesterol (mg/dL)	66.5 ± 6.1a	61.6 ± 9.9ab	56.2 ± 5.9b	57.8 ± 10.7ab	55–79	< 200
HDL (mg/dL)	39.8 ± 3.9a	40.0 ± 5.2a	37.8 ± 3.8a	38.0 ± 5.0a	NR	> 60
LDL (mg/dL)	18.7 ± 3.6a	14.0 ± 5.6 ab	11.5 ± 2.7b	11.1 ± 7.6b	NR	< 100
VLDL (mg/dL)	8.0 ± 1.8a	7.6 ± 1.8a	6.9 ± 2.5a	8.7 ± 3.1a	NR	< 30
Triglycerides (mg/dL)	40.2 ± 8.9a	37.8 ± 9.0a	34.6 ± 12.7a	43.3 ± 15.4a	42–160	< 150
AST (U/L)	145.8 ± 23.5ab	146.9 ± 15.8ab	165.2 ± 23.2a	130.8 ± 14.4b	81–180	< 31
ALT (U/L)	64.7 ± 15.9a	67.4 ± 12.5a	71.7 ± 8.9a	59.4 ± 5.8a	36–58	< 31
HIS	3,2 ± 0,16a	3,19 ± 0,12a	3,12 ± 0,13a	3,10 ± 0,18a	NR	NR

Source: * Melo et al. (2012); ** Pagana & Pagana (2001); Kahn (2003); Bastos (2011); Xavier et al. (2013); Group 1 = Negative control (basal feed); Group 2 = Positive control (basal feed + DMH); Group 3 = Juice + added L. *rhamnosus* GG + DMH; Group 4 = Juice plus added L. *rhamnosus* GG. NR: values not reported. Values expressed as the mean ± standard deviation, with n = 10. Means followed by the same letter in the same line do not differ from each other according to the Tukey test at 5% of probability.

drug was not administered to group G4, which received only feed and the probiotic juice.

There was no difference ($p > .05$) in the weights of the organs or in the hepatosomal Index (HSI) between the animal groups (Table 5), reinforcing the positive results obtained in the biochemical analyses of the blood. The biochemical blood analyses and the HSI evaluation suggest that the probiotic juice did not induce hepato- and/or nephrotoxicity, and was capable of regulating the cholesterolemic index in Wistar rats.

3.10. Lactic bacteria and LGG counts in the fecal samples from the Wistar rats

There was no difference (p > .05) in the standard plate count for lactic bacteria between the four groups of animals, or in the differential LGG count between the groups which received the probiotic juice (Table 6). Typical LGG colonies were confirmed by PCR to ensure the result of the standard plate count of this probiotic bacterium in the rats feces (Fig. 3).

FAO/WHO (2001a) determined the evaluation of the functionality of probiotic strains by way of comparative survival tests to bile salts in *in vitro* and *in vivo* trials, animal model tests being recommended before the human trials. The mean viability of LGG in the mixed pineapple and jussara juice prior to GIT simulation remained at 7.99 log CFU/mL, not differing ($p > .05$) from the viability of the bacterium in the product administered to groups G3 and G4 during the 10 weeks of the experiment, which had 7.96 log CFU/mL. Comparing the *in vitro* and *in vivo* trials, a reduction of approximately 2.3 log cycles of viable bacterial

Table 6
Lactic bacteria count and differential count for L. *rhamnosus* GG in the feces cultures.

Experimental groups	Lactic bacteria (log CFU/g)	*L. rhamnosus* GG (log CFU/g)
Group 1	6.9 ± 1.1a	< 1.0a
Group 2	7.1 ± 0.4a	< 1.0a
Group 3	6.7 ± 0.7a	5.6 ± 0.7b
Group 4	6.6 ± 0.6a	5.8 ± 0.4b

Group 1 = Negative control (basal feed); Group 2 = Positive control (basal feed + DMH); Group 3 = Basal feed + pineapple plus jussara juice fermented by L. *rhamnosus* GG + DMH; Group 4 = Basal feed + pineapple plus jussara juice fermented by *L. rhamnosus* GG. Values expressed as the mean ± standard deviation, with n = 10. Means followed by the same letter in the same column do not differ from each other according to the Tukey test at 5% of probability.

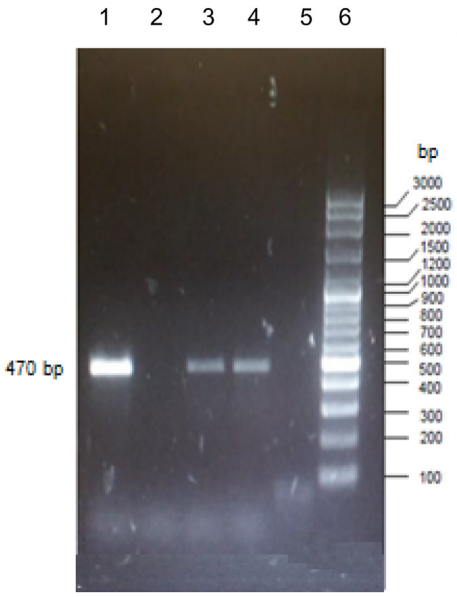

Fig. 3. Agarose gel electrophoresis 1.5% of PCR reaction products: (1) LGG; (2) *L. rhamnosus* Lc 705bacteria isolated from feces of the samples under study confirmed as LGG; (5) negative control - without DNA; (6) molecular weight marker (100 bp Plus DNA Ladder - gbtbio).

probiotic cells was found, in relation to the quantification of the probiotic in the product (Fig. 4). There was no difference (p > .05) between the mean LGG counts at the end of the *in vitro* GIT (enteric phase II) simulation and in the fecal samples (Fig. 4), indicating that the *in vitro* trial was efficient in proving that the mixed pineapple and jussara juice was an excellent carrier for the probiotic.

The capacity for intestinal adhesion and colonization of LGG stands out. According to Lebeer et al. (2012), the SpaCBA pilus of this bacterium was shown to be a fundamental structure for the efficient adhesion to the intestinal epithelium, favoring the formation of a biofilm on the Caco-2 epithelial cells. It should be emphasized that part of the LGG cells ingested by the animals in the mixed pineapple and jussara juice might not have been eliminated in the feces since they were adhered to the epithelium, resisting the intestinal peristalsis of the rats.

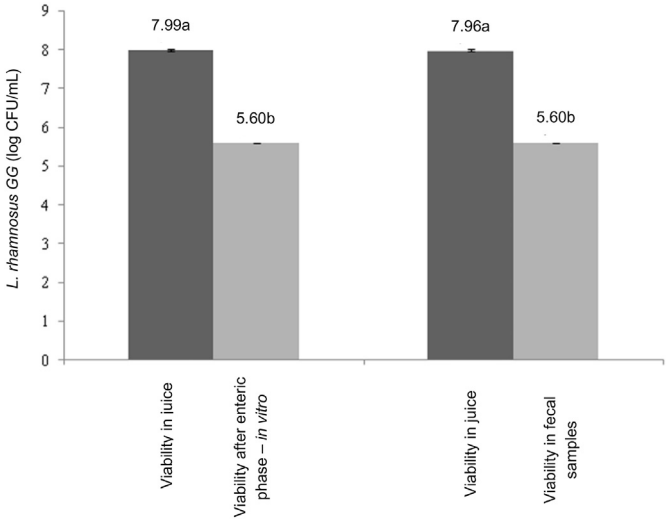

Fig. 4. Comparison between the survival of L. *rhamnosus* GG before and after the *in vitro* trial and before and after passage through the GIT in the *in vivo* trial. The same letters indicate the treatments do not differ between themselves at 5% of probability according to the Tukey test.

3.11. Evaluation of the fecal pH and quantification of the short chain fatty acids

A difference ($p < .05$) was observed between the initial and final fecal pH values for the groups of animals, obtaining results of 5.9 and 8.0, respectively, during the experiment. These results were different from the expected values, since the basal fecal pH of the animals (prior to starting to administer the juice) was lower than that determined at the end of the tenth week of the study (Fig. 5). Raman et al. (2013) stated that the lowering of intestinal pH by probiotics has been reported as one of the anti-mutagenic effects for the prevention of colon and rectum cancers, being cited in association with the inactivation of carcinogenic substances, immune-modulating effects, modulation of the intestinal flora, modulation of apoptosis and cell differentiation.

It was shown that for groups G1 and G4, the pH value of the sample collected directly from the caecum at the moment of euthanasia was 7.5. According to Osuka et al. (2012), the pH of the caecum is in the range from 5.5 to 7.5, leading to the consideration that although the probiotic juice did not cause a lowering of the intestinal pH, it also did not lead to an alteration in the physiological pH of the animal species.

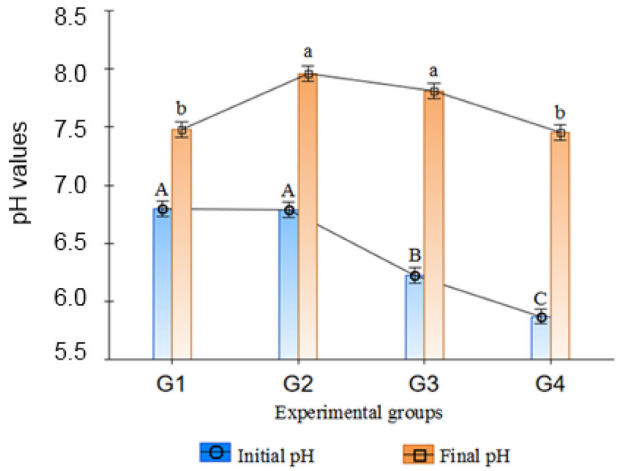

Fig. 5. Initial and final values for the fecal pH of the experimental groups: G1 = Negative control (basal feed); G2 = Positive control (basal feed + DMH); G3 = Basal feed + pineapple plus jussara juice fermented by L. *rhamnosus* GG + DMH; G4 = Basal feed + pineapple plus jussara juice fermented by *L. rhamnosus* GG. Means followed by the same letter do not differ from each other according to the Tukey test at 5% of probability.

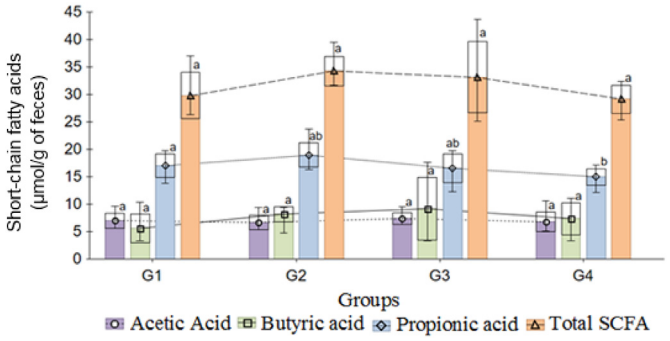

Fig. 6. Short chain fatty acid concentrations in the animal feces. G1 = Negative control (basal feed); G2 = Positive control (basal feed + DMH); G3 = Basal feed + pineapple plus jussara juice fermented by *L. rhamnosus* GG + DMH; G4 = Basal feed + pineapple plus jussara juice fermented by *L. rhamnosus* GG. Means followed by the same letter do not differ from each other according to the Tukey test at 5% of probability.

In general, the lowering of the intestinal pH value is attributed to the colonic fermentation of carbohydrates, giving rise to the short chain fatty acids (SCFA) acetate, propionate and butyrate. There was no difference ($p > .05$) between the groups of animals for the concentrations of the SCFA acetic and butyric acids. However there was a difference ($p < .05$) for the concentration of propionic acid between groups G1 and G4 (Fig. 6). Propionate is involved in the hepatic metabolism, acting as a modulator in the production of cholesterol and a precursor of gluconeogenesis, also being an inducer of apoptosis, although to a lower degree than butyrate (Cook & Sellin, 1998; Roy, Kien, Bouthillier, & Levy, 2006; Topping & Clifton, 2001).

The genus *Lactobacillus* and *Bifidobacterium* are responsible for the first fermentation stage of the prebiotics, giving rise to lactic, pyruvic and acetic acids, which are used as substrates by other colonic bacteria to metabolize butyric and propionic acids (Fernández et al., 2016). Walker, Duncan, Leitch, Child, and Flint (2005) stated that at pH values above 6.5, the bacteria responsible for the production of butyrate, *Roseburia* spp. and *Faecalibacterium prausnitzii* are found in reduced numbers, and the propionate-producing *Bacteroides* become dominant. This condition could explain why the propionic acid concentration was higher than the acetic and butyric acids concentrations for all the animal groups in this study, since the minimal fecal pH value found at the end of the experiment was 7.5. Thus, in order to promote an increase in the production of SCFA, the authors believe that the addition of prebiotics to the juice formulation elaborated would exert a promising effect.

3.12. Quantification and differentiation of the aberrant crypt foci (ACF)

No differences were observed ($p > .05$) in the multiplicity of the crypts independent of the cut evaluated, for the categories ACF ≤ 3 and ACF > 3 (Fig. 7). The ACFs are biomarkers of colon cancer (Fig. 8), being the first detectable abnormalities in the intestine of model animals chemically induced for carcinogenesis (Khare, Chaudhary, Bissonnette, & Carroll, 2006). Characterization of the number of crypts is considered an important parameter to evaluate the advance of a lesion, since the increase in size of an ACF appears to be a process of division of the crypt itself (Cheng & Lai, 2003).

Raju (2008) affirmed that after 8 weeks ACF with 1, 2 and 3 crypts were more frequent, evolving in the following weeks to an increase in multiplicity of the crypts and a regression of the lesions with smaller numbers of crypts. Therefore, the duration of the experiment after the induction of carcinogenesis was significant to determine the

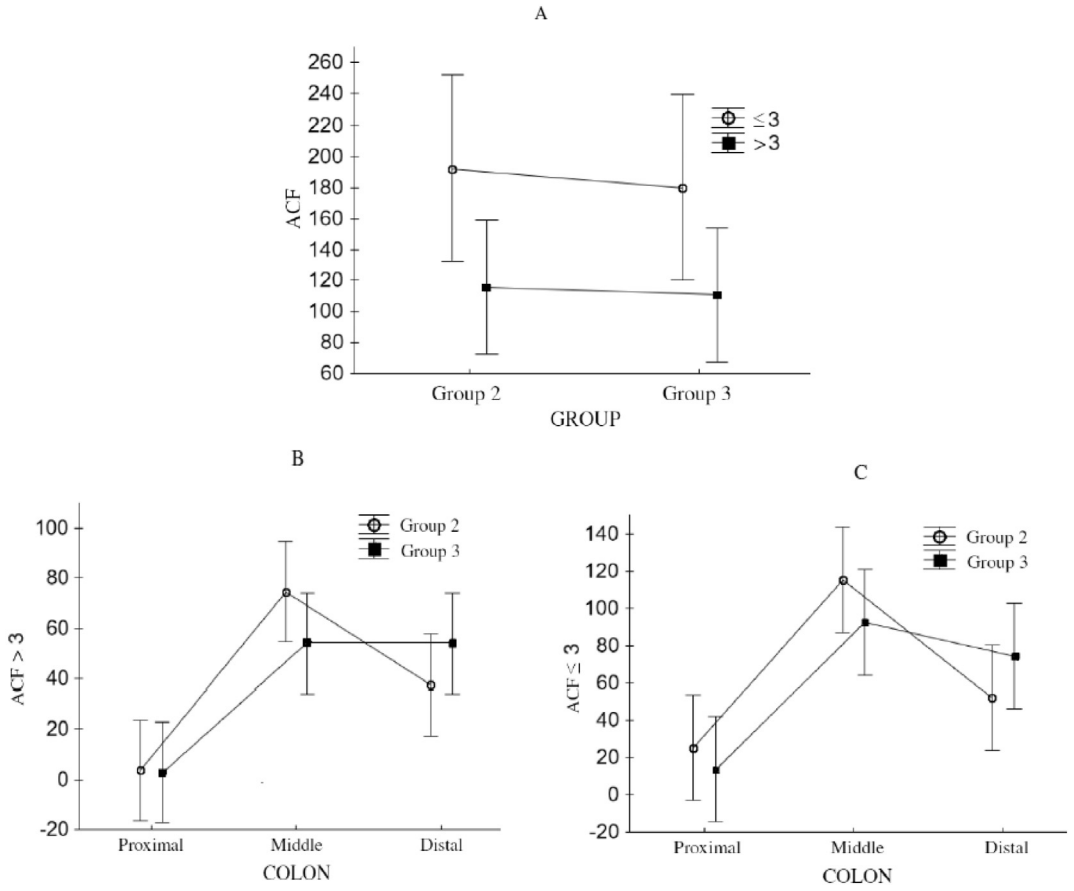

Fig. 7. Mean number of ACF in the colons of the Wistar rats, 10 weeks after treatment. (A) Mean total number of ACF categorized as ≤3 and > 3, differentiated per group; (B) Mean number of foci with ≤3 crypts, differentiated according to group and intestinal cut; (C) Mean number of foci with > 3 crypts, differentiated according to group and intestinal cut;. Group 2 = Positive control (basal feed + DMH) and Group 3 = basal feed + pineapple plus jussara juice fermented by *L. rhamnosus* GG + DMH. Values expressed as the means with a confidence interval, $n = 5$.

preventative character or control capacity that certain foods or nutritive agents exerted on the disease. Liboredo et al. (2013) and Gomides et al. (2015), on studying the action of probiotics and of defatted linseed flour, respectively, during 14 weeks, also observed the protective effect against the appearance of ACF in animals with carcinogenesis induced by DMH.

The absence of a protective effect on the intestinal epithelium in the group with induced carcinogenesis, which received a daily dose of probiotic (G3), could also be associated with the fact that no increase in the production of SCFA was observed, and consequently no reduction in the intestinal pH value. Pathogenic and putrefactive bacteria specific to the intestine, inhibited by intestinal acidification, are initiators and promotors of inflammatory processes that favor continuity of the aggression to the tissue, due to debility of the barrier function of the intestinal epithelium (Jobin, 2012; Uronis et al., 2009). Thus, the addition of prebiotics to the juice could favor a smaller incidence of ACF due to a reduction in aggression of the intestinal epithelium by pro-inflammatory mediators.

4. Conclusions

The juices elaborated were considered products with elevated

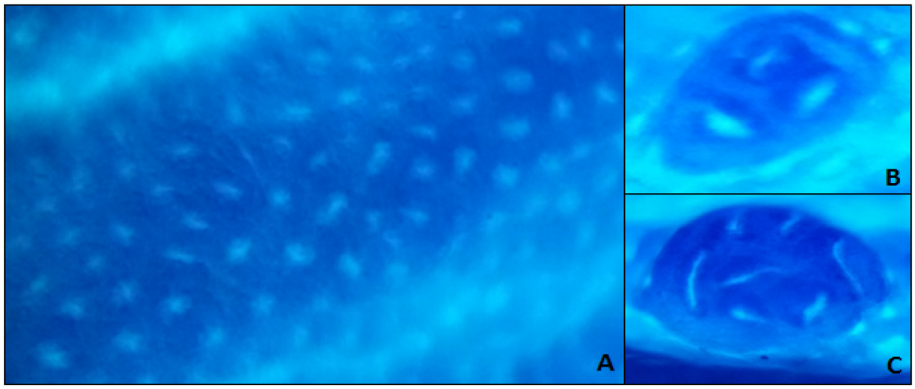

Fig. 8. Intestinal epithelium dyed with 0.1% methylene blue, magnification of x10. (A) healthy epithelium; (B) focus with 3 crypts; (C) focus with 8 crypts. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

antioxidant potential and the LGG remained viable throughout the shelf life of the product, with counts above 9.2 log CFU per 100 mL portion. A comparison of the results of the *in vitro* and *in vivo* trials showed that the *in vitro* simulation of GIT was an efficient methodology to prove that the mixed pineapple and jussara juice was an excellent vehicle for LGG. At least 5.6 log CFU/mL of LGG reached the simulated large intestine, not differing from the total number of live cells that survived the digestive tracts of the animals. The elaborated product conformed with the survival requirements of a probiotic to bile salts, both *in vitro* and *in vivo*, as recommended by FAO, and remained apt for human consumption since it conformed to the microbiological standards recommended by Brazilian legislation.

Based on the reference values for the renal markers urea and creatinine and the hepatic markers ALT and AST for Wistar rats, the juices did not show hepato- or nephrotoxicity. As the *in vivo* assay was performed, these evaluations were important to demonstrate to consumers the safety of the product obtained.

The probiotic juice was not able to promote an increase in the production of short chain fatty acids and consequent acidification of the intestinal pH. It was suggested that the fecal pH value above 6.5 favored predominance of propionic acid and suppressed colonic production of butyric acid, this being the main energy source for the intestinal epithelium, contributing to the barrier fuction it exerts. The addition of prebiotic fibers to the formulation could contribute to an increase in production of short chain fatty acids, promoting intestinal acidification.

The ingestion of the juice fermented by LGG did not exert a protective effect against the development of pre-neoplastic lesions in the Wistar rats, nevertheless the product can be consumed with the objective of avoiding coronary diseases, since it contributes to a reduction in the LDL fraction of cholesterol.

Acknowledgements

The authors are grateful to the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (grant number: MPR-00005-13), to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (grant number: 467824/2014-2), and to the Grupo PET Ciências Agrárias (grant number: Edital n° 09/2010) for their financial support.

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