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Development and characterization of probiotic fermented smoothie beverage[1]

Desenvolvimento e caracterização de bebida fermentada probiótica *smoothie*

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ABSTRACT - Dairy products are the primary delivery for probiotics, with great popularity among the public looking for functional foods. The addition of probiotics to new food matrices requires studies about the stability and viability of these microorganisms in the final product. This study aimed to develop a probiotic smoothie beverage and to evaluate the microbiological quality, pH, acidity, syneresis, probiotics viability, sensory acceptance, and consumers' preference. For that, thirty-six beverage formulations were prepared using yogurt and different fruit pulps. Six formulations were selected for the study, as follows: probiotic yogurt with the addition of mango pulp, grape, red fruits (containing 8 and 10% sucrose), mango/ passion fruit, and red fruits/açaí, using a yogurt/pulp ratio of 60/40 (w/w). The beverages presented adequate hygienic-sanitary quality, with an initial probiotics viability around 7 log CFU mL^{-1}. An increase in acidity and a decrease in pH, as well as a higher rate of syneresis and a small decline in probiotic viability were observed for all formulations throughout 30 days of storage. The probiotic smoothie made with red fruit pulp (10% sugar) was selected due to its better sensory acceptance, and maintenance of probiotic counts to confer health benefits.

Key words: Yogurt. Bifidobacteria. Viability. Acceptance. Fruit Pulp.

RESUMO - Produtos lácteos são os principais veículos para incorporação de probióticos, e têm grande popularidade entre o público que procura alimentos funcionais. A aplicação probióticos em novas matrizes alimentares necessita de estudos a fim de verificar a estabilidade e viabilidade dos probióticos no produto. O objetivo deste trabalho foi desenvolver uma bebida probiótica *smoothie* e avaliar a qualidade microbiológica, pH, acidez, sinérese, viabilidade dos probióticos e aceitação e preferência sensorial. Para tal foram elaboradas 36 formulações, empregando-se iogurte e diferentes polpas de frutas. Seis formulações foram selecionadas sensorialmente contendo iogurte probiótico e as polpas de manga, uva, frutas vermelhas (com 8 e 10% açúcar), manga/maracujá, frutas vermelhas/açaí, na proporção 60/40 (m/m), as quais foram avaliadas. As bebidas apresentaram adequada qualidade higiênico-sanitária e a viabilidade inicial dos probióticos foi em torno de 7 log UFC mL^{-1}. Durante 30 dias de estocagem verificou-se o aumento da acidez e a diminuição no pH, o aumento na sinérese e uma pequena diminuição na viabilidade dos probióticos, em todas as formulações. A bebida *smoothie* contendo iogurte probiótico e polpa de frutas vermelhas (10% açúcar), foi selecionada devido à aceitação e preferência sensorial e a manutenção dos níveis de probióticos para conferir benefícios à saúde do consumidor.

Palavras-chave: Iogurte. Bifidobactérias. Viabilidade. Aceitação. Polpa de frutas.

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INTRODUCTION

Consumers' behavior in relation to food has changed, once consumers are more concerned about the relationship between diet and health (SHORI, 2016). Fermented dairy products containing probiotics have great popularity among the public looking for functional foods due to the presence of viable microorganisms that confer health benefits (BALLUS *et al*., 2010).

Yogurt is obtained by the fermentation of whole, skimmed, or standardized milk, through the action of *Lactobacillus delbrueckii* ssp *bulgaricus* and *Streptococcus thermophilus*, which can be accompanied by other lactic acid bacteria that confer specific characteristics to the final product (BRASIL, 2007). Yogurt is a widely consumed food all over the world for its therapeutic, nutritional, and sensory benefits (ALMEIDA *et al*., 2008; GONZALEZ; ADHIKARI; SANCHO-MADRIZ, 2011). It is recognized as a healthy product with a growing consumer demand based on healthy eating trends. In addition, the yogurt market is characterized by innovations that have attracted new consumers (MACBEAN, 2010).

Currently, there is a growing trend to fortify dairy products with fruit to improve the nutritional and sensory profile of the products (KAILASAPATHY; HARMSTORF; PHILLIPS, 2008). The incorporation of fruits into the yogurt has contributed significantly to a healthy image and increased yogurt consumption (HERNÁNDEZ-HERRERO; FRUTOS, 2014). Fruits are recognized sources of vitamins, minerals, and fibers, and its protective effect is due to phytochemicals with antioxidant action in its composition (MELO *et al*., 2008). Fruit juices are consumed and appreciated worldwide, not only for their flavor but also because they are nutritionally important in the human diet. Some studies have reported that fruit juice can be a good delivery for functional ingredients, such as probiotics (NAZZARO *et al*., 2008).

Smoothie is defined as a consistent soft drink of a fresh fruit puree with milk, yogurt, or ice cream (RANI; KUMAR; SABIKHI, 2016). The combination of dairy products and fruit beverages is gaining a lot of interest among the consumers as juiceceuticals, like fruit-yogurt beverages that are typical examples of hybrid dairy products offering health, flavor, and convenience (KRUPA; JANA ATANU; PATEL, 2011). The fruits can be added to yogurt alone or in the form of blends, chilled or frozen, and as juice or syrups (HERNÁNDEZ-HERRERO; FRUTOS, 2014).

Probiotics are defined as live microorganisms which when administered in suitable amounts confer a health benefit to the host (KORBEKANDI *et al*., 2015). Several authors have reported the clinical and therapeutic effects of the benefits attributed to the ingestion of food products containing these microorganisms (ALMEIDA *et al*., 2008). However, some factors can affect the probiotic viability, including the acidity of the substrate, dissolved oxygen, and the post-acidification of fermented beverages, which vary according to the different bacterial species. The viability of bacteria is an important characteristic of the use of probiotics in beverages, once they should survive during the shelf life, with minimum viable cells of 10^6-10^7 CFU/mL (SHORI, 2016), thus, the use of probiotic cultures in different food matrices should be investigated. The objective of this study was to develop a probiotic fermented beverage and to evaluate the microbiological, physicochemical, and sensory aspects and the probiotic viability during the storage.

MATERIAL AND METHODS

The study was carried out in the pilot plant and laboratory in the Dairy Research Center (Tecnolat), at the Institute of Food Technology, Campinas, SP.

The following ingredients were used: mixed probiotic culture, Kit Bifi, CSL, Italy (*Bifidobacterium longum*, *B. infantis* and *B. breve*) and a thermophilic culture, Jointec X3, CSL, Italy (*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*); skim milk powder (Molico, Nestlé); pasteurized skim milk (Xandô); pasteurized fruit pulp (DeMarchi); sucrose (refined sugar, União).

For the preparation of 36 smoothie formulations, yogurt was made using reconstituted skim milk (12%) and sucrose (8%). This base mixture was heat-treated at 85 °C for 20-30 minutes, cooled (42-44 °C) and the thermophilic culture Jointec X3 was inoculated. The fermentation was performed in a chamber at 42 °C until reaching pH 4.7 ± 0.1. Then, the pasteurized fruit pulps (mango, acerola, grape, acerola/mango, açaí, acerola/cocoa, acerola/mango/ pineapple, acerola/passion fruit, red fruits (RF), strawberry, strawberry/blackberry, and strawberry/açaí/blackberry) were added to the yogurt formulations at yogurt/pulp ratios of 60/40, 55/45, and 50/50 (w/w). An additional amount of sucrose (heat-treated at 85 °C / 10 min.) was added to the yogurt to reach 8% sugar in the final product. The beverages were stored at 8 ± 2 °C and evaluated for pH, acidity, and soluble solids (ºBrix). The samples were subjected to sensory evaluation after 1-2 days of manufacture by six panelists selected for sensory acuity according to ISO-13299 (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 2016). The appearance, odor, flavor, texture/mouth feel, and overall quality of the samples were evaluated using a linear 10 cm structured scale to quantify the intensities of the characteristics perceived, in which the scores 0, 5, and 10 corresponded to nonexistent/very weak, moderate, and very strong, respectively. After the sensory evaluation, the panelists consensually described the overall impression using a structured linear scale of 10 cm (0 = extremely bad, 1 = very bad, 3 = bad, 5 = regular, 7 = good, 9 = very good and 10 = excellent). The six formulations with the highest scores in the sensory evaluation were selected, as well as the better yogurt/fruit pulp ratio.

Six formulations were prepared as described above, with the addition of Kit Bifi and the yogurt culture to promote fermentation. Then, the selected pulps (mango, grape, mango/passion fruit (80/20), red fruits (RF, corresponding to a blend containing blackberry, strawberry, and raspberry), and red fruits/açaí (95/5) were added, using a yogurt/pulp ratio of 60/40 (w/w). Sucrose was added to reach a final concentration of 8% sugar in the final product (except for the formulation containing red fruits with 10% sucrose). The preserving agent potassium sorbate was also used in the concentration of 0.03%.

The beverages were stored in a cold room at 8 ± 2 °C and evaluated for microbiological characterization (total and thermotolerant coliforms, molds and yeasts) after 1 day of manufacture, and probiotic counts (Bifidobacteria), pH, titratable acidity and syneresis at 1, 15, and 30 days of storage. From 7 to 10 days of storage, the formulations were submitted to the sensory evaluation through the acceptance tests and preference ranking.

The methodology proposed by the International Dairy Federation (INTERNATIONAL DAIRY FEDERATION, 2007) was used for the selective enumeration of the probiotic microorganisms (*Bifidobacterium* spp.), with modifications. For that, MRS agar was supplemented with lithium chloride (0.1%), L-cysteine (0.05%) and dicloxacillin (0.5 mg / L), using depth plating and incubation under anaerobic conditions at 37 ± 1 °C for 72 ± 3 hours. The coliforms at 30 ºC and thermotolerant coliforms (at 45 ºC) were determined by the most probable number using lauryl sulfate tryptose broth (LST), incubated at 30 ± 1 ºC for 24 ± 2 hours (ISO 4831) (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 2006). To confirm the presence of coliforms, aliquots from the LST tubes exhibiting microbial growth and gas production were transferred to tubes containing 2% brilliant green broth (BGB, Difco) and EC broth (Difco). The tubes with BGB broth were incubated at 30 ± 1 °C for 24 ± 2 h to confirm the presence of coliforms at 30 °C, while the tubes containing EC broth were incubated for up to 48 ± 2 hours at 44 ± 1 °C for confirmation of coliforms at 45 °C (ISO 7251) (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 2005). Molds and yeasts were enumerated using dichloran rose bengal chloramphenicol agar (DRBC, Difco), incubated for 5 days at 25 ± 1 °C (FRANK; YOUSEF, 2004).

The pH values of the pulps and beverages were measured in a digital pH meter (B-375, Micronal, São Paulo, Brazil). The titratable acidity of the pulps was determined by titration with 0.1 N sodium hydroxide to pH 8.3 (INSTITUTO ADOLFO LUTZ, 2008), and the results were expressed as % citric acid per 100 g sample. The total soluble solids content (ºBrix) of the pulps was measured in a digital refractometer (HI96801, Hanna, Barueri, Brazil). The titratable acidity of the beverages was determined by titration with 0.1 N NaOH and the results were expressed as % lactic acid per 100 grams of the product (BRASIL, 2006).

Spontaneous syneresis of beverages was determined according to Silva, Pacheco. and Antunes (2010). For that, 10 mL aliquots were maintained at 8 ± 2 °C in 15 mL conical tubes, and syneresis or whey drainage was measured in centimeters.

Regarding the acceptance and preference tests, sixty consumers of fruit juice and yogurt evaluated six smoothie beverages (mango, grape, mango/passion fruit, red fruits with 8% sucrose, red fruits/açaí, and red fruits with 10% sucrose) according to the overall acceptability, appearance, aroma, and flavor using a 9-point hedonic scale (9=like extremely, 5= not like or dislike, and 1= dislike extremely) (MEILGAARD; CIVILLE; CARR, 2006). The samples were also evaluated according to the ideal intensity for the attributes fermented milk flavor, fruit flavor, sweetness and consistency using a 5-point just about right scale (5 = much too strong/sweet/consistent, 3 = just about right, 1 = much too weak/sweet/consistent).

The samples (40 mL/12 °C) were presented to the consumers in a sequential monadic form following a randomized complete block design. Mineral water was offered before and between the samples for palate cleansing. The tests were conducted in individual booths with fluorescent lamps, equipped with the computerized system *Compusense Five* 4.8 version (Compusense Inc., Guelph, ON, Canada). Data were submitted to analysis of variance (ANOVA) and Tukey’s Test. To evaluate the preference ranking, each consumer was asked to rank the samples from the most to the least preferred, and the results were analyzed by the Friedman and Fischer tests.

The pH, acidity, rate of syneresis, and probiotic counts were determined for all samples during 30 days of storage. The analysis of variance (ANOVA) was used to evaluate the differences between the formulations and the effect of time on the samples. The Tukey’s test at the 5% probability level was used to compare means. Data were analyzed using Minitab software, version 16.1.1.

RESULTS AND DISCUSSION

The total soluble solids (ºBrix), pH, and titratable acidity of mango, acerola, grape, and strawberry pulps were in accordance with the Normative Instruction # 1 (BRASIL, 2000). There are no standards in the current legislation for the other pulps used, alone or in combination.

The pH of the 36 smoothie formulations ranged from 3.84 to 4.65; the total soluble solids content (ºBrix) from 12.1 to 18.7, and the titratable acidity from 0.492 to 1.160 g lactic acid per 100 g sample. The beverages containing the acerola/passion fruit pulp exhibited the higher acidity and lower pH, and the beverages made with grape and mango pulps presented the higher ºBrix. The variation in pH and acidity of the beverages was due to the natural acidity and the vitamin C content of the fruit pulps. A great variation was also observed in the total soluble solids content of the beverages, probably due to the pulp composition, which contains high total sugar levels.

After the consensual sensory evaluation, six formulations were selected, as follows: mango, grape, mango/passion fruit (80/20), red fruits (8 and 10% sucrose), and red fruits/açaí (95/5) in a yogurt/ fruit pulp ratio of 60/40 (w/w).

The pH, ºBrix, and titratable acidity (%) of the pulps used in the preparation of the six smoothie formulations were respectively 4.06; 14.6; and 0.643 for mango pulp, 3.03; 18.0; and 0.911 for grape pulp, 3.44; 14.0; and 1.492 for the mango/passion fruit (80/20), 3.19; 7.0; and 1.494 for the red fruits, and 3.21; 6.1; and 1.328 for the red fruits/açaí pulp (95/5).

Table 1 shows the results of the microbiological characterization of all samples after one day of manufacture. Total and thermotolerant coliforms counts were less than 0.3 MPN/mL, while yeasts and molds counts ranged from 3 to 6 CFU / mL, which is probably from the fruit pulps used in the beverages preparation.

The results are within the limits established by the Brazilian legislation for similar products, such as fermented milk and fermented milk beverages (BRASIL, 2007). Therefore, the beverages of this study presented adequate microbiological quality, guaranteeing the safety of the product for consumption.

Table 1 - Microbiological characterization of the six smoothie beverages

Smoothie beverages	Coliforms at 30 ºC (MPN/mL)	Coliforms at 45 ºC (MPN/mL)	Molds and Yeasts (CFU/mL)
Mango	<0.3	<0.3	<10.0
Grape	<0.3	<0.3	6.0
Mango-Passion fruit	<0.3	<0.3	5.0
[1,2]RF 8%	<0.3	<0.3	3.0
[1,3]RF 10%	<0.3	<0.3	3.0
[1]RF-açaí	<0.3	<0.3	3.0

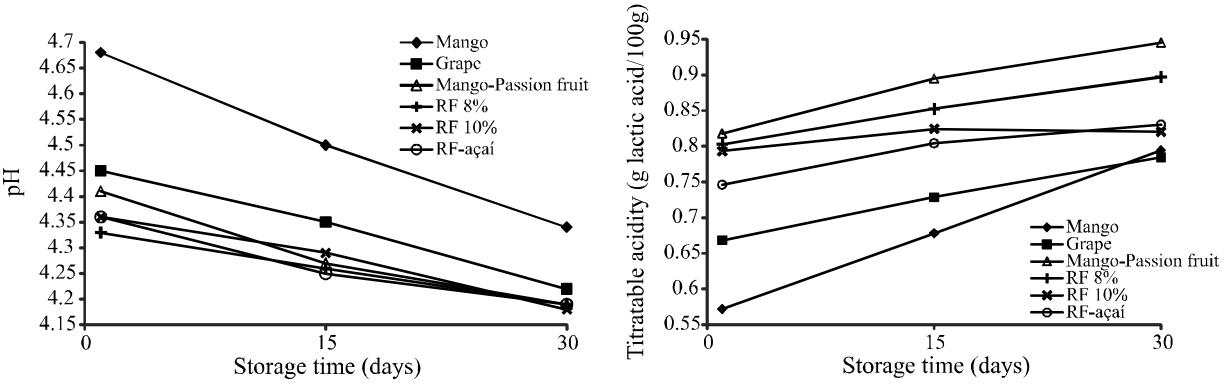
[1]RF = Red fruits; [2] Formulation containing red fruits (RF) with the addition of 8% sucrose; [3]Formulation containing red fruits (RF) with the addition of 10% sucrose

The initial and final pH of the beverages ranged from 4.67 to 4.33, and 4.33 to 4.17, respectively. Figure 1 shows the pH and acidity of the beverages throughout the storage time.

A significant (P<0.05) and linear decrease in pH was observed over time, which is common during the storage of fermented dairy products, due to the production of organic acids by lactic acid bacteria. The post-acidification of yogurts during the refrigerated storage is due to the production of lactic acid, especially by *L. bulgaricus* (KORBEKANDI *et al*., 2015). Donkor *et al*. (2006) reported that the pH of yogurts stored under refrigeration can vary to a greater or lesser extent, depending on the initial pH value, the refrigeration temperature, the storage time, and the post-acidification behavior of the microorganisms. In yogurts, the main microorganisms responsible for post-acidification during the storage *are L. delbrueckii subsp. bulgaricus* and *S.thermophilus*.

Despite the significant differences in pH of the beverages throughout the storage, initial and final pH values of 4.4 and 4.2 were observed, except for the beverage made with mango pulp, which exhibited a higher initial and final pH when compared to the other formulations, probably due to the lower acidity of this pulp when compared to the others. Gallina *et al*. (2012) also obtained initial pH value near 4.40 in a beverage formulation made from guava

Figure 1 - pH and titratable acidity of smoothie beverages during 30 days of storage at 8 °C: mango (♦), grape (■), mango/passion fruit (Δ), red fruits 8% (+), red fruits 10% (x), and red fruits/açaí (○)



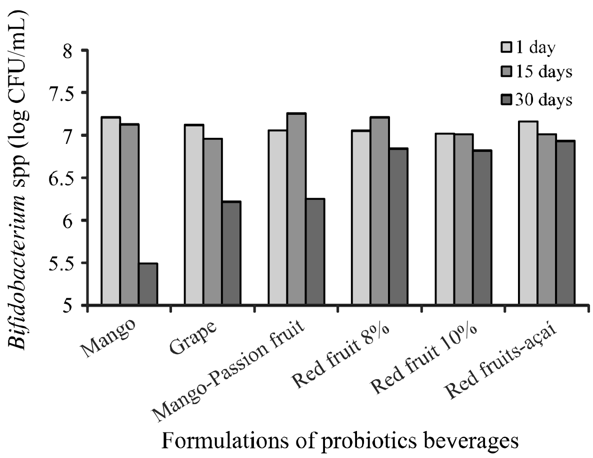
pulp and milk fermented (50/50%) with yogurt starter culture and bifidobacteria, with and without the addition of prebiotics (FOS). Kailasapathy, Harmstorf. and Phillips (2008) also found a decline in pH in yogurt with the addition of commercial fruit pulps, with a value of 4.45 in the first day of manufacture and 4.25 after 35 days at 4 ºC, which is consistent with the results observed in this study.

Figure 1 shows the values of titratable acidity of the beverages throughout the storage. The acidity ranged from 0.5716 to 0.8180 g lactic acid/100 g sample after one day of manufacture and 0.7958 to 0.9454 g lactic acid/100 g at the end of the storage. A significant increase in acidity (P<0.05) was observed for the beverages with the addition of mango, red fruits (RF 8%) and grape pulps, which was not observed for the beverages made with mango/passion fruit, red fruits (RF 10%) and red fruits/açaí pulps. Yogurts supplemented with fruits rich in antioxidants have lower pH and higher titratable acidity when compared to yogurts without the addition of fruits (KUMAR; KUMAR, 2016). Hossain, Fakruddin. and Islam (2012), Gallina *et al.* (2012) and Barbosa and Gallina (2017) also observed an increase in acidity and a decrease in pH of yogurts containing fruit pulp. The beverages presented titratable acidity values similar to those observed for fermented milk, usually between 0.6 and 1.0 g of lactic acid per 100 g of product (BRASIL, 2007). The parameters pH and acidity play an important role on the sensory characteristics and probiotics viability of the products (BARBOSA; GALLINA, 2017).

For the success of probiotic beverages, it is very important that probiotics maintain their viability and functionality during the shelf life of the product (SHORI, 2016). The probiotics viability in yoghurts depends on several factors such as the probiotic strain used, the yoghurt culture, the interaction between species, culture conditions, fermentation time, storage conditions, yoghurt pH (post-acidification during the storage), sugar content (osmotic pressure), milk solids content, nutrient availability, presence of hydrogen peroxide, dissolved oxygen, buffer capacity, concentration of metabolites (lactic and acetic acid), β-galactosidase concentration, temperature, among others (DONKOR *et al.*, 2006; KAILASAPATHY; HARMSTORF; PHILLIPS, 2008; SHORI, 2016). A significant reduction (P<0.05) of the probiotics viability was observed for all beverages during the storage, with counts ranging from 5.49 to 6.93 log CFU mL^{-1}) (Figure 2).

The sensitivity of the probiotic culture is affected by low pH values and is dependent on the strain, especially for *Bifidobacteria* (ALMEIDA *et al.*, 2008). It is worth mentioning that pH values lower than 4.30 in yogurts

Figure 2 - Viable counts (log $CFU.mL^{-1}$) of the probiotic microorganisms (*Bifidobacterium* spp.) on smoothie beverage during 30 days storage at 8 ± 2 °C



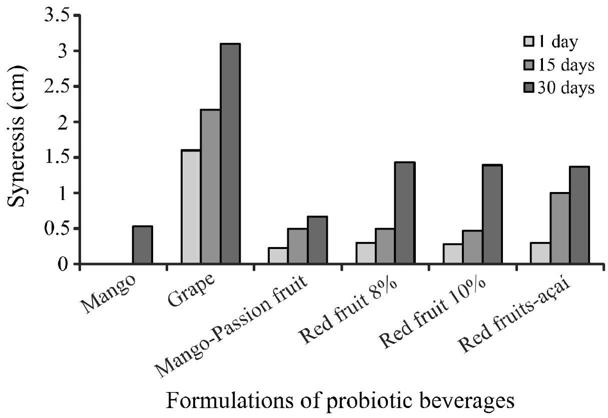
can affect the probiotics viability, and bifidobacteria are sensitive to pH of fruit juices (pH 3-4) (BARBOSA; GALLINA, 2017). However, *B. animalis* strains are more resistant to acids when compared to strains of other species. Studies have reported that the addition of vegetables and fruit juices or pulps to dairy beverages may be deleterious to the viability of some probiotic species, due to the acidity and presence of antimicrobial compounds such as organic acids (benzoic acid) and flavor compounds (SHORI, 2016).

Barbosa and Gallina (2017) found probiotic counts around 6 log CFU/mL in beverages made with probiotic yogurt and mango pulp, and Gallina *et al.* (2011) found *Bifidobacteria* counts of 6 log CFU/mL in fermented probiotic milk containing FOS during 28 days of refrigerated storage. In addition, Gallina *et al.* (2012) observed counts of 10^6-10^7 CFU/mL in a beverage containing probiotic yogurt and guava pulp during 30 days of storage. Kumar and Kumar (2016) observed a significant reduction in the number of viable probiotic cells during storage in yogurts, probably due to the processing conditions and the low pH and high acidity of the products. Kailasapathy, Harmstorf. and Phillips (2008) reported a correlation between pH of yogurts and the probiotics viability during storage, which was affected by the addition of fruit pulp, indicating that fruit-based yogurts and the properties of fruit preparations, such as pH, can affect the viability of probiotic bacteria.

Several authors have reported the importance of the survival of the probiotic bacteria in populations high enough to confer health benefits to the consumer. Shori (2016) reported that the beverage must exhibit a minimum number of viable probiotic cells during the shelf life (10^6-10^7 CFU/mL), and Kumar and Kumar (2016) reported that probiotics must reach the intestine in sufficient numbers between 6 and 7 log CFU/gram of product to confer health benefits. For the six beverages of the present study, probiotics counts remained in appropriate numbers during the storage, except for the formulation with the addition of mango pulp, which presented different viability when compared to the beverages containing red fruits (8 and 10%) and red fruits/açaí pulp, with no significant differences from the other formulations (P>0.05). Although the beverage made with mango pulp presented the highest initial and final pH, it exhibited the lowest probiotics counts, indicating that pH was not the main factor to reduce the viability of probiotics during the storage. Almeida *et al.* (2008) also reported that the growth of probiotic cultures (*Lb acidophilus* and *Bifidobacterium bifidum*) in yogurts made with açaí pulp was not inhibited by the low pH values during the storage, which exhibited a log reduction after 21 days at 4 ºC, with the probiotic counts still in accordance with the Brazilian legislation (BRASIL, 2007), which recommends minimum bifidobacteria counts of 10^6 CFU/g. Therefore, among the six beverages of this study, only the mango beverage did not comply with this legislation after 30 days of refrigerated storage.

Syneresis is a relevant technological problem and a common defect in yogurt, which can cause a consumer’s rejection of the product. The syneresis is defined as the shrinkage of the gel, which occurs with the expulsion of liquid or whey separation and is related to the instability of the gel network, resulting in the loss of the ability to entrap all the serum phase (VARELTZIS *et al*., 2016). Figure 3 shows the rate of syneresis of the six beverages over 30 days of storage, with a significant increase (P<0.05) for all formulations. Barbosa and Gallina (2017) also observed an increase in syneresis in beverages made with yogurt and mango pulp during the refrigerated storage, especially after 30 days of storage.

Figure 3 - Rate of syneresis (cm) of the smoothie beverage formulations during 30 days of storage at 8 ± 2 °C



Several factors can affect syneresis in yogurts, including the total solids content, milk composition (proteins, salts), changes in temperature, pH, and mechanical factors (agitation, vibration, homogenization, etc), type of culture, low acid production during fermentation, and high acidity during the storage (SILVA; PACHECO; ANTUNES, 2010; VARELTZIS *et al*., 2016). The addition of fruits to yogurt formulations may increase the rate of syneresis and result in a weak gel, due to the acidity of the pulps and the decrease of total solids (NARAYANA; GUPTA, 2013), leading to changes in the structure of protein network thus promoting syneresis. The beverages made

Table 2 - Sensory evaluation of smoothie beverages for the overall acceptability, appearance, aroma, and flavor, and the ideal intensity of fermented milk flavor, fruit flavor, sweetness, and consistency as well as the preference ranking

Attribute		Smoothie beverages of yogurt and fruit pulps						
		Mango	Grape	Mango/Passion fruit	RF 8%	RF/Açaí	RF 10%	LSD
Acceptability	Overall	7.5(1.2) a	7.0(1.4) a	7.4(1.1) a	5.3(1.8) b	5.2(2.1) b	7.2(1.2) a	0.47
	Appearance	7.5 (1.0) a	7.3(1.1) a	7.4(1.1) a	6.1(1.5) b	6.4(1.7) b	7.5(0.9) a	0.54
	Aroma	6.7(1.3) ab	6,8(1.3) a	7.3(1.1) a	6.3(1.6) bc	5.9(1.4) c	7.2(0.9) a	0.56
	Flavor	7.3 (1.5) a	7.0(1.5) a	7.3(1.5) a	5.0(2.1) b	4.4(2.2) b	7.2(1.5) a	0.78
Intensity	Fermented milk flavor	3.0 (0.5) a	3.0(0.6) a	2.9(0.6) a	3.2(1.1) a	3.0(1.1) a	3.0(0.7) a	0.36
	Fruit flavor	2.9(0.4) abc	2.6(0.8) c	3.1(0.5) a	2.7(1.1) bc	3.1(1.1) a	3.0(0.5) ab	0.39
	Sweetness	3.1(0.4) a	3.1(0.7) a	2.9(0.6) a	2.3(1.1) b	2.2(0.9) b	3.0(0.6) a	0.30
	Consistency	3.5(0.7) a	2.9(0.7) b	3.3(0.6) a	2.7(1.0) b	2.7(1.0) b	3.4(0.7) a	0.33
Ranking		143 a	185 b	147 ab	307 c	309 c	169 ab	40

*Results of 60 evaluations expressed as mean (standard deviation) of acceptability/intensity and the rank sum values of all samples LSD: Least significant difference at 5% level of significance. Mean/Ranking sum values followed by the same lowercase letter on the same line do not differ statistically ($p>0.05$)

with the addition of mango pulp and mango/passion fruit pulp had the lowest rate of syneresis, with no significant differences between them ($P>0.05$) after 30 days of storage. In contrast, the beverage made with grape pulp exhibited the highest syneresis, differing significantly from the others. Regarding the acidity and pH, they were not the factors affecting syneresis, once the beverages containing mango and mango/passion fruit pulps presented the highest and lowest pH and acidity values, respectively.

With respect to the sensory evaluation, 88.3% of consumers were female and 11.7% male, aged from 18 and 60 years. Concerning the frequency of yogurt consumption, 11.7% of consumers consumed at least once during 15 days, 10% consumed once a week, 28.3% consumed 2 to 6 times a week, and 50% of the assessors reported consuming yogurt daily. The results of the acceptance test and the preference ranking are shown in Table 2.

Regarding the overall acceptability, especially for the attributes appearance, aroma, and flavor, the beverages containing mango, grape, mango/passion fruit, and red fruits 10% presented scores ranging from “like moderately” to “like very much”, and were the more accepted by consumers. For the intensity of fermented milk flavor, no significant differences were observed for all samples, which were close to the ideal intensity. Regarding the intensity of fruit flavor, the samples containing mango, mango/passion fruit, red fruits/açaí, and red fruits (10%) presented scores close to the just-right point. For sweetness, the beverages with mango, grape, mango/passion fruit, and red fruits (10%) presented scores close to the just-right point. Concerning the consistency of the samples, all scores were close to the ideal, and the beverages made with mango, mango/passion fruit and red fruits (10%) pulps showed scores between just-right point and “more consistent”, and were significantly different from the beverages made with grape, red fruits (8%), and red fruits/ açaí, with scores between just-right point and “less consistent”. The smoothie probiotic beverages made with mango, mango/passion fruit, and red fruits (10%) pulps were preferred in the ranking test when compared to the others.

CONCLUSIONS

1. The smoothie beverage made with probiotic yogurt and red fruits pulp (60/40, w/w) and 10% sugar stood out for the sensory acceptance, the preference ranking, and the maintenance of probiotic viability during storage when compared to the other formulations;
2. The selected probiotic smoothie beverages maintained the probiotic counts required for the health claim, thus it can be considered an appropriate vehicle for the incorporation of probiotics into a new functional product.

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