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Probiotic Flavored Fermented Goat Milk as An Adequate Vehicle for Beneficial Bacteria and Higher Total Phenolic and Antioxidant Activity

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Abstract

The study aimed to evaluate two grape flavored fermented goat milk produced with or without probiotics. Physicochemical characteristics, sensory analysis, antioxidant profile and probiotics viability of the dairy beverages were analyzed. The phenolic contents and antioxidant activity of probiotic milk were higher than conventional milk. A higher loss in cell viability was observed for *L. acidophilus* than for the *B. animalis*. The average sensory acceptability scores obtained by both dairy beverages were higher than 6.5. Therefore, the probiotic fermented milk showed an adequate vehicle for the probiotics with good viability, a higher total phenolic and antioxidant activity and good acceptability.

Keywords: Goat milk; Probiotic; Dairy beverage; Gastrointestinal simulation

Introduction

Probiotic functional foods are reported to provide several health benefits. Their efficacy are influenced by the selection of microbial cultures and the concentration of viable population in the product [32]. *Lactobacillus* and *Bifidobacterium* are associated with maintaining an optimum gut microbiota balance [35], decreasing the lactose intolerance symptoms [30], immune system stimulation [25], and presents antioxidative properties [26].

Probiotics have become an integral part of the complex world as biologics, pharmaceuticals, food and nutritional supplements due to their potential of providing health benefits. Currently, there is a notable increase in the consumption of non-bovine milk in substitution to conventional milk [40]. The specific nutritional composition of goat mill is related to higher protein digestibility, during digestion or technological processing, which can exert beneficial properties to the organism [37]. However, caprine milk products it is not widely accepted by consumers, mainly due to its typical flavor derived from their capric, caproic and caprylic acids content [16]. It is noted that the association of probiotic fermented milk with functional ingredients, such as juice, improves its nutritional profile as well as its sensory attributes [40]. In this way, purple grape juice stands out due to its pleasant flavor and flavonoids concentration, specifically anthocyanidins and resveratrol [13]. These compounds have important biological functions and health benefits, acting on the oxidative and inflammatory process [28].

Reactive Oxygen Species (ROS) mediated oxidative stress are known to play vital role in the development of chronic diseas-

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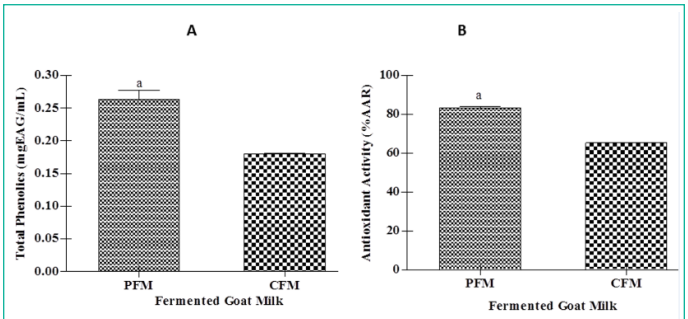

Figure 1: Total phenolic contents (A) and antioxidant activity (B) of Conventional Fermented Milk (CFM) and Probiotic Fermented Milk (PFM). Values are means and errors bars indicate standard deviations (n=3). [a] p<0.01 from unpaired *t* test.

es such as cancer, diabetes, heart disease, stroke, Alzheimer's disease, rheumatoid arthritis, cataract and aging [3]. Thus, a novel approach is represented by the development of probiotic products exerting antioxidant activity. To neutralize the oxidant molecules, the human body synthesizes antioxidant enzymes and molecules that, together with the antioxidants contained in food, form a biological antioxidant barrier to chemicals that induce oxidative stress, either by generating ROS or by inhibiting antioxidant system [22]. Furthermore, the administration of probiotic lactic acid bacteria can modulate the gut microbiota composition and activity, influencing the metabolism of polyphenols and the release of bioactive metabolites at the intestinal level. However, there are a limited number of studies about the contribution of probiotic bacteria to the antioxidant activity of probiotic beverages [23].

The viability of probiotic microorganisms in food products and their resistance during the gastrointestinal transit is necessary to obtain health benefits related to activities exerted at the intestinal level [31]. In the production of flavored fermented milks, the addition of fruit juices or pulps may interfere in the survival of probiotic microorganisms [12], and should be investigated. Few publications on probiotic flavored fermented goat milk are available in literature [33,34,40]. Thus, the present study aimed to evaluate the viability and *in vitro* gastrointestinal tolerance of probiotics *Bifidobacterium animalis* and *Lactobacillus acidophilus* in flavored fermented milk produced with goat milk and grape juice, and their influence on the antioxidant activity, total phenolic content, texture and sensory features of the beverages during refrigerated storage.

Table 1: Texture analyses of fermented goat milk beverages during 28 days of storage at 4±1 ºC.

Beverages	Time (days)	Firmness N x10²	Consistency N x 10² s	Cohesiveness N x10²	Viscosity index N x 10² s
CFM	1	12.19±0.18^{A}	241.70±5.96^{A}	8.06±0.16^{A}	14.94±0.87^{A,a}
	14	15.99±0.40^{B}	369.52±5.35^{B}	10.75±0.30^{B}	17.59±1.31^{B}
	28	16.41±0.77^{B}	369.60±4.32^{B}	11.71±0.59^{B,a}	19.42±1.06^{B}
	Overall mean	14.87±2.03	326.60±62.88	10.17±1.64	17.31±2.16
PFM	1	12.06±0.23^{A}	234.86±2.70^{A}	8.01±0.13^{A}	13.26±0.48^{A,b}
	14	15.99±0.20^{B}	369.61±2.63^{B}	10.75±0.15^{B}	17.63±0.55^{B}
	28	16.15±0.29^{B}	369.81±3.60^{B}	10.95±0.20^{B,b}	18.50±0.75^{B}
	Overall mean	14.73±2.10	324.76±66.61	9.90±1.43	16.46±2.43

Values are mean ± SD of four replicate determinations. $^{A-C}$ Different superscript capital letters in a column for a same beverage denote significant differences (p<0.05) between sampling days. $^{a-b}$ Different superscript letters in a column denote significant differences (p<0.05) between trials for a same moment. There was no significant difference between trials for overall mean. CFM: Conventional Fermented Milk; PFM: Probiotic Fermented Milk.

Materials and Methods

Production of the Flavored Fermented Milk

The Probiotic Fermented Milk (PFM) and Conventional Fermented Milk (CFM) were produced using the commercial starter culture *Streptococcus thermophilus* TA-40 (Danisco, Sassenage, France; 0.003 g/100 g). The PFM was added by *B. animalis* subsp. *lactis* BB-12 and *L. acidophilus* La-5 (Chr. Hansen, Hørsholm, Denmark; 0.024 g/100 g). Goat milk provided by Embrapa Goats and Sheep (Sobral, Ceará, Brazil) was supplemented with 5% (w/v) sucrose and pasteurized at 90ºC for 15 min, then cooled to 43±2ºC for the addition of the starter and probiotic cultures. The fermentation process was conducted at 40±1ºC until reaching pH 5.0±0.1. Next, the fermented milk temperature was decreased to 4ºC up to the following day, and then the beverages were flavored with 20% (w/v) of purple grape juice obtained from Embrapa Grapes and Wine (Bento Gonçalves, Rio Grande do Sul, Brazil). All ingredients were mixed with a blender to form a homogeneous product. The final product was packed in polypropylene bottles and stored at 4±1ºC for further analysis. The CFM was used for analysis of physicochemical properties and sensory quality in comparison with PFM. Total solids, total dietary fiber, ash, fat, and protein content were determined for PFM on the seventh day of storage (AOAC, 2012). All the analyses were performed in triplicate and were expressed as g/100g of whole matter.

Physicochemical Parameters and Instrumental Analysis

During fermentation and at 1, 7, 14, 21 e 28 days of storage at 4ºC, PFM samples were taken to determine pH (pH meter Jenway 3510, Staffordshire, UK) and titratable acidity. Titratable acidity was determined according to standard methods and expressed as g/100 g lactic acid [21]. Firmness, consistency, cohesiveness and viscosity index were evaluated in CFM and PFM samples after 1, 14 and 21 days of storage using a back extrusion cell (A/BE) on a Texture Analyzer TA-XTPlus (Stable Micro Systems, Surrey, UK), as described by Buriti *et al*. (2014). The analyses were performed in quadruplicate.

Table 2: Viability of *S. thermophilus* TA-40, *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12 in the Probiotic Fermented Milk (PFM) during 28 days of storage at 4±1ºC.

Microorganism (log CFU/mL)	1 day	7 days	14 days	21 days	28 days
S. thermophilus	8.92±0.01	8.39±0.42	8.90±0.02	8.88±0.02	9.20±0.49
L. acidophilus	7.89±0.02^{A}	7.76±0.10^{A}	6.18±0.05^{B}	6.16±0.03^{B}	6.11±0.02^{B}
B. animalis	8.65±0.02^{A}	7.26±0.01^{B}	7.19±0.01^{C}	7.19±0.01^{C}	7.98±0.02^{D}

Values are mean ± SD of three batches, in duplicate, at each sampling day. $^{A-E}$ Different superscript capital letters in a same row denote significant differences (p<0.01) between sampling days.

Table 3: Survival of *L. acidophilus* La-5 and *B. animalis* BB-12 under simulated gastrointestinal conditions in the fermented milk at 7 days of storage at 4±1 ºC.

Microorganism (log CFU/mL)	0 h	2 h	4 h	6 h
L. acidophilus	6.95±0.01^{A,a}	5.75±0.08^{B,a}	5.55±0.02^{B,a}	5.25±0.15^{B,a}
B. animalis	8.75±0.20^{A,b}	6.94±0.01^{B,b}	5.68±0.03^{C,b}	5.12±0.02^{D,a}

Values are mean ± SD of three replicate determinations. $^{A-D}$ Different superscript capital letters in a same row denote significant differences (p<0.01) between different sampling periods of the in vitro assay. $^{a-b}$ Different superscript letters in a column denote significant differences (p<0.05) between probiotics for a same moment. pH at 0, 2, 4 and 6h=4.39; 2.46; 5.0 and 6.3, respectively.

Table 4: Sensory evaluation scores of the fermented goat milk.

Fermented milk	Flavor	Appearance	Texture	Color	Overall acceptability
Conventional	6.76±1.99	6.87±1.46	7.08±1.41	7.43±1.34	6.65±1.79
Probiotic	6.86±1.89	7.14±1.67	7.06±1.50	7.27±1.73	7.00±1.53

Values are mean ± SD. Scores vary between 1 (dislike extremely) and 9 (like extremely). There were no significant difference between fermented milk.

Total Phenolic Content and Antioxidant Activity

The total phenolic compounds content in CFM and PFM were determined using the Folin-Ciocalteu method [45]. Aliquots of 0.5 mL of each fermented milk extract were added to 0.5 mL of Folin-Ciocalteu reagent (20%). After homogenization, 0.5 mL of sodium carbonate (7.5%) was added. The reaction mixture was homogenized by vortex (2865 g, 10 s) and incubated at room temperature (30 min). The reading of absorbance was performed in spectrophotometer (Thermo scientific, Evolution 606, USA) at 765 nm. Analytical curve of gallic acid (0.0 to 250 µg/mL, R_2=0.999) was used to quantify the compounds. The results were expressed in mg of gallic acid equivalents/mL (mg GAE/mL). In a test tube, protected from light, aliquots of samples (PFM and CFM) were added to 1.5 mL of methanolic DPPH solution (1.1-diphenyl-2-picrylhydrazyl) and stirred by vortex (3000 rpm) for 30 s. After 30 min of standing, the absorbance of the solution was read in a spectrophotometer (Thermo scientific, 606 Evolution, USA) at 517 nm (Bloor, 2001). The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation: Scavenging ability (%) = [(control absorbance – sample absorbance) / (control absorbance)] x 100

Microbial Viability

Populations of *S. thermophillus, B. animalis* and *L. acidophilus* in PFM was determined after 1, 7, 14, 21 and 28 days of storage at 4 ºC. The samples were serially diluted in sterile peptone water (1g/L) and subsequently plated, in duplicate. *S. thermophilus* enumeration was performed on M17 agar, containing lactose (Vetec, Duque de Caxias, Brazil; 5g/L), and incubated at 37ºC for 48h. Populations of *L. acidophilus* were enumerated by pour plating 1 mL of adequate dilutions into MRS agar (Oxoid Basingstoke, UK), followed by incubation at 37ºC for 72 h. For the selective enumeration of *B. animalis*, 1mL of adequate dilutions were pour plated in modified DeMan-Rogosa-Sharpe (MRS) agar (Oxoid, Basingstoke, UK), prepared with dicloxacillin (Sigma, St. Louis, US), cysteine hydrochloride (Cromoline, Diadema, Brazil), and lithium chloride (Cinética®, Jandira, Brazil) to reach a concentration of 0.5 mg/L, 0.5 g/L and 1 g/L, respectively, followed by incubation at 37ºC for 72h. All bacteria were incubated in anaerobic jars (Anaerobic System Anaerogen, Oxoid, UK), except *S. thermophilus,* which was incubated under aerobic conditions. The results were expressed as of log colony forming units per gram (CFU/mL).

Resistance to Simulated Gastrointestinal Conditions

PFM samples were collected at 7 days of storage for the evaluation of *L. acidophilus* and *B. animalis* survival to gastric and enteric simulated conditions according to the method described by Liserre and Franco (2007), with modifications. Samples were decimally diluted in a sterile 0.85% (w/v) NaCl solution. For the gastric phase simulation, the pH was set at 2.5 with 1 N HCl solution. Pepsin (Sigma-Aldrich, St. Louis, USA) and lipase (Aldrich Chemical Company, Milwaukee, USA) solutions were added to reach final concentrations of 3.0 g/L and 0.9 g/L, respectively. The flasks were incubated at 37°C for 2 h under agitation (150 rpm). Subsequently, enteric conditions were simulated in two phases. In the enteric phase 1, the pH was increased to 5.0 with a sterile alkaline solution (150 mL of 1 N NaOH, 14 g of $PO_4H_2Na.2H_20$ and distilled water up to one 1 L), and bovine bile and pancreatin (Sigma-Aldrich, St. Louis, USA) were added to reach a concentration of 10 g/L and of 1 g/L, respectively. After 2 h of incubation at the same conditions, the pH was adjusted to 6.5 - 7.0, and the respective bile and pancreatin concentrations were adjusted to 10 g/L and 1 g/L for the second enteric phase, followed by an additional incubation period of 2 h. In order to enumerate the viable *L. acidophilus* and *B. animalis* cells, aliquots were taken at the assay baseline (0 h) and after 2, 4 and 6 h and serially diluted in peptone water solution. Adequate dilutions (1 mL) were pour plated in acidified MRS agar, followed by anaerobic incubation at 37°C for 48 h. A survival ratio (SR%) was calculated based on the initial and final populations to estimate the relative resistance of each strain to the simulated TGI conditions. All results are presented as log CFU/mL.

Sensory Analysis

The sensory evaluation of the flavored fermented goat milk was approved by the Federal University of Viçosa Human Ethics Research Committee, Brazil (Process No. 219.644; CAAE: 13380413.8.0000.5153) and was carried out at the Laboratory of Sensory Analysis of UNINTA College. Sensory evaluation was performed with CFM and PFM samples after 7 days of cold storage (4±1ºC) through acceptability tests, using the hybrid hedonic scale (1 = disliked extremely, 5=neither liked nor disliked, 9=liked extremely) focusing on attributes of taste, flavor, color, consistency and overall acceptability [38]. The samples were maintained under refrigeration prior the tests and served, monadically, in individual disposable plastic cups (approximately 30 mL) codified with three random digits. The sensory test was carried out with 63 untrained panelists aged 19 – 40 years old recruited among potential consumers of the beverages, and performed in individual booths. Water and unsalted crackers were available during a 1 min rest period between sample sets to refresh the palate. The consumers were also instructed to report the sensory attributes that they liked and disliked most in the samples.

Statistical Analysis

The experimental data were analyzed by SPSS software version 20 (IBM, Armonk, NY) and the results were expressed as mean±Standard Deviation (SD). Values were the average of quadruplicate/triplicate/duplicate experiments. Before analysis, data were checked for the normality, homogeneity of variances and sphericity using the Shapiro-Wilk, Levene's and Mauchly's tests, respectively. Differences between trials (CFM and PFM) in a single moment were tested using unpaired *t* test or Mann-Whitney test. Differences between experimental storage periods were statistically analyzed using repeated measures Analysis of Variance (ANOVA), followed by the post hoc Bonferroni test, taking on $p<0.05$. When normality was not found, the equivalent non-parametric tests were applied. Differences at $p<0.05$ were considered to be significant.

Results and Discussion

Composition, Physicochemical and Instrumental Analysis

Probiotic Flavored Fermented goat Milk (PFM) had the following chemical composition: total solids 17.3±0.04 g/100g, protein 2.47±0.02 g/100g, fat 2.64±0.02 g/100g, ash 0.74±0.01 g/100g and total dietary fiber 0.14±0.01 g/100g. Total solids, protein and fat contents of PFM were found to be lower than other study [29], reflecting the higher moisture content in flavored fermented milk due to the addition of grape juice. Changes in these parameters, especially total solids and fat content may affect physical-chemical properties such as viscosity, syneresis and water holding capacity [39].

The texture parameters analyse of the dairy beverages are presented in Table 1. The measured firmness, consistency, cohesiveness and viscosity index of Conventional Fermented Milk (CFM) showed an increase during the 28 days of storage, but the values registered at 14 and 28 days did not differ significantly ($p>0.05$). Interestingly, these parameters values increased only until day 14 ($p<0.05$) for PFM, however, did not affect the sensory acceptability scores. PFM presented a lower viscosity index on the first day of storage ($p=0.04$) compared to CFM, and no significant difference was detected for firmness and consistency ($p>0.05$) when the two trials were compared at the same sampling period. However, little information exists about the influence of probiotic strains on physicochemical properties of dairy beverages. Fermented milk prepared using only probiotic strains, such as *L. acidophilus* and *Bifidobacterium* spp. are often characterized by the undesirable sensory proprieties and texture [40], whereas physical properties such as firmness and ability to retain water are important factors for quality assessment [18]. As we report below, these parameters were not affected in the present study, probable due to the presence of starter culture *S. thermophilus* in both beverages.

The use of a combination of starter and probiotic cultures requires an adequate formulation that should guarantee rapid acidification during fermentation and reproducibility of the fermented milks features. The time needed to reach pH 5.0±0.1 during the fermentation was lower for PFM as compared to CFM (2h 30 min *vs* 5 h; $p<0.001$). In this way, we obtained a PFM with adequate technological parameters, with time of fermentation lower than others study developed with non-flavored fermented goat milk containing lactobacillus cultures (48 h to reach pH 3.06) [36] or *S. thermophilus* ST-20Y, *L. acidophilus* La-5 and *B. animalis* BB-12 (6 h to reach pH 5.0) [29].

During refrigerated storage, the pH of PFM ranged from 4.10 to 4.19 ($p< 0.001$), indicating no further acidification, probably due to the presence of three bacteria that show a lower proteolytic activity [43]. Values of pH below 4.0 are generally considered detrimental to the survival of probiotic organisms [47]. Titratable acidity remained stable during the storage period, ranged from 0.82 to 0.85 mg lactic acid g^{-1}. Others studies have been reported small reductions in the pH and increase in the titratable acidity for goat's milk beverages [12,39].

Total Phenolic Content and Antioxidant Activity of Dairy Beverages

The Total Phenolic (TP) contents and antioxidant activity of PFM were significantly higher ($p<0.01$) than CFM. The TP of fermented milk was 0.179±0.001 mg GAE/mL in CFM and 0.264±0.01 mg GAE/mL in PFM. The antioxidant activity ranged from 65.17±0.24 for CFM to 83.30±0.68 % for PFM (Figure 1). Studies have shown that selected strains of bifidobacteria and lactobacilli present antioxidants properties and can be used to elaborate fermented dairy beverages that improve total antioxidant status and decrease markers of oxidative stress [10].

A high proportion of polyphenols from the diet are not directly absorbed and the transformation these compounds in the gut depends on microbial esterase and glucosidase activities [20]. In this way, gut bacteria may play a major role in the production of new phenolic compounds "*in situ*", which could have better bioavailability and higher biological activity than their parent compounds [41]. Besides, the lactic acid fermentation can increase concentrations of total phenols, flavonoids and anthocyanins in food [24]. This can explain, partially, the higher TP contents and antioxidant activity observed in PFM in this study.

Interestingly, Balakrishnan and Agrawal (2014) reported a higher antioxidant activity in probiotic fermented milk obtained from goat milk (93%) followed by a product from camel milk (86%) and then a product from cow milk (79%), suggesting that probiotic bacteria are able to utilize the nutrients in goat and camel milk more efficiently, in comparison to cow milk. Several studies have highlighted the association between the consumption of foods with high antioxidant activity and the development and progression of chronic low-grade inflammation and metabolic diseases [7,8].

Microbiological Analysis

The *S. thermophilus* population of the PFM remained stable during the entire period of cold storage, presenting a viability of 8.91 log CFU/mL at day 1 and 9.2 log CFU/mL at day 28. On the other hand, *L. acidophilus* and *B. animalis* subsp. *lactis* populations reduced significantly during the storage ($p<0.001$), reaching concentrations of 6.10 and 7.98 log CFU/mL at day 28, respectively. *L. acidophilus* maintained viable cell counts ≥7 log CFU/mL for 2 weeks, whereas *S. thermophilus* and *B. animalis* subsp. *lactis* maintained this concentration throughout the 28 days. The viability values of bacteria in PFM during the cold storage period are shown in Table 2.

The health benefit of fermented milk containing probiotics depends on the viability of the probiotic microorganisms in the refrigerated product [44]. In spite of having no agreement about the effective dose, many authors have been suggesting a minimum dose between 10^6 - 10^9 CFU/day to assure the therapeutic effect [50]. The Codex Alimentarius (2010) states that the minimum viable quantity of probiotic culture should be 10^6 CFU/mL and 10^7 CFU/mL for starter culture. In the present study, despite the registered variations in viability during cold storage, probiotic bacteria maintained suitable amounts during the completely studied period. In agreement with other studies, a higher loss in cell viability was observed for *L. acidophilus* La-5 than for the bifidobacteria strain [39,51]. However, *L. acidophilus* La-5 was able to maintain the minimum therapeutic level (>10^6 CFU/mL) up to 4 weeks storage, in contrast to Ranadheera *et al*. (2012) in flavored goat`s milk yogurts.

The viability of the probiotic strain depends on several factors, being the drop in pH the most important cause of the decrease in the viability of the probiotic culture [31]. However, the pH values registered for PFM in the present study did not affect the viability of *L. acidophilus* La-5 and *B. animalis* BB-12, and there was no pH reduction during the storage period. In addition, it has been supposed that mixed cultures of probiotics in fermented milk may result in poor growth and subsequently poor viability in storage compared to pure cultures, most probably due to competition for nutrients [48]. Thus, it seems that *B. animalis* subsp. *lactis* has an advantage compared to the *L. acidophilus* [4.39]. Additionally, other study has reported that food polyphenols are able to selectively modify the growth of susceptible microorganisms. *L. acidophilus* and *B. animals subsp. lactis* BB-12 showed an inhibition of growth by the phenolic extracts. On the other hand, *L. plantarum*, *L. casei*, and *L. bulgaricus* strains reached maximal growth in the presence of polyphenol extracts (343 mg/g of total phenolic content), being most appropriate for use in fermented beverages containing high proportion of polyphenols [46].

Probiotic Resistance to Simulated Gastrointestinal Conditions

The survival of a probiotic bacteria during the gastrointestinal transit should be investigated in each food matrix, complementing the study of probiotic viability, since most probiotic effects depends on the viable cells action at intestinal level and the food matrix might exert an important role in probiotic protection or inhibition. Overall, moderate reductions in *L. acidophilus* and *B. animalis* subsp. *lactis* counts were observed throughout exposition to simulated gastrointestinal conditions. In the present study, *L. acidophilus* and *B. animalis* populations were within the detection limits throughout the entire assay, in contrast to previous studies [9,11,15].

During the simulated gastric phase, the populations of *L. acidophilus* and *B. animalis* subsp. *lactis* decreased significantly ($p<0.001$ and $p<0.01$, respectively). At gastric conditions, populations of *L. acidophilus* reduced 1.2 log CFU/mL, in average, after 2 h of exposure, whereas *B. animalis* subsp. *lactis* populations reduced 1.8 log CFU/mL. Similarly to the present study, Fávaro-Trindade and Grosso (2002) reported a reduction of only 1 log cycle for *L. acidophilus* La-5 after 2 h of exposure to pH 2. Population reductions varying from 0.1 to 2.5 log cycles was reported for 6 strains of *L. acidophilus* isolated from commercial probiotic yoghurts, after 90 min in simulated gastric juice containing pepsin at pH 2.0. However, strains of *L. acidophilus* were more tolerant to the low pH 2.0 than strains of *L. paracasei* and *L. rhamnosus,* which rapidly lost viability (Schillinger, Guigas, Heinrich 2005). *B. animalis* subsp. *lactis* has also exhibited a higher sensitivity to simulated gastric conditions [4,11,19].

During the simulated enteric phase, the *L. acidophilus* La-5 survived well, maintaining almost the same population. On the other hand, populations of *B. animalis* BB-12 reduced significantly (Table 3). It is possible that the presence of bile salts affects the phospholipids and proteins of bacterial cell membranes, and gram-positive bacteria seem to be more susceptible to the deleterious effects of bile than Gram-negative. However, the tolerance to the bile is a strain-dependent characteristic that should not be generalized in terms of species [5]. In the present study, *B. animalis* BB-12 showed a higher sensitivity to the simulated enteric conditions.

Overall, the minimum populations of probiotic bacteria registered after the *in vitro* digestion assay remained above 5 log CFU/mL, for both bacteria. However, the survival rate of *L. acidophilus* (75.71%) was higher than *B. animalis* (58.55%) considering the entire assay ($p<0.01$). A different study reported that *L. acidophilus* La-5 exhibited greater survival rates than *B. animalis* supsp. *lactis* BB-12 in the more acidic stirred fruit yogurts, confirming their capacity for acid tolerance [39]. Thus, the microenvironments produced by the food matrix or ingredients on the intestine level may protect the probiotic microorganism from the harsh conditions of the gastrointestinal tract. Moreover, food components could bind to bile acids, reducing their toxic effect on probiotic cells [5].

Interestingly, the observed patterns of survival after exposure to simulated gastrointestinal conditions were in contrast with the trends observed in the viable counts recorded during the cold storage of PFM, since *B. animalis* subsp *lactis* showed higher viability than *L. acidophilus*. However, considering the usual portion of dairy beverages consumed, at least 100 mL, the results obtained *in vitro* suggests that a relevant number of probiotic viable cells, ensures in the beverages a minimum dose of 10^6 CFU/mL [14]. Furthermore, the population of *B. animalis* subsp *lactis* was higher than *L. acidophilus* ($p<0.01$) until 4h at pH 5.0 of assay.

Sensory Evaluation of Dairy Beverages

The results of the sensory evaluation of the dairy beverages are shown in Table 4. Sixty-three panelists (41 females and 22 males; mean age = 24.2±4.40 years old) participated in the study. The average sensory acceptability scores obtained by CFM and PFM for flavor, appearance, texture, color, and overall impression were higher than 6.5 in a 9-point hedonic scale. There was no significant difference between the average sensory acceptability scores attributed to PFM and CFM, although the overall acceptability score of PFM was slightly higher than CFM (7.0 *vs* 6.6). Among the tested sensory attributes, flavor received the lowest scores for both fermented milks; meanwhile the color was the most appreciated.

It has been suggested that probiotic bacteria addition creates sensory advantages in dairy products [17], especially, the *L. acidophilus* La-5 may produce flavor compounds, such as acetaldehyde, which are recognized as important flavor components. However, in this study, despite the good flavor score, no increase in consumer's acceptance was observed for PFM compared to CFM, probably reflecting the contribution of the grape juice flavor compounds. Martín-Diana *et al*. (2003) obtained lower scores for all sensory attributes for a non-flavored fermented goat's milk containing *L. acidophilus* La-5 and *B. animalis* BB-12. The incorporation of natural sugars into the dairy beverages base through addition of fruit juice has been proposed a key factor to achieve higher consumer acceptability for goat's milk beverages [39,49], besides contributing with nutrients and bioactive compounds not found in milk, particularly, polyphenols.

The general comments by the panelists regarding sensory attributes were also evaluated. The most common criticisms were related to the semi-liquid texture of the beverages and the "goaty" flavor. Ranadheera *et al*. (2012) related that complaints regarding the characteristic unpleasant ''goaty'' taste were not recorded for the 10% and 15% stirred fruit yogurts.

Conclusion

The probiotic flavored fermented goat milk evaluated in the present study showed to be an adequate vehicle for the probiotics *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12 and exhibited a good sensory quality. Furthermore, the antioxidant activity of the probiotic fermented milk was higher than conventional fermented milk. Thus, this study presents relevant information on physicochemical, sensory, microbial and antioxidant properties of a probiotic flavored fermented goat milk, which could be exploited to formulate novel probiotic foods that can exert a role in the prevention of oxidative stress and related diseases.

Author Statements

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Conflict of Interest

The authors declare no conflict of interest.

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