

Development and Physicochemical, Sensory and Microbiological Characterization of Ferment Milk With Addition of Cupuaçu

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Abstract

The development of dairy products using probiotic microorganisms has been an important focus in the food industry. Thus, the present study aimed to evaluate the physicochemical, sensory and microbiological quality of fermented milk with addition of cupuaçu (*Theobroma grandiflorum*) flavor. The experimental design used was completely randomized, with five replicates, arranged in a $4 \times 2 \times 2$ factorial scheme, consisting of fermented milk with addition of four different strains of probiotic bacteria (*Lactobacillus casei shirota*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Lactobacillus paracasei*), presence and absence of cupuaçu flavor, and two evaluation times (1 and 7 days of storage). The results allowed us to conclude that the bacterial strain B (*Lactobacillus casei*) led to higher values of L*, b*, %Brix and pH. In the presence of cupuaçu flavor, the values of pH, L*, b* and %Brix were significantly higher, which allows us to infer that cupuaçu pulp contributes to decreasing the values of pH and increasing the acidity, lightness, b* and %Brix of fermented milk. Fermented milk with addition of bacteria A (*Lactobacillus casei shirota*) in the presence of cupuaçu flavor showed reduction in pH and %Brix and, therefore, better acceptance by consumers and bioconservation. The presence of cupuaçu flavor in fermented milk with bacteria B (*Lactobacillus casei*) alters L* and b* at seven days of storage. Fermented milk with addition of cupuaçu flavor constitutes an opportunity and possibility for developing new flavors from Amazonian fruits, strongly contributing to the durability of dairy products, since the physicochemical, sensory and microbiological characteristics are enhanced within the standards of food safety.

Keywords: amazonian fruits, probiotic bacteria, production, technology, *Theobroma grandiflorum*

1. Introduction

Milk is a food composed of a combination of nutrients that are fundamental for consumption, such as water, carbohydrates, lipids, proteins, minerals and vitamins, such as A and D (fat-soluble) and B and C (water-soluble), which together provide important elements to health (Silva et al., 2008). Habitual consumption of this food is recommended, especially to achieve the recommended daily intake of calcium, a nutrient that, among other functions, is fundamental for the formation and maintenance of the body's bone structure (Muniz et al., 2013). Due to its nutritional profile, milk has been investigated in studies addressing the importance of its intake in the different phases of life, as well as potential health benefits attributed to its consumption and the use of probiotic bacteria.

The development of dairy products using probiotic microorganisms has been an important focus in the food industry. Currently, the production of foods containing specific probiotic strains with appropriate concentrations of viable cells along the shelf life is a technological challenge. Bacteria belonging to the genus *Lactobacillus*, such as those of the group *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus acidophilus*, are very often used as probiotic supplements for food, especially for fermented milk, since they have been isolated from all portions of the gastrointestinal tract of healthy humans. The beneficial influence of probiotics on human intestinal microbiota includes factors such as antagonistic effects, competition and immunological effects, resulting in increased resistance against pathogens (Saad, 2006). Thus, the use of probiotic bacterial cultures stimulates the multiplication of beneficial bacteria, to the detriment of the proliferation of potentially harmful bacteria, reinforcing the natural defense mechanisms of the host (Puupponen-Pimiä et al., 2002).

Fermented milks, according to the Technical Regulation of Identity and Quality of Fermented Milk (Brazil, 2007), are products with or without the addition of other food substances, obtained by coagulation and reduction

of milk pH, or reconstituted, with or without the addition of other dairy products, by lactic fermentation through the action of cultures of specific microorganisms, and these must be viable, active and abundant in the final product during its shelf life. Such fermentation is performed with one or more of the following cultures: *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium sp.*, *Streptococcus salivarius subsp. thermophilus* and/or other lactic acid bacteria that, through their activity, contribute to determining the characteristics of the final product (Brazil, 2007). However, the production of fermented milk, such as fermented milk with addition of fruits, is a considerable alternative for dairy industries and can constitute a rational and logical form of different types of processing, with various flavors, being part of a promising market within the Brazilian scenario.

The Brazilian Amazon is rich in diversity of plants that are still little explored by most of the population and that can become a food alternative of high nutritional value and with alleged presence of nutraceutical compounds. Among the fruits of great importance for the Amazon region, cupuaçu (*Theobroma grandiflorum*) stands out as the most commercially exploited and is part of the group of tropical fruits with excellent commercial value. The pulp is the most consumed part of this fruit and, due to the characteristics of acidity, pectin content, active aroma, pleasant taste, high contents of volatile compounds and mineral salts (Martins, 2008), it is an important raw material for the processing industry. Data from the Food and Agriculture Organization (FAO) have shown that the worldwide marketing of fruit products has significantly grown in recent years. The use of fruit pulp in the formulations of dairy beverages offers some advantages, such as giving pleasant taste and contributing to improving the nutritional value of foods (Zulueta, 2007).

Several studies have been carried out in the different segments aimed at discovering new nutritional and functional sources, and the diversity of bioactive compounds in foods justifies the endless search for information on their physicochemical, physiological or functional and sensory properties. Thus, considering the potential of the Amazon region regarding the investment in food technology, the production of fermented milk with addition of Amazonian fruits creates the opportunity for discovering new horizons for products with different treatments and that meet both national and international consumers, through the creation and exploration of Amazonian products and species. In addition, research should be based on the fact that the strategic decisions to be made by the agents of the milk and dairy production chain depend on a deep understanding of consumer behavior, which has not yet been sufficiently investigated in the country.

In view of the above, the present study aimed to evaluate the physicochemical, sensory and microbiological quality of fermented milk with addition of cupuaçu (*Theobroma grandiflorum*) flavor.

2. Methods

The experiment was conducted at the premises of the Food Agroindustry of the Federal Institute of Education, Science and Technology of Rondônia, Colorado do Oeste Campus, in the municipality of Colorado do Oeste, RO, Brazil, at the geographic coordinates 13°06' S and 60°29' W, and average altitude of 407 meters.

The experimental design used was completely randomized, with five replicates, arranged in a $4 \times 2 \times 2$ factorial scheme, consisting of fermented milk with addition of four different strains of probiotic bacteria (*Lactobacillus casei shirota*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Lactobacillus paracasei*), presence and absence of cupuaçu flavor, and two times of evaluation (1 and 7 days of storage), in a total of 80 experimental units.

The milk used in the test was produced in the Animal Science sector of the Federal Institute of Education, Science and Technology of Rondônia, Colorado do Oeste Campus, and provided for the Food Agroindustry. Initially, this milk was skimmed to have a final maximum fat content of 0.5 g/100 g, according to the Normative Instruction n° 62 of 2011, in slow pasteurization, raising the temperature to 65 °C for thirty minutes, followed by cooling to the temperature of incubation of fermenting microorganisms (37 °C), in a rigorously controlled environment for a period of 7 hours. Milk fermentation occurred until the pH of the medium reached values of 4.0 (± 0.25). This pH interval was determined considering the sensory and conservation characteristics of the product. The fermenting microorganisms used came from different commercial products and their samples were identified as treatments A, B, C and D, which correspond to: A (*Lactobacillus casei shirota*), B (*Lactobacillus casei*), C (*Lactobacillus acidophilus*) and D (*Lactobacillus paracasei*).

Fermented milk with and without cupuaçu flavor was produced by adding 9% sugar in each treatment. Subsequently, the milk was heated to prepare the syrup, and part of it received 5% of natural pulp of cupuaçu (*Theobroma grandiflorum*).

Treatments that, after processing, showed apparent viscosity visually similar to/compatible with that of fermented milk existing on the market were subjected to physicochemical analyses: pH, acidity, colorimetric

parameters L^* (lightness ranging from 0 [totally black] to 100 [totally white]), a^* (-80 green to +100 red) and b^* (intensity from -50 blue to + 70 yellow), and %Brix, analyzed at the first and seventh days of storage.

The sensory acceptance test (attributes: color, texture, consistency, aroma, taste, appearance and overall aspect) was performed at seven and fifteen days of storage, in triplicates. Sensory evaluation of acceptance was carried out by untrained tasters and considering the scores according to the hedonic scale with values from 1 to 9 points (1-disliked extremely, 2-disliked very much, 3-disliked moderately, 4-disliked slightly, 5-Neither liked nor disliked, 6-liked slightly, 7-liked moderately, 8-liked very much and 9-liked extremely), indicating how much they liked or disliked the color, texture, consistency, aroma, taste, appearance and overall aspect of the products. Purchase intention was evaluated by means of a mixed structured scale with 5 points (1-would buy, 2-possibly would buy; 3-maybe would buy, 4-possibly would not buy and 5-would not buy).

Microbiological determinations were performed according to the methodology described by Brazil (2003), evaluating the presence of total coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella sp.*, based on the microbiological parameters recommended by the legislation for fermented dairy beverages (Brazil, 2005).

The physicochemical, sensory and microbiological results were subjected to analysis of variance and the differences in the means were compared by Tukey test at 5% significance level, using Sisvar software.

3. Results and Discussion

The results showed significant effects ($p \leq 0.05$) of the interaction between bacteria (B) and flavor (F) on b^* , pH and %Brix (Table 1), while the interaction between bacteria (B) and time (T) influenced only the colorimetric parameter L^* (Table 2), and the interaction between flavor (F) and time (T) only affected L^* and b^* (Table 3). There was no significant effect of the triple interaction of the factors on any of the physicochemical variables analyzed.

Further analysis of the double interaction between bacteria (B) and flavor (F) showed that the bacterial strain A was statistically superior to the probiotic bacteria B, C and D, both in the presence and absence of cupuaçu flavor for pH values (Table 1). In the presence of cupuaçu flavor, the bacterial strain A led to pH values on the order of 4.02, statistically differing ($p \leq 0.05$) from the treatment without cupuaçu flavor (pH of 4.32), which showed a 7.46% reduction in pH values in the presence of cupuaçu flavor and reduction in %Brix (Table 1). This probably occurred because of the low pH and high acidity of cupuaçu pulp. For the same analysis, bacteria D led to the lowest values of pH (pH 3.32) and, therefore, the highest values of acidity compared to the other bacteria (A, B and C), as well as the highest values of %Brix, both in the absence and presence of cupuaçu flavor (Table 1).

There was no statistical difference in pH values in the presence and absence of cupuaçu flavor for the treatment with bacteria D; the pH values remained stable, around 3.32, both in the absence and presence of cupuaçu flavor. In addition, there was no statistical difference in %Brix. Such higher acidity caused by bacteria D is related to the metabolites produced by these lactic acid bacteria, which contribute to inhibiting the growth of microorganisms. It is known that, at the beginning of fermentation, milk pH favors the development of *Streptococcus salivarius subspecies thermophilus*, which tends to be inhibited when pH reaches values from 4.2 to 4.4. However, the increase in acidification, that is, in lactic acid content, favors the growth of *Lactobacillus delbrueckii subspecies bulgaricus*, which can tolerate pH values within the range from 3.5 to 3.8 (Andrade, 2010). From the obtained data, it can be inferred that the pH values observed in the treatments meet the minimum value established for fermented milk recommended by the Technical Regulation of Identity and Quality (RTIQ) of Fermented Milks (Brazil, 2007).

The colorimetric parameter L^* was higher in fermented milk with bacteria B in the presence of cupuaçu flavor, differing statistically from the other bacteria and in the absence of cupuaçu flavor. This means that the addition of bacteria B in milk fermentation promoted greater lightness in the product (Table 1), corroborating the data of the colorimetric variable b^* , which pointed to a slightly yellowish color in the fermented milk, as shown in Table 2.

Table 1. Colorimetric parameter L*, pH and %Brix of fermented milk with different strains of probiotic bacteria in the absence and presence of cupuaçu flavor

Bacteria	Flavor	
	Absence	Presence
<i>Colorimetric L*</i>		
A	40.14 aB	58.66 bA
B	43.21 aB	60.56 aA
C	36.44 aB	57.11 bA
D	40.99 aB	57.27 bA
<i>pH</i>		
A	4.32 aA	4.02 aB
B	3.44 cB	3.52 cA
C	3.85 bA	3.86 bA
D	3.32 dA	3.32 dA
<i>% Brix</i>		
A	14.77 cA	14.82 bA
B	15.00 bcA	15.15 aA
C	15.22 abA	14.65 bB
D	15.27 aA	15.12 aA

Note. Lowercase letters separate the means within each column, and the capitals separate the means within the row. Equal letters do not differ from each other by the tukey test at 5% probability.

There were significant effects of the different strains of probiotic bacteria at both times of storage of fermented milk on the colorimetric variables L* and b*. At seven days of storage, the bacteria B led to the highest values of L* and b*, compared to the other probiotic bacteria, and differed statistically ($p \leq 0.05$) when compared to the first storage time (Table 2). At the first storage time, bacteria A differed significantly from the others, promoting the highest values of L* and lowest values of b* (Table 2).

Table 2. Colorimetric parameters L* and b* of fermented milk with different strains of probiotic bacteria on the first and seventh days of storage

Bacteria	1° Day	7° Day
<i>Colorimetric L*</i>		
A	44.59 aB	56.21 bA
B	33.80 bB	65.97 aA
C	37.26 bB	56.29 bA
D	30.83 bB	57.41 bA
<i>Colorimetric b*</i>		
A	9.12 bB	12.12 bA
B	12.13 aB	14.65 aA
C	8.65 bB	12.22 bA
D	10.17 abB	11.86 bA

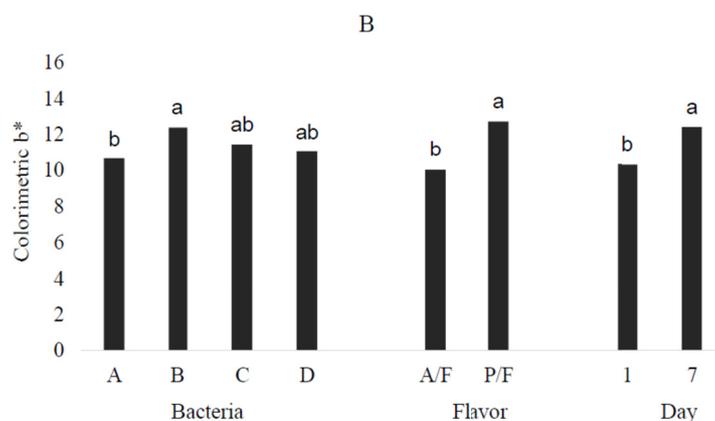
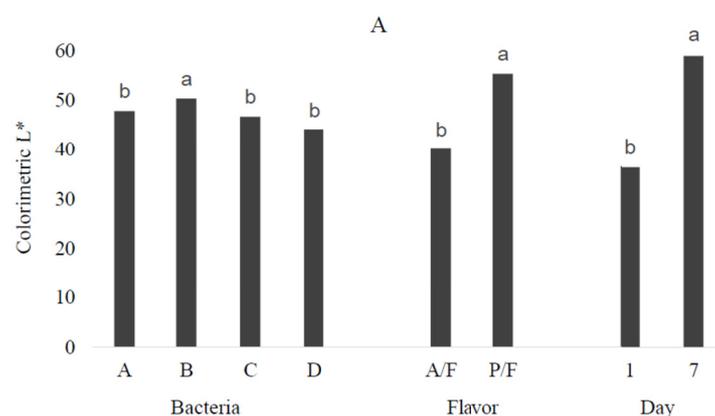
Note. Lowercase letters separate the means within each column, and the capitals separate the means within the row. Equal letters do not differ from each other by the tukey test at 5% probability.

Table 3. Colorimetric parameters L* and b* of fermented milk in the absence and presence of cupuaçu flavor on the first and seventh days of storage

Sabor	1° Day	7° Day
<i>Colorimetric L*</i>		
Absence	20.23 bB	57.78 aA
Presence	53.00 aB	60.15 aA
<i>Colorimetric b*</i>		
Absence	7.36 bB	12.14 aA
Presence	13.28 aA	12.65 aA

Note. Lowercase letters separate the means within each column, and the capitals separate the means within the row. Equal letters do not differ from each other by the tukey test at 5% probability.

The other results did not show significant effect of the interaction and are presented independently for each factor (Figures 1 and 2). For the independent effect of the studied factors, there was significant difference ($p \leq 0.05$) between the different strains of probiotic bacteria for the variables L^* , a^* , b^* , pH and %Brix. Compared to the strains A, C and D, the bacteria B led to significantly higher values of L^* , b^* and %Brix (Figures 1A, 1B and 1C), lower values of pH and consequently higher acidity (Figure 2B). These data reveal good lightness, yellowish color and slight sweetness of the fermented milk with addition of probiotic bacteria B. In the presence of cupuaçu flavor and at seven days of storage, there were statistical differences ($p \leq 0.05$) for L^* , b^* and %Brix when compared to treatments in the absence of cupuaçu flavor and at the first day of storage (Figures 1A, 1B and 1C). The percentage increments in relation to the absence and presence of cupuaçu flavor corresponded to 37.82%, 4.21% and 26.97%, respectively, for L^* , b^* and %Brix, while the increments corresponding to the storage time were 61%, 20.15% and 4.88% for the same variables mentioned above. The pH value in the absence of cupuaçu flavor was higher than that of the treatment in the presence of cupuaçu flavor, which allows us to infer that cupuaçu pulp contributed to decreasing the values of pH and increasing the acidity of fermented milk (Figure 2B).



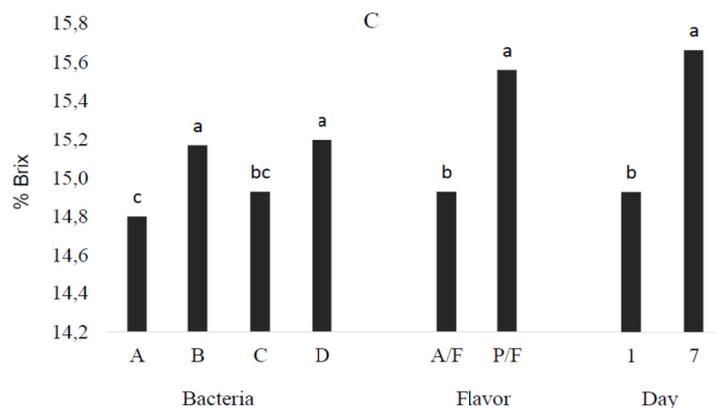


Figure 1. Colorimetric parameters L*(A) and b* (B) and %Brix (C) of fermented milk with different strains of probiotic bacteria, in the absence and presence of cupuaçu flavor and at different storage times. Means followed by the same letter on the bars do not differ statistically by Tukey test at 5% probability level

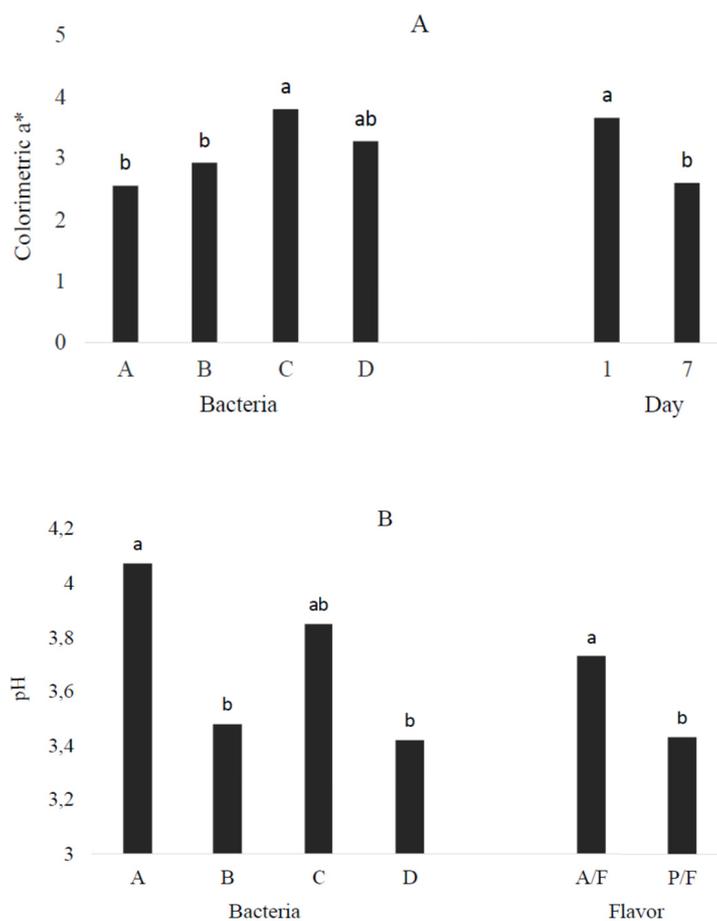


Figure 2. Colorimetric parameter a* (A) and pH (B) of fermented milk with different strains of probiotic bacteria and at different storage times and in the absence and presence of cupuaçu flavor. Means followed by the same letter on the bars do not differ statistically by Tukey test at 5% probability level

Regarding sensory variables, there were no significant effects ($p \geq 0.05$) of the interaction between bacteria (B) and flavor (F), and the independent results for each factor, when significant ($p \leq 0.05$), are presented in Table 4 and Figure 3.

The most accepted treatments with the different probiotic bacteria in terms of the attributes of color, aroma, appearance and overall aspect, considering the values of the means closest to the descriptive terms “liked slightly” and “liked moderately”, were those promoted by the addition of bacteria A, which led to the highest values of acceptance for all attributes mentioned above and did not differ statistically from the bacteria C and D, indicating that these products have good acceptance and can be marketed. According to Dutcosky (2011), a product reaching a percentage equal to or greater than 70% is considered accepted by the tasters. For the same analysis, the use of bacteria B resulted in the lowest values of acceptance, averaging around 5.42, so it fits in the acceptance class “Neither liked nor disliked” (Table 4).

It is relevant to mention that bacteria A promoted the best results in the analysis of bacteria (B) × flavor (F) interactions for the physicochemical evaluations of L*, pH and %Brix, and that the results corroborate those found in the acceptance test (Figures 3A and 3B).

Table 4. Sensory analysis of fermented milk in the absence and presence of cupuaçu flavor

Bacteria	Color	Odor	Appearance	Aspect
A	7.35 a	7.20 a	7.00 a	7.17 a
B	5.25 b	5.57 b	5.87 b	5.02 b
C	6.80 ab	6.25 ab	6.97 ab	7.32 a
D	6.67 ab	6.40 ab	6.45 ab	6.57 ab

Lowercase letters separate the averages within each column. Equal letters do not differ from each other by the Tukey test at 5% probability. n = 80 judges. 1 Structured hedonic scale of nine points (1 = i greatly disliked; 9 = i really liked it). The values correspond to the mean with estimate of the standard deviation.

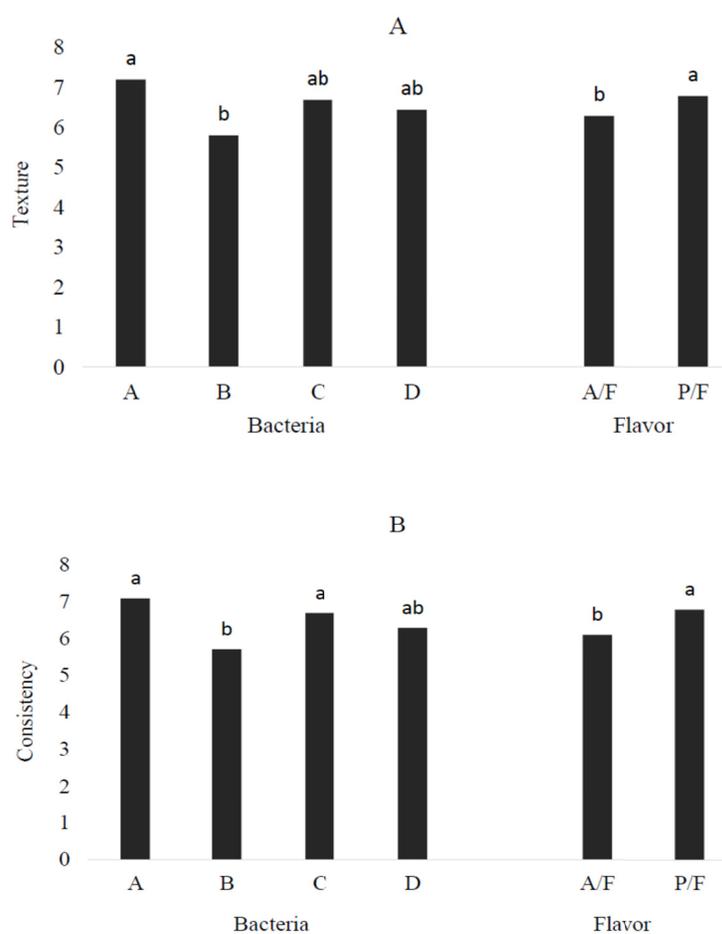


Figure 3. Texture (A) and consistency (B) of fermented milk with different strains of probiotic bacteria, in the presence and absence of cupuaçu flavor. Means followed by the same letter on the bars do not differ statistically by Tukey test at 5% probability level

In all samples of fermented milk, the Most Probable Number/mL (MPN/mL) of coliforms at 35 °C and coliforms at 45 °C were lower than 0.3 MPN/mL. According to the RTIQ of Fermented Milk (Brazil, 2005), the MPN/mL of coliforms allowed for fermented dairy beverages is up to 100 MPN/mL of coliforms at 35 °C and 10 MPN/mL of coliforms at 45 °C for indicative samples. The results indicate adequate hygienic-sanitary quality throughout the process of production and storage of the fermented beverages and demonstrate microbiological quality above the level required by the current legislation, therefore suitable for consumption (Table 5). According to the Brazilian legislation, *Salmonella* spp. must be completely absent (Brazil, 2001). The shelf life of fermented milk was longer than 60 days, with maintenance of quality of the sensory characteristics of the product.

Table 5. Microbiological analysis of fermented milk in the absence and presence of cupuaçu flavor

Bacteria	Total C.	Thermotolerant	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella</i> sp.
<i>Absence cupuaçu</i>					
A	0.1	0.1	Absence	Absence	Absence
B	0.1	0.1	Absence	Absence	Absence
C	0.1	0.1	Absence	Absence	Absence
D	0.1	0.1	Absence	Absence	Absence
<i>Presence cupuaçu</i>					
A	0.3	0.1	Absence	Absence	Absence
B	0.3	0.1	Absence	Absence	Absence
C	0.3	0.1	Absence	Absence	Absence
D	0.3	0.1	Absence	Absence	Absence

Note. The values correspond to the average of three replicates. *Reference standards according to the Normative Instruction n° 16 of 08/23/2005 of the Ministry of Livestock, Agriculture and Food Supply. Total C. (Total coliforms), *E. coli* (*Escherichia coli*), *S. aureus* (*Staphylococcus aureus*).

4. Conclusions

A bacteria B (*Lactobacillus casei*) apresentou-se valores de colorimetria L*, colorimetria b*, % Brix and pH superiores Bacteria B (*Lactobacillus casei*) led to higher values of L*, b*, %Brix and pH.

In the presence of cupuaçu flavor, the values of pH, L*, b* and %Brix were significantly higher, which allows us to infer that cupuaçu pulp contributes to decreasing the values of pH and increasing the acidity, lightness, b* and %Brix of fermented milk.

Fermented milk with addition of bacteria A (*Lactobacillus casei shirota*) in the presence of cupuaçu flavor showed reduction in pH and %Brix values and, therefore, better acceptance by consumers and bioconservation.

The presence of cupuaçu flavor in fermented milk with bacteria B (*Lactobacillus casei*) alters the colorimetric parameters L* and b* at seven days of storage.

The microbiological quality was higher than that required by the current legislation, indicating adequate hygienic-sanitary quality throughout the process of production and storage of the fermented beverages.

Fermented milk with addition of cupuaçu flavor constitutes an opportunity and possibility for developing new flavors from Amazonian fruits, strongly contributing to the durability of dairy products, since the physicochemical, sensory and microbiological characteristics are enhanced within the standards of food safety.

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