Chemical, microbiological and sensory viability of low-calorie, dairy-free kefir beverages from tropical mixed fruit juices

Pedro Paulo Lordelo Guimarães Tavares, Emanuele Araújo dos Anjos, Renata Quartieri Nascimento, Larissa Farias da Silva Cruz, Paulo Vitor França Lemos, Janice Izabel Druzian, Thâmilla Thalline Batista de Oliveira, Roberta Barreto de Andrade, Adriana Lúcia da Costa Souza, Karina Teixeira Magalhães-Guedes and Maria Eugênia de Oliveira Mamede

*Graduate Program in Food Science, Faculty of Pharmacy, Federal University of Bahia (UFBA), Salvador, Brazil; Sensory Analysis Laboratory, Faculty of Pharmacy, Federal University of Bahia (UFBA), Salvador, Brazil; Graduate Program in Biotechnology (Renorbio), Health Sciences Institute, Federal University of Bahia (UFBA), Salvador, Brazil; Department of Nutrition, Federal University of Sergipe (UFS), Lagarto, Brazil

ABSTRACT
The use of non-dairy matrices for probiotic delivery can be beneficial for vegetarian, vegan and lactose intolerant consumers. Stevia rebaudiana extract can be used as sweetener to replace sucrose for glucose intolerants. This research evaluated the use of kefir grains to prepare a low-calorie, fruit-based kefir beverage. Fermentation was carried out by inoculating kefir grains into a mixture of mango and umbu pulps. Two beverages, one containing sucrose, and the other, stevia, were characterized according to their proximate composition and submitted to a 30-day shelf-life period at 5°C, evaluating lactic acid bacteria, yeasts, pH, acidity, color parameters, soluble solids, ascorbic acid, sensory acceptance, and purchase intention every 10 days. Lactic and acetic acids were analyzed at the beginning and end of the shelf-life. Both beverages showed probiotic potential. The stevia-sweetened beverage had lower calorie content and good sensory acceptance. These results support the possibility to develop low-calorie, fruit-based kefir beverages.

Viabilidad química, microbiológica y sensorial de bebidas de kéfir bajas en calorías y sin lácteos preparadas a partir de jugos de frutas tropicales mixtas

RESUMEN
El uso de matrices no lácteas para la administración de probióticos puede ser beneficioso para consumidores vegetarianos, veganos e intolerantes a la lactosa. El extracto de Stevia rebaudiana puede utilizarse como edulcorante substituto de la sacarosa en el caso de intolerantes a la glucosa. Esta investigación se propuso evaluar el uso de granos de kéfir para preparar una bebida de kéfir baja en calorías y a base de frutas. La fermentación se llevó a cabo inoculando granos de kéfir en una mezcla de pulpas de mango y umbú. Posteriormente, se caracterizaron dos bebidas, una con sacarosa y otra con stevia, según su composición proximal, sometiéndolas a un periodo de vida útil de 30 días a 5°C. Luego, se evaluaron cada 10 días las bacterias lácticas, las levaduras, el pH, la acidez, los parámetros de color, los sólidos solubles, el ácido ascórbico, la aceptación sensorial y la intención de compra. Asimismo, se analizó el contenido de ácidos láctico y acético al inicio y al final de la vida útil. Se constató que ambas bebidas mostraron potencial probiótico. La bebida endulzada con stevia posee menor contenido calórico y buena aceptación sensorial. En conclusión, estos resultados fundamentan la posibilidad de elaborar bebidas de kéfir bajas en calorías y a base de frutas.

1. Introduction
Kefir is a fermented, probiotic beverage from eastern Europe (Arslan, 2015). Kefir grains are an aggregation of yeasts, lactic acid and acetic acid bacteria that coexist in symbiosis and are enveloped by a matrix of polysaccharides (Alsayadi et al., 2014). The most common substrates utilized for kefir are water with brown sugar and cow’s milk. On the other hand, few studies have evaluated the viability of fermenting alternative substrates such as fruit juices for those that suffer from intolerance to dairy products (Laureys & De Vuyst, 2017). Umbu (Spondias tuberosa) and mango (Mangifera indica L.) are common fruits in the northeastern region of Brazil, are rich in nutrients such as carotenoids and ascorbic acid, and are used to produce juices for their highly appreciated sensory characteristics (Jahurul et al., 2015; Teixeira et al., 2019). The use of such tropical fruits as fermentation substrates has the potential to insert new kefir beverages onto the food market.

The development of new beverages by combining prebiotic carbohydrates with probiotic microorganisms shows potential on the food market. Fructooligosaccharides (FOS) are considered as prebiotic carbohydrates for their potential to serve as substrates for gut microbiome and for the probiotic microorganisms present in food products, when used in combination (Freire et al., 2017). Elaborating a symbiotic beverage by combining FOS with kefir shows the promise to

CONTACT Pedro Paulo Lordelo Guimarães Tavares (pplordelo@gmail.com) Faculty of Pharmacy, Federal University of Bahia (UFBA), S/n, Ondina Campus, Salvador, BA 40170-115, Brazil.

© 2021 The Author(s). Published with license by Taylor & Francis Group, LLC. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
provide the food market with a product rich in beneficial effects.

The high consumption of sugar-sweetened beverages is a result of the abundant use of sucrose in the Brazilian diet and is associated with the risk of developing obesity. Studies have found that the use of artificially sweetened beverages could reduce weight and promote overall health (Borges et al., 2017) since artificial sweeteners usually have a zero-calorie content.

*Stevia rebaudiana* is a plant found in South America that contains glycosides, which has sweetening potential up to 300 times higher than that of sucrose and a zero-calorie content (Narayanan et al., 2014). Weber and Hekmat (2013) evaluated the influence of stevia extract on the survival of *lactobacillus* in yogurt and found that the presence of the sweetener did not result in impairment of the probiotic potential of the beverage. On the other hand, Denina et al. (2014) found that stevia had inhibitory potential in the multiplication of *Lactobacillus reuteri*, which leads to the need for further evaluation of the use of stevia in probiotic foods.

To ensure the probiotic effects of a product, a minimum value of viable cells is recommended, ranging from 10^6 to 10^9 CFU L^-1 at the expiry date (Shori, 2016), which can be a challenge when using a dairy-free food matrix. Therefore, the objective of the present work was to evaluate the viability of producing low-calorie, probiotic mixed fruit flavored kefir beverages with umbu and mango.

2. Materials and methods

Pasteurized umbu and mango pulps were bought from Doce Mel (Brazil), Fructooligosaccharide was bought from New Nutrition (Brazil), sucrose (União, Brazil) was bought from the local market, water kefir grains were donated by the Federal University of Bahia Recôncavo (Brazil), stevia powder extract (96.50% total stevioside, 60% rebaudioside A) was donated by Sweetmix (Brazil), xanthan gum and ascorbic acid were donated by Sigma-Aldrich (United States of America) and Granolab (Brazil), respectively.

2.1. Preparation of the kefir beverages

Each beverage formulation was prepared according to the fruit pulp proportions (Table 1). The frozen pasteurized fruit pulps were thawed and homogenized with the water volume defined for each beverage (Table 1). The substrates were packed in sterile glass vials, sealed with screw caps and refrigerated for further fermentation. Cultivation of the kefir grains was carried out according to Tavares et al. (2018). During a seven-day period, the water kefir grains were activated at room temperature in a solution containing water and brown sugar at 12 °Bx. After this period, the grains were inoculated in a proportion equivalent to 10% of the volume of the beverages and allowed to ferment at 25°C in a biological oxygen demand (BOD) incubator (CE-300/350 model, Cienlab, Brazil) for a period of 24 hours. After the fermentation process, the water kefir grains were removed from the fermented beverages and the remaining ingredients (sucrose/stevia, xanthan gum, fructooligosacharide and ascorbic acid) were added and homogenized. The xanthan gum was added to avoid phase separation, fructooligosacharide was added to provide the beverages with symbiotic potential (Brazilian Health Regulatory Agency (Anvisa), 2019), and ascorbic acid was added as a preservative. Stevia was added considering its maximum daily ingestion of 4 mg/kg of body weight, as proposed by Food and Agriculture Organization of the United Nations (FAO) (2019).

2.2. Acceptance test

The sensory analysis of the kefir beverages was carried out by 65 untrained tasters, both male and female. The tasters were asked to indicate how much they liked or disliked each beverage on a hybrid 10 cm hedonic scale (Villanueva et al., 2005). The samples were served in clear, 50 mL tulip-shaped glasses. The two beverages with the highest acceptance scores were chosen to carry out the following analyses as shown below (one sample from each group).

2.3. Proximate composition

The two most accepted kefir samples were characterized for their moisture, ash, carbohydrate, protein, fat, and total calories contents, according to the techniques established by Association of Official Analytical Chemists (AOAC) (2019). The moisture content was determined using an infrared moisture analyzer (IV3100 model, Gehaka, Brazil). For the ash content, the samples were incinerated and then weighed. The protein content was determined by the Kjeldahl method and the fat content was determined using the Bligh and Dyer (1959) method. The carbohydrates were measured as the difference from 100% of the sum of the other macromolecules and the calories were calculated considering 4 kcal per 1 g of carbohydrates and proteins and 9 kcal per 1 g of fat.

2.4. Shelf-life analysis

The two most accepted kefir beverages were evaluated for their physicochemical, microbial, and sensory stability during a 30-day period. Each beverage was produced, packed

| Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------|---|---|---|---|---|---|---|---|---|----|----|----|
| Mango (%) | 15.00 | 20.00 | 25.00 | 30.00 | 35.00 | 40.00 | 15.00 | 20.00 | 25.00 | 30.00 | 35.00 | 40.00 |
| Umbu (%) | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Water (%) | 65.87 | 60.87 | 52.87 | 47.87 | 38.87 | 33.87 | 68.86 | 63.86 | 58.85 | 53.85 | 48.84 | 43.84 |
| Sugar (%) | 3.00 | 3.00 | 6.00 | 6.00 | 10.00 | 10.00 | - | - | - | - | - | - |
| Stevia (%) | - | - | - | - | - | - | 0.01 | 0.01 | 0.02 | 0.02 | 0.03 | 0.03 |
| Xanthan gum (%) | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Fructooligosacharide (%) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ascorbic Acid (%) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Total (%) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
in sterile glass vials and maintained under refrigeration at 5°C in a BOD incubator (CE-300/350 model, Cienlab, Brazil). Samples were taken every 10 days to carry out the following analyses: pH, titratable acidity, vitamin C, total soluble solids, color (AOAC, 2019), lactic acid bacteria count, yeast count (American Public Health Association (APHA), 2015), sensory acceptance and purchasing intent (Meligaard et al., 2007).

The titratable acidity was determined by titration with 0.01 N NaOH solution (AOAC, 2019). The pH was determined by direct reading using a potentiometer (K39-1014B model, Kasvi, Brazil). The vitamin C content was determined by a redox titration using an iodine solution (AOAC, 2019). The total soluble solids were determined using an analogical refractometer (DR 201–95 model, Kruss, Germany). The color was analyzed using a benchtop colorimeter (CR 5 model, Konica Minolta, Japan) in the transmittance mode, obtaining values for L*, a* and b*. The total color difference ($\Delta E_{ordE}$) was also calculated, using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}.$$  

For the Lactic Acid Bacteria (LAB) count, serial dilutions were prepared in saline solution, inoculated onto MRS agar plates (Kasvi, Brazil) supplemented with cycloheximide (100 mg/L), incubated for 72 hours at 30°C and the colony formation units (CFU) counted. For yeasts, serial dilutions were prepared, inoculated onto Saboraud Agar (Kasvi, Brazil) containing chloramphenicol (500 mg/L) and incubated for 120 hours at 30°C.

Lactic and acetic acids were identified and quantified by High Performance Liquid Chromatography (Series 200 model, Perkin Elmer, United Kingdom) using a 220 mm-× 4.6 mm × 10 μm polypropylene H column (Perkin Elmer, United Kingdom), injection volume: 10 μL, UV-Vis detector at 220 nm, flow: 0.8 mL/min and mobile phase: ultra-pure water acidified with H2SO4 to pH 2.0. The peaks corresponding to each acid were identified from the retention times according to the standards.

A sensory acceptance test was carried out every 10 days by the same tasters who took part in the preliminary analysis, again using a hybrid 10 cm hedonic scale (Villanueva et al., 2005), and also determining the purchasing intent of the products using a hedonic scale ranging from 1 (would definitely not buy the beverage) to 5 (would definitely buy the beverage). The sensory analysis was approved by the Ethic Committee of the Nutrition School – Federal University of Bahia (project number 1.759.169).

### 2.5. Statistical analysis

A one-way ANOVA was carried out to compare the means of the 12 samples in the preliminary acceptance test, and to compare the mean results for each beverage individually during the shelf-life. The student T test was used to compare the mean values obtained for the samples in the physico-chemical characterization, and to compare the means obtained for the two beverages at the same shelf-life time. Pearson’s correlation test was also applied between all shelf-life analyses. The software STATISTICA 7.0 was used considering a 95% significance level.

### 3. Results and discussion

Figure 1 shows the mean acceptance scores awarded to each sample in the preliminary sensory analysis. The beverages with the lowest fruit pulp and sweetener concentrations showed the worst results. Samples 6 and 12 had the highest concentrations of fruit pulp and sweetener and the highest scores amongst the samples, both with scores above 6.50 points and were therefore chosen for the characterization and shelf-life analyses.

The beverages sweetened with stevia obtained lower scores when compared with the beverages sweetened with sugar. This finding suggests that the sweetening capacity of stevia may incur undesirable effects such as a slight bitter taste, that could lower the overall score of the beverage (Singla & Jaitak, 2016).

Table 2 reports the results of the proximate composition for the two beverages with highest acceptance. The results for moisture and carbohydrate showed statistical differences between the two samples, probably due to the addition of sucrose to sample 6, which impacted on the total calorie count.

The low number of calories in sample 12 is of commercial interest since the demand for sweetened beverages and low-calorie products has been increasing on the international market. Studies have also shown that a high consumption of sucrose-sweetened beverages may be associated with an increased chance of acquiring type 2 diabetes mellitus and other chronic diseases (Singh et al., 2015). In addition, the evidence suggests a beneficial effect on the body mass index when sucrose-sweetened beverages are replaced by lower-calorie beverages, which may contribute to a reduction in the risk of developing chronic non-communicable diseases (Zheng et al., 2015).

The use of stevia shows the promise of contributing to a lower energy intake and the promotion of a balanced calorie consumption, while being a relatively safe sweetener considered as GRAS (Generally Recognized as Safe) (Borges et al., 2017). Both samples had low fat and protein contents, since fruits are usually not rich in these nutrients (O’Shea et al., 2015). Kefir fermentation is known to produce peptides, which have been associated with some beneficial

![Figure 1](#)
effects in the organism, such as the inhibition of lipogenesis and modulation of oxidative damage (Tung et al., 2017). Regarding the ash content, the results found were similar to those described by Natal et al. (2017), while working with mango juices.

Figure 2 shows the physicochemical behavior of the beverages during the 30-day shelf-life period. Regarding the pH and acidity, as expected, these parameters showed opposite behaviors during the period, with a strong negative correlation between them for both the sucrose sweetened kefir (−0.97, p < .05) and the stevia sweetened kefir (−0.81, p < .05). The microorganisms present in the kefir grains are responsible for the production of acetic and lactic acids. Tavares et al. (2018) reported that the production of lactic acid during the fermentation of kefir is of great importance, due to its inhibitory effect on both spoilage and pathogenic microorganisms.

Regarding the total soluble solids (TSS), there was a significant difference between the samples, which can be explained by the fact that stevia is considered a non-caloric sweetener. On the other hand, no statistical difference was observed between different shelf-life periods for the same sample. Different results were identified by Khatoon and Gupta (2015), who found a lower TSS count after 7 days of storage. A strong positive correlation with lactic-acid bacteria count (0.89, p < .05) was only observed for the stevia sweetened kefir which could be associated with the fact that the soluble solids serve as an important substrate for the survival of these microorganisms.

Regarding the ascorbic acid, Oliveira et al. (2013) suggested that a loss of more than 50% of the initial content of ascorbic acid at the end of a product’s shelf-life is a determinant of low-quality storage. In the present work, the loss of ascorbic acid was lower for both beverages (23.63% for the sucrose sweetened beverage and 15.57% for the stevia sweetened beverage), which is a good predictor.

Negative correlations were observed between ascorbic acid, ΔE (−0.94 for both samples, p < .05) and total acidity (−0.92 for sucrose and −0.95 for stevia sweetened kefirs, p < .05). A positive correlation was found with pH (0.95 for sucrose and 0.90 for stevia sweetened kefirs, p < .05). These correlations are justified since ascorbic acid can degrade into browning compounds due to both aerobic and anaerobic reactions in aqueous acidic solutions (Oliveira et al., 2013).

![Table 2. Results from the proximate composition for the two samples with the best acceptance.](image)

Table 2. Resultados de la composición proximal de las dos muestras que obtuvieron mejor aceptación.

| Sample                  | Moisture (%) | Ashes (g/100 mL) | Carbohydrates (g/100 mL) | Fats (g/100 mL) | Proteins (g/100 mL) | Calories (kcal/100 mL) |
|-------------------------|--------------|------------------|--------------------------|---------------|-------------------|------------------------|
| Sugar-sweetened kefir   | 64.37 ± 0.14*| 0.19 ± 0.01*     | 14.70 ± 0.18*            | 0.12 ± 0.01*  | 0.62 ± 0.02*      | 62.61 ± 0.67*          |
| Stevia-sweetened kefir  | 93.01 ± 0.10*| 0.13 ± 0.02*     | 6.18 ± 0.06*             | 0.11 ± 0.01*  | 0.63 ± 0.02*      | 28.17 ± 0.19*          |

Results are shown as means ± standard deviation. Equal lower-case letters indicate that there was no significative difference between results in columns at p ≤ 0.05.

Los resultados están indicados como medias ± desviación estándar. Las letras minúsculas iguales señalan que no hubo diferencias significativas entre los resultados de las columnas a p ≤ 0.05.

![Figure 2. pH (a), Acidity (b), Total Soluble Solids (TSS) (c) and Ascorbic Acid (d) concentrations during the 30 days of shelf-life. Error bars show standard deviations. Same lower-case letters indicate no significative difference for the same sample at different shelf-life periods at p ≤ 0.05. Same capital letter indicates no significative difference for the different beverages for the same shelf-life period analyzed at p ≤ 0.05.][image]

Figura 2. Concentraciones de pH (a), acidez (b), sólidos solubles totales (TSS) (c) y ácido ascórbico (d) durante los 30 días de vida útil. Las barras de error representan las desviaciones estándar. Las mismas letras minúsculas señalan que no hay diferencias significativas para la misma muestra en distintos periodos de vida útil a p ≤ 0.05. La misma letra mayúscula indica que no hay diferencias significativas para las distintas bebidas durante el mismo periodo de vida útil analizado a p ≤ 0.05. —— Representa la bebida endulzada con stevia y —— representa la bebida endulzada con sacarosa.
Figure 3. Yeast (a) and Lactic Acid Bacteria (b) count per mL and Lactic Acid Bacteria (c) count per 250 mL during the 30 days of shelf-life and lactic/acetic acid (d) concentrations (g/L) at the beginning and end of shelf-life period. Error bars show standard deviations. Same lower-case letters indicate no significant difference for the same sample at different shelf-life periods at $p \leq 0.05$. Same capital letter indicates no significant difference for the different beverages for the same shelf-life period analyzed at $p \leq 0.05$. For Figures a,b and c, ----- Represents the stevia sweetened beverage and ----- represents the sucrose sweetened beverage. For Figure d, - and - represent results for stevia and sucrose-sweetened beverages, respectively, regarding lactic acid concentration; and ------ and ------ represent results for stevia and sucrose-sweetened beverages, respectively, regarding acetic acid concentration.

Figura 3. Recuento de levaduras (a) y bacterias del ácido láctico (b) por mL; recuento de bacterias del ácido láctico (c) por 250 mL durante los 30 días de vida útil y concentraciones de ácido láctico/acético (d) (g/L) al inicio y al final del periodo de vida útil. Las barras de error representan las desviaciones estándar. Las mismas letras minúsculas señalan que no hay diferencias significativas para la misma muestra en distintos periodos de vida útil $p \leq 0.05$. La misma letra mayúscula indica que no hay diferencias significativas para las distintas bebidas durante el mismo periodo de vida útil analizado $p \leq 0.05$. Para las figuras a, b y c, ----- representa la bebida endulzada con stevia y ----- representa la bebida endulzada con sacarosa. Para la figura d, - y - representan los resultados de las bebidas endulzadas con stevia y sacarosa, respectivamente, en cuanto a la concentración de ácido láctico; y ------ y ------ representan los resultados de las bebidas endulzadas con stevia y sacarosa, respectivamente, en cuanto a la concentración de ácido acético.

Figure 3 shows the microbial counts during the 30-day shelf-life period and the lactic/acetic acid concentrations. Regarding the lactic acid bacteria count, for both samples, the T30 count was statistically lower than that at the other periods analyzed. The increased acidity level (correlation of $−0.97$ for the sucrose and $−0.82$ for the stevia sweetened beverages, $p < 0.05$) and the decrease in pH (correlation of $0.99$ for both samples, $p < 0.05$) could justify the reducing levels of microorganisms. The counts for all the periods analyzed showed more than $7.00 \log^{10} CFU$ per recommended portion of the product (250 mL), and thus the products can be considered as potentially probiotic up to the end of the shelf-life period, according to Minelli and Benini (2008). The main benefits associated with the consumption of the probiotic microorganisms in kefir are improved digestion, a hypocholesterolemic effect, glycemic control, an anti-hypertensive effect, anti-inflammatory effect, antioxidant activity, anti-carcinogenic activity and anti-allergenic effect (Rosa et al., 2017).

The period found for the shelf-life of the probiotics in the present work can be considered in accordance with data found in the literature, with results ranging from 7 (Nualkaekul et al., 2013) to 28 (Da Costa et al., 2017) or more days in similar studies. The use of stevia as a sweetener apparently did not affect the overall shelf-life period of the beverage studied, even though a statistical difference was observed for the LAB counts on the 20<sup>th</sup> and 30<sup>th</sup> days of analysis. Esmerino et al. (2013) obtained similar results.

With respect to the behavior of the yeasts, the results obtained at the different times of analysis were similar to those obtained by other authors (Corona et al., 2016; Randazzo et al., 2016). The T30 yeast counts were also statistically lower than at the other times analyzed for both samples.

Saccharomyces cerevisiae is the most common yeast found in kefir beverages and has been associated with some beneficial effects, such as inhibition of the growth of Shigella sp. and the cytotoxicity of Clostridium difficile toxins (Bolla et al., 2013). Strong correlations were found with pH (0.91, $p < 0.05$) and acidity ($−0.96$, $p < 0.05$), but only for the sucrose-sweetened kefir, which could be due to the fact that other variables, such as the soluble solids content could have a more important correlation with the yeast count in the stevia sweetened kefir.

Figure 3(d) shows the behavior of the lactic and acetic acids at the beginning and end of the shelf-life period. The lactic acid concentration was highest at the end of the fermentation period and decreased at the end of the shelf-life and similar results were found by Puerari et al. (2012). Acetic acid showed the opposite behavior, with its concentration increasing at the end of the shelf-life for both samples. Lactic and acetic acids are responsible for the unique kefir flavor and they also protect the beverage from spoilage by microorganisms (Puerari et al., 2012; Viana et al., 2017). The modification in the concentration of these acids could be justified by the fact that some lactic acid bacteria are not only capable of producing lactic acid, but of using it as a substrate for fermentation, producing acetic acid (Elferink et al., 2001).

As expected, the LAB count had a strong positive correlation with the lactic acid concentration ($0.99$, $p < 0.05$), since
they are its main producers (Magalhães et al., 2010), while the acetic acid concentration had strong correlations with the pH value (−0.99, p < .05) and total acidity (0.98, p < .05).

It must be pointed that the fruit juices were also evaluated before fermentation in relation to the pH, lactic acid bacteria and yeast counts. The medium value for pH was 3.81 ± 0.17 and there was no lactic acid bacteria nor yeast found. Immediately after the inoculation of the water kefir grains, the beverages, whilst not fermented, already showed microbial counts, with a 5.21 ± 0.07 log10CFU/mL of LAB and 5.79 ± 0.10 log10CFU/mL of yeast. These findings may indicate a very mild fermentation process, in comparison with results reported by Randazzo et al. (2016).

Figure 4 shows the results for the color parameters and total color difference for both samples during the shelf-life period. The sugar sweetened sample showed different overall color values than the stevia beverage, probably due to the different formulations, with the stevia beverage containing more water and no added sucrose. The samples presented a decrease in all the color parameters during the shelf-life period and the same result was observed by M.G.M. Costa et al. (2013).

Table 3 shows the results of the sensory analyses carried out during the shelf-life of the beverages. There was a statistical difference between the samples, with the sucrose beverage presenting greater acceptance and higher purchase intention scores, which suggests more familiarity with its taste as compared to stevia. Similar results were found by Alizadeh et al. (2014).

The longer the shelf-life, the lower the score found for both beverages in both analyses. K.K.F.D. Costa et al. (2017) obtained similar results studying a probiotic beverage, and some tasters described an acidic taste in both samples in the last shelf-life analysis.

The changes observed on the 10th day of the shelf-life in the instrumental color analyses did not appear to influence acceptance of the beverage, since a statistical difference was only observed for global acceptance on the last day of analysis and a noticeable difference in ΔE was already present on the 20th day. Dias et al. (2012) discussed the fact that, although color is an important sensory characteristic, product flavor may have a greater impact on its acceptance. The acidity and pH could be the parameters responsible for the decrease in beverage acceptance, since strong correlations between acceptance and acidity (−0.98 for sucrose and −0.85 for stevia, p < .05) and pH (0.99, p < .05 for both

| Shelf-life (days) | Sugar Sweetened Kefir Beverage | Stevia Sweetened Kefir Beverage |
|-------------------|-------------------------------|--------------------------------|
|                   | Global Acceptance | Purchasing Intention | Global Acceptance | Purchasing Intention |
| 0                 | 8.07ab (1.71) | 4.34ab (0.78) | 6.52ab (2.56) | 3.58ab (1.30) |
| 10                | 7.80ab (1.24) | 4.45ab (0.71) | 5.92ab (1.41) | 2.94ab (1.04) |
| 20                | 7.35ab (1.17) | 3.91ab (0.70) | 5.82ab (1.25) | 2.57ab (1.05) |
| 30                | 7.12ab (1.08) | 3.48ab (0.80) | 5.70ab (1.21) | 2.12ab (0.93) |

Results shown as means (standard deviation). Same lower-case letters indicate no significant difference for the same sample at different shelf-life periods at p ≤ 0.05. Same capital letter indicates no significant difference for the different beverages for the same shelf-life period analyzed at p ≤ 0.05.

Los resultados están indicados como medias (desviación estándar). Las mismas letras minúsculas señalan que no existen diferencias significativas para la misma muestra en distintos periodos de vida útil a p ≤ 0.05. La misma letra mayúscula indica que no hay diferencias significativas para las distintas bebidas durante el mismo período de vida útil analizado a p ≤ 0.05.

The changes observed on the 10th day of the shelf-life in the instrumental color analyses did not appear to influence acceptance of the beverage, since a statistical difference was only observed for global acceptance on the last day of analysis and a noticeable difference in ΔE was already present on the 20th day. Dias et al. (2012) discussed the fact that, although color is an important sensory characteristic, product flavor may have a greater impact on its acceptance. The acidity and pH could be the parameters responsible for the decrease in beverage acceptance, since strong correlations between acceptance and acidity (−0.98 for sucrose and −0.85 for stevia, p < .05) and pH (0.99, p < .05 for both

![Figure 4](image-url) **Figure 4.** L* (a), a* (b), b* (c) and ΔE (d) analyses during the 30 days of shelf-life. Error bars show standard deviations. Same lower-case letters indicate no significant difference for the same sample at different shelf-life periods at p ≤ 0.05. Same capital letter indicates no significant difference for the different beverages for the same shelf-life period analyzed at p ≤ 0.05. Representa la stevia sweetened beverage and represents the sucrose sweetened beverage.

![Figura 4](image-url) **Figura 4.** Análisis de L* (a), a* (b), b* (c) y ΔE (d) durante los 30 días de vida útil. Las barras de error representan las desviaciones estándar. Las mismas letras minúsculas señalan que no hay diferencias significativas para la misma muestra en distintos periodos de vida útil a p ≤ 0.05. La misma letra mayúscula indica que no hay diferencias significativas para las distintas bebidas durante el mismo periodo de vida útil analizado a p ≤ 0.05. Representa la bebida endulzada con stevia y representa la bebida endulzada con sacarosa.
samples) could indicate that the tasters who took part in the sensory tests preferred less acidic beverages. Salmerón et al. (2015) discuss that the production of acetic and lactic acids by the kefir microorganisms potentially affect both physicochemical parameters and the overall sensory characteristics of the beverages.

The stevia-sweetened kefir beverage showed global acceptance above 5.00 throughout the whole shelf-life and buying intention above 2.50 for the first three periods analyzed, which could show a willingness to accept stevia as a substitute for sucrose. Similar scores were found by Randazzo et al. (2016) when testing different fruit juices as substrates for kefir fermentation. Pimentel et al. (2015) also found similar values when working with probiotics sweetened with sucralose, however sucralose is derived from substances considered toxic, so the use of stevia may benefit from being a natural substance with less risk of toxicity (Tandel, 2011).

Functional foods are part of a new conception of food, since, in addition to their nutritional compositions, they beneficially affect one or more functions of the organism. Within this context, the relevance of the present study was to respond to an increasing demand for functional products on the food market (Markowick & Slizewska, 2017). The interaction between the in vivo probiotic and prebiotic functions in a synergistic way is of interest, since prebiotics are known to increase the viability of some microbial strains (Pandey et al., 2015) and promote even greater beneficial effects, other than those intrinsic to them separately.

4. Conclusion

The beverages differed in their total calories, due to the non-caloric quality of the stevia extract, giving it commercial potential. Both beverages could be considered potentially probiotic due to their lactic acid bacteria and yeast count during the storage period and had good sensory acceptance. Although the instrumental color analyses showed significative differences, these differences were only perceived in the sensory evaluations after 20 days of storage. The results showed that both beverages functioned as viable matrices for kefir and could be implemented on the food market as new synbiotic products, with the stevia beverage having the advantage of its low caloric content and that it is a natural sweetener with low toxicity. The beverages could serve as promoters of a healthier diet and a balanced gut microbiome, especially to those suffering from dairy intolerance since they contain beneficial microorganisms and non-digestible carbohydrates functioning in a synergic way. The use of a fruit-based matrix can increment the daily consumption of vitamin-rich products and promote an adequate fruit ingestion.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Alizadeh, M., Azizi-Lalabadi, M., & Kheirouri, S. (2014). Impact of using stevia on physicochemical, sensory, rheology and glycemic index of soft ice cream. Food and Nutrition Sciences, 5(4), 390–396. https://doi.org/10.4236/fns.2014.54047
Alisayadi, M., Jawfi, Y., Belarbi, M., Soualem-Mami, Z., Merzouk, H., Sari, D., Sabri, F., & Ghailim, M. (2014). Evaluation of anti-hyperglycemic and anti-hyperlipidemic activities of water kefir as probiotic on streptozotocin-induced diabetic wistar rats. Journal of Diabetes Mellitus, 04(2), 85–95. https://doi.org/10.4236/jdsm.2014.42015
American Public Health Association. (2015). Compendium of methods for the microbiological examination of foods (5th ed.). APHA Press.
Arslan, S. (2015). A review: Chemical, microbiological and nutritional characteristics of kefir. CYTA: Journal of Food, 13(3), 340–345. https://doi.org/10.1002/cyta.2014.981588
Association of Official Analytical Chemists. (2019). Official methods of analysis of AOAC international (21st ed.). AOAC International.
Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37 (8), 911–917. https://doi.org/10.1139/o59-099
Bolla, P. A., Carasi, P., Bolla, L., De Antoni, G. L., & Serradell, L. (2013). Protective effect of a mixture of kefir-isolated lactic acid bacteria and yeasts in a hamster model of Clostridium difficile infection. Anaerobe, 21, 260–263. https://doi.org/10.1016/j.anaerobe.2013.03.010
Borges, M. C., Louzada, M. L., De Sá, T. H., Lavery, A. A., Paran, D. C., Garzillo, J. M. F., Monteiro, C. A., & Millett, C. (2017). Artificially sweetened beverages and the response to the global obesity crisis. PLOS Medicine, 14(1), 1–9. https://doi.org/10.1371/journal.pmed.1002195
Brazilian Health Regulatory Agency (Anvisa). (2019). Alegações de propriedade funcional aprovadas. Retrieved April 29, 2021, from https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-1/biblioteca-de-normas-vinhos-e-bebidas/apecagemes-de-propriedade-funcional-aprovadas_anvisa.pdf
Corona, O., Randazzo, W., Micelli, A., Guarcello, R., Francesca, N., Erten, H., Moschetti, G., & Settanni, L. (2016). Characterization of kefir-like beverages produced from vegetable juices. LWT - Food Science and Technology, 66, 572–581. https://doi.org/10.1016/j.lwt.2015.11.014
Costa, K. K. F. D., Junior, M. S. S., Rosa, S. I. R., Callari, M., & Pimentel, T. C. (2017). Changes of probiotic fermented drink obtained from soy acid byproducts during cold storage. LWT - Food Science and Technology, 78, 23–30. https://doi.org/10.1016/j.lwt.2016.12.017
Costa, M. G. M., Fonteles, T. V., De Jesus, A. L. T., & Rodrigues, S. (2013). Sonicated pineapple juice as substrate for L. casei cultivation for probiotic beverage development: Process optimisation and product stability. Food Chemistry, 139(1–4), 261–266. https://doi.org/10.1016/j.foodchem.2013.01.059
Da Costa, G. M., Silva, J. V. C., Mingotti, J. D., Barão, C. E., Klososki, S. J., & Pimentel, T. C. (2017). Effect of ascorbic acid or oligofructose supplementation on L. paracasei viability, physicochemical characteristics and acceptance of probiotic orange juice. LWT - Food Science and Technology, 75, 195–201. https://doi.org/10.1016/j.lwt.2016.08.051
Deina, I., Semjonovs, P., Fomina, A., Trimane, R., & Linde, R. (2014). The influence of stevia glycosides on the growth of Lactobacillus reuteri strains. Letters in Applied Microbiology, 58(3), 278–284. https://doi.org/10.1111/lam.12187
Dias, N. A. A., Sara, S. B., Miranda, L. S., Pires, I. S. C., Pires, C. V., & Halboth, N. V. (2012). Influence of color on acceptance and identification of flavor of foods by adults. Food Science and Technology (Campinas), 32(2), 296–301. https://doi.org/10.1590/S0101-206120120005000059
Elferink, S. J. W. H., Krooneman, J., Gottschal, J. C., Spoelstra, S. F., Faber, F., & Driehuis, F. (2001). Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by Lactobacillus buchneri. Physiological and Biotechnology, 67(1), 125–132. https://doi.org/10.1128/AEM.67.1.125-132.2001
Esmerino, E. A., Cruz, A. G., Pereira, E. P. R., Rodrigues, J. B., Faria, J. A. F., & Bolini, H. M. A. (2013). The influence of sweeteners in probiotic petit suisse cheese in concentrations equivalent to that of sucrose.

Acknowledgments

The authors are grateful to the Graduate Program in Food Science of the Federal University of Bahia for its funding support, to the company Sweetmix for donating the powdered stevia extract, to the company Sigma-Aldrich for donating the xanthan gum, to the company Granolab for donating the ascorbic acid and to CAPES for providing a scholarship.
Food and Agriculture Organization of the United Nations. (2019). Joint FAO/WHO expert committee on food additives. Retrieved February 8, 2020, from http://www.fao.org/3/ca5270en/ca5270en.pdf

Freire, A. L., Ramos, C. L., & Schwan, R. F. (2017). Effect of symbiotic interaction between a fructooligosaccharide and probiotic on the kinetic fermentation and chemical profile of maize blended rice beverages. Food Research International, 100(P1), 698–707. https://doi.org/10.1016/j.foodres.2017.07.070

Jahurul, M. H. A., Zaidul, I. S. M., Ghafoor, K., Al-Juhaimi, F. Y., Nyam, K., Norulaini, N. A. N., Sahena, F., & Omar, A. K. M. (2015). Mango (Mangifera indica L.) by-products and their valuable components: A review. Food Chemistry, 183(15), 173–180. https://doi.org/10.1016/j.foodchem.2015.03.046

Khatoon, N., & Gupta, R. K. (2015). Probiotics beverages of sweet lime and sugarcane juices and its physicochemical, microbiological & shelflife studies. Journal of Pharmacognosy and Phytochemistry, 4(3), 25–34. https://www.phytojournal.com/articles/2015/vol4Issue3/PartA-3-4-28.pdf

Laureys, D., & De Vuyst, L. (2017). The water kefir grain inoculum determines the characteristics of the resulting water kefir fermentation process. Journal of Applied Microbiology, 122(3), 719–732. https://doi.org/10.1111/jam.13370

Magalhães, K. T., Pereira, G. V. M., Dias, D. R., & Schwan, R. F. (2010). Microbical communities and chemical changes during fermentation of sugary brazilian kefir. World Journal of Microbiology & Biotechnology, 26(7), 1241–1250. https://doi.org/10.1007/s11274-009-0294-x

Markowiak, P., & Sliżewska, K. (2017). Effects of probiotics, prebiotics, and symbiotics on human health. Nutrients, 9(9), 1–30. https://doi.org/10.3390/nu90901021

Meilgaard, G. K., Civille, G. V., & Carr, B. I. (2007). Sensory evaluation techniques (4th ed.). CRC Press.

Minelli, E., & Benini, A. (2008). Relationship between number of bacteria and their probiotic effects. Microbial Ecology in Health and Disease, 20 (4), 180–183. https://doi.org/10.1080/09168750802040805

Narayanan, P., Chinnasamy, B., Jin, L., & Clark, S. (2014). Use of just-about-right scales and penalty analysis to determine appropriate concentrations of stevia sweeteners for vanilla yogurt. Journal of Dairy Science, 97(6), 97. https://doi.org/10.3168/jds.2013-3765

Natal, D. I. G., Rodrigues, K. C. C., Moreira, M. E. C., Queiróz, J. H., Benjamin, L. A., Santos, M. H., Sant’Ana, H. M. P., & Martino, H. S. D. (2017). Bioactive compounds of the Ubá mango juices decrease inflammation and hepatic steatosis in obese Wistar rats. Journal of Functional Foods, 32, 409–418. https://doi.org/10.1016/j.jff.2017.03.023

Nualkaekul, N., Zaidul, I. S. M., Ghafoor, K., Al-Juhaimi, F. Y., Nyam, K., Norulaini, N. A. N., Sahena, F., & Omar, A. K. M. (2015). Mango (Mangifera indica L.) by-products and their valuable components: A review. Food Chemistry, 183(15), 173–180. https://doi.org/10.1016/j.foodchem.2015.03.046

Pande, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics- a review. Journal of Food Science and Technology, 52(12), 7577–7587. https://doi.org/10.1007/s13197-015-1921-1

Pimentel, T. C., Madrona, G. S., & Prudêncio, S. H. (2015). Probiotic clarified apple juice with oligofructose or sucralose as sugar substitutes: Sensory profile and acceptability. LWT - Food Science and Technology, 62(1), 838–846. https://doi.org/10.1016/j.lwt.2014.08.001

Puerari, C., Magalhães, K. T., & Schwan, R. F. (2012). New cocoa pulp-based kefir beverages: Microbiological, chemical composition and sensory analysis. Food Research International, 48(2), 634–640. https://doi.org/10.1016/j.foodres.2012.06.005

Randazzo, W., Corona, O., Guarcello, R., Francesca, N., Germana, M. A., Erten, H., Moschetti, G., & Settanni, L. (2016). Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. Food Microbiology, 54, 40–51. https://doi.org/10.1016/j.fm.2015.10.018

Rosa, D. D., Dias, M. M. S., Greszkowski, L. M., Reis, S. A., Conceição, L. L., & Peluzio, M. C. G. (2017). Milk kefir: Nutritional, microbiological and health benefits. Nutrition Research Reviews, 30(1), 82–96. https://doi.org/10.1097/01.NRR.0000545242160000275

Salmérón, I., Thomas, K., & Pandiella, S. S. (2015). Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. Journal of Functional Foods, 15, 106–115. https://doi.org/10.1016/j.jff.2015.03.012

Shori, A. B. (2016). Influence of food matrix on the viability of probiotic bacteria: A review based on dairy and non-dairy beverages. Food Bioscience, 13, 1–8. https://doi.org/10.1016/j.fbio.2015.11.001

Singh, G. G., Micha, R., Khatibzadeh, S., Lim, S., Ezzati, M., & Mozaffarian, D. (2015). Estimated global, regional, and national disease burdens related to sugar-sweetened beverage consumption in 2010. Circulation, 132(8), 639–666. https://doi.org/10.1161/CIRCULATIONAHA.114.016066

Simpola, R., & Jaitak, V. (2016). Synthesis of rebioaidioside A from stevioside and their interaction model with hTAS2R4 bitter taste receptor. Phytochemistry, 125, 106–111. https://doi.org/10.1016/j.phytochem.2016.03.004

Tandel, K. R. (2011). Sugar substitutes: Health controversy over perceived benefits. Journal of Pharmacology and Pharmacotherapeutics, 2(4), 236–243. https://doi.org/10.4103/0976-500X.58936

Tavares, P. P. L. G., Silva, M. R., Santos, L. F. P., Nunes, I. L., & Magalhães-Guedes, K. T. (2018). Produção de bebida fermentada kefir de quinoa (Chenopodium quinoa) saborizada com cana (Theobroma cacao) em pó. Revista Brasileira de Ciências Agrárias, 13(4), 1–7. https://doi.org/10.5039/agraria.v13i4a5593

Teixeira, N., Melo, J. C. S., Batista, L. F. P., Paula-Souca, J., Fronza, P., & Brandão, M. G. L. (2019). Edible fruits from Brazilian biodiversity: A review on their sensorial characteristics versus bioactivity as tool to select research. Food Research International, 119, 325–348. https://doi.org/10.1016/j.foodres.2019.01.058

Tung, Y., Chen, H., Wu, H., Ho, M., Cheng, K., & Chen, C. (2017). Kefir peptides prevent hyperlipidemia and obesity in high-fat-diet-induced obese rats via lipid metabolism modulation. Molecular Nutrition and Food Research, 62(3), 1–9. https://doi.org/10.1002/mnfr.201700505

Viana, R. O., Magalhães-Guedes, K. T., Braga Junior, R. A., Dias, D. R., & Schwan, R. F. (2017). Fermentation process for production of apple-based kefir vinegar: Microbial, chemical and sensory analysis. Brazilian Journal of Microbiology, 48(3), 592–601. https://doi.org/10.1016/j.bjm.2016.11.006

Villanueva, N. D. M., Petenate, A. J., & Silva, M. A. A. P. (2005). Performance of the hybrid hedonic scale as compared to the traditional hedonic, adjusting-and-ranking scales. Food Quality and Preference, 16(8), 691–703. https://doi.org/10.1016/j.foodqual.2005.03.013

Weber, A., & Hekmat, S. (2013). The effect of Stevia rebaudiana on the growth and survival of Lactobacillus rhamnosus GR-1 and sensory properties of probiotic yogurt. Journal of Food Research, 2(2), 1–8. https://doi.org/10.5539/fr.v2n2p136

Zheng, M., Allman-Farinelli, M., Heitmann, B. L., & Rangan, A. (2015). Substitution of sugar-sweetened beverages with other beverage alternatives: A review of long-term health outcomes. Journal of the Academy of Nutrition and Dietetics, 115(5), 767–779. https://doi.org/10.1016/j.jand.2015.01.006