Chemical Evaluation and Probiotic Potential of Kefir Different Nutrient Media

Suzana Casaes de Jesus Teodoro[1*], Matheus Chaves de Jesus[1], Crissia dos Santos Rocha[1], Roseane Santos Oliveira[1], Ingrid Lessa Leal[1]
[1]SENAI CIMATEC University Center; Salvador, Bahia, Brazil

Kefir is a gelatinous mass composed of bacteria and yeast. This study aims to develop beverages obtained from kefir grains' fermentation in different nutrient media. Thus, kefir fermentation was performed in an aqueous solution of sucrose, whole milk, whole grape juice, and water-soluble coconut extract. The fermentation process was analyzed from grain growth, beverage yield, lactic acid determination, pH, total soluble solids, and microbiological analysis. The results showed the beverages had pH and lactic acid values following the legislation. Furthermore, it identified the probiotic potential of the coconut and milk water-soluble extract beverages.
Keywords: Probiotic Beverage. Kefir Benefits. Fermented Beverage. Gut Microbiota.

Introduction

Consumers are increasingly committed and concerned with issues associated with health and quality of life [1]. Thus, they opt daily for products that have functionality claims. In this sense, the food industry progressively invests in developing lines with these functional and nutritional characteristics to satisfy the public's preferences [2].

An excellent example of foods that present functional characteristics is probiotics. They are defined as a food supplementation composed of microorganisms that regulate the intestinal microbiota, conferring good health to the user [3]. In addition, to maintain the advantages of these supplements, it should be consumed regularly [3].

We performed this study using kefir probiotic drink, which according to Brazilian legislation, is defined by the food in lactic acid cultures, fermented by kefir grains composed of bacteria of *Lactobacillus* and diverse species of the genera *Lactococcus, Acetobacter,* and *Leuconostoc*. In addition, they can have yeasts of the type *Kluyveromyces marxianus*, which are lactose fermenting agents, and *Saccharomyces omnisporus, Saccharomyces cerevisiae, Saccharomyces exiguus*, which do not ferment lactose. We emphasize that for the beverage to be considered probiotic, the microorganisms must be abundant in the final product [4].

There is a tendency to add fruit pulp to improve the acceptance of probiotic beverages [2] because these fruit pulps add better characteristics to the product, such as flavor and color. In addition, it increases the nutritional value of these products since many fruits contain bioactive compounds in their composition, which can help prevent some diseases [5]. Thus, innovation with new substrates for fermented products has proved attractive because it adds satisfactory sensory characteristics to the product, helping consumer acceptance [6].

This probiotic drink strengthens the immune system because, according to Pereira and colleagues (2011) [7], kefir has live microorganisms that can improve the intestinal microbial balance, helping to eliminate pathogenic microorganisms and resulting in health benefits to those who consume it [7].

Based on the benefits and thinking of people who are looking for functional foods, this work was carried out to produce the fermented kefir drink in different nutrient media to do a physicochemical analysis and perform microbiological analysis of molds and yeasts, lactic acid bacteria and aerobic bacteria, to evaluate the probiotic potential of the drinks.

Received on 26 November 2022; revised 18 January 2023.
Address for correspondence: Suzana Casaes de Jesus Teodoro. Rua Primeira Travessa José Araújo, 27 - Itapuã, Salvador - BA - Brasil. Zipcode: 41620850. E-mail: suzanacasaes123@gmail.com.

J Bioeng. Tech. Health 2023;6(1):10-14

Materials and Methods

After the survey of methodologies for producing kefir, the fermentation process was established in the nutrient media: water kefir in water with sucrose, water kefir from water-soluble coconut extract, water kefir in whole grape juice, and milk kefir in milk.

In previously cleaned recipients, we prepared different culture media. We mixed water (1L) with organic brown sugar (100g) at room temperature to prepare the water with sucrose solution. In another container, 1L of grape juice was placed. For the water-soluble coconut extract, 1 coconut (229.61g), water (1L), and organic brown sugar (100g) were used, and the material was processed in a blender and filtered, and finally, pasteurized whole milk (1L).

We inoculated sucrose, grape juice, water-soluble coconut extract, and water kefir grains (200 g) for the media of the water solution. Moreover, we inserted milk kefir grains (13.192 g) for milk. The containers were closed with a thin cloth, and the fermentation process was carried out at room temperature in a place with little light for 24-48h. Then, the kefir was filtered, and the beverage was stored in the refrigerator at approximately 4°C [8-10]. Finally, the grains were stored for further use. We used the Nogueira and colleagues' method (2016) [11] to cell growth of fermented kefir grains (▲m) on different substrates. For the results, the grains were weighed before and after fermentation; after that, the numbers obtained were applied to the following equation [11]:

$$\blacktriangle m(\%)=(mkf-mk0)/mk0*100 \quad (1)$$

In equation 1, ▲m(%) is the change in mass of kefir grains, (mk0) is the weight at the beginning, and (mkf) after fermentation. The Nogueira and colleagues' method (2016) [11] determined the yield, the beverage mass was measured before and after fermentation, and the results were applied to the following equation [11]:

$$R(\%)=msf/ms0*100 \quad (2)$$

In equation 2, R(%) corresponds to the beverage yield, ms0 corresponds to the initial mass, and msf to the final mass of the fermented kefir beverage in the substrates, respectively [11].

The titratable acidity method of Adolfo Lutz (2005) [12] was performed twice after 24 hours of the fermentation process. The results were expressed in g/100mL of lactic acid. For the pH evaluation, direct reading was done using a calibrated ph meter. In addition, the soluble solids content (°Brix) was measured by direct reading in a digital refractometer [10].

Microbiological analysis was performed on the beverages after 24h of fermentation to quantify the presence of different microorganisms.

For lactic acid bacteria analysis, samples were used at a dilution of 10^{-7} and 10^{-8}, in duplicates in 3MTH PetrifilmTM Lactic Acid Bacteria Count (LAG) Plates, following the manufacturer's guidance [13].

Mold and yeast analysis was done at 10^{-3}, 10^{-7}, and 10^{-8} dilutions in duplicates on 3MTN PetrifilmTM Rapid Yeast and Mold Count (Rym) plates, following manufacturer's guidance (3M Petrifilm, 2021). In addition, aerobic mesophilic bacteria analysis was done at 10^{-7} and 10^{-8} dilutions in duplicates on 3MTM PetrifilmTM Aerobic Count (AC) plates, following the manufacturer's guidance [13].

Results and Discussion

We analyzed the grains' growth, comparing the final and initial mass of the grains grown in each medium separately. Table 1 shows the variation in mass and the percentage of mass acquired.

After the analysis of the kefir grains' yield (Table1), we observed that the grains of water kefir fermented in water-soluble coconut extract and whole grape juice grew, on average, 12.9% more than the grains of water kefir in water with sucrose, which is its standard culture medium. The results showed that adding these 2 substrates in the kefir fermentation made the medium more nutritious, benefiting the grains. The grains of

Table 1. After fermentation in different nutrient media, the yield of kefir grains and kefir drinks.

Grain	Nutrient Media	Yield Grains (%)	Yield Drink (%)
Water kefir	Water solution with sucrose	8.7	100
Water kefir	Entire grape juice	21.3	100
Water kefir	Water-soluble coconut extract	21.9	85
Milk kefir	Milk	36.2	100

milk kefir also had a good yield. So, for producing the fermented milk kefir drink, a smaller amount of grains is necessary compared to the number of grains needed to produce the water kefir drink. In the study directed by Pereira and colleagues (2011) [7], the kefir grains obtained an average growth of 20% concerning the initial weight of the grains, similar to the present study, in which the average grains' growth was 22% [7].

The yield of the drinks was analyzed with the drinks before and after fermentation, comparing the final and initial volume of the drinks (Table 1). Table 1 shows that the yield of kefir fermented beverages in the different nutrient media was good (100%), except for the water-soluble coconut extract, which had an estimated loss of 15%. The high content of lipids in the coconut caused this loss. These lipids solidified in the water-soluble coconut extract during fermentation, so they were removed from the beverage through filtration.

The soluble solids index was checked before and after the fermentation process, in the 4 different culture media, with the help of a digital refractometer. Table 2 presents the results of the brix degree.

Table 2 presents the number of soluble solids (basically as sugar or sucrose) consumed by the grains in each culture medium. The water kefir grains that obtained the highest sugar consumption were those fermented in water-soluble coconut extract. This higher consumption of sucrose in this medium was responsible for causing a higher yield of the grains in this substrate. Tu and colleagues (2019) [14] found a significant reduction in the contents of soluble solids from 9.20 to 4.43 ºBrix in 48 hours of water kefir grains' fermentation. The results found in the literature were similar to those exposed in this work, considering that the fermentation in this study was only for 24h. Tu's study also observed that the grape juice presented a very high result of soluble solids at the beginning and end of the fermentation. This high sugar content in this substrate was not favorable for fermentation and resulted in a compromise in the grains' health, similar to the present study, in which the average growth of the grains was 22% [14].

Our results indicated the quantification of lactic acid in the samples, and the pH analysis was performed separately on each medium after fermentation (Table 3).

Table 3 presents the fermented beverages' percentages of lactic acid lower than 1%. According to the legislation, Resolution No. 46 determines that kefir should have a maximum acidity (expressed as lactic acid) of 1% [4]. Thus, the fermented beverages

Table 2. Quantity of soluble solids per degree Brix.

Grain	Nutrient Media	Total Soluble Solids Before Fermentation (°Brix)	Total Soluble Solids After Fermentation (°Brix)
Water kefir	Water solution with sucrose	9.8	7.5
Water kefir	Entire grape juice	22.2	19.5
Water kefir	Water-soluble coconut extract	8.7	5.7
Milk kefir	Milk	8.0	7.6

prepared in this study showed percentages of lactic acid within the limit established by legislation. A study conducted by Alves (2020) [10] regarding the production of lactic acid found a variation of acidity from 0.68 to 1.01 g/100mL in 24 hours of kefir grains' fermentation [4].

The pH values found in the study of physicochemical characterization of fermented products by kefir grains are usually in the range between 4.2 and 4.5. In the present study, we noted more excellent proximity of the pH level found in the products based on milk, water and sucrose, and water-soluble coconut extract, which showed more intense changes in the fermentative process. On the other hand, the product developed from whole grape juice showed a pH level farther from the range found in previous studies, and the average found in the analyses (mean pH = 4.00, with a value of 3.41) may indicate one of the reasons for the low effectiveness of microorganisms in the medium.

The microbiological analysis indicated the microorganisms' colony-forming units desired to evaluate each beverage's probiotic potential. Table 4 shows the values found for each order.

Following the amount of UFC presented for each drink (Table 4), the milk kefir and water kefir drinks in water-soluble coconut extract are probiotics because they meet the minimum viable amount of lactic acid bacteria in probiotic foods, which should be between the range of 10^8 to 10^9 UFC in the daily recommendation of the product ready for consumption [4]. On the other hand, the water kefir drinks in sugar water and grape juice did not show probiotic potential. This result in the grape drink may have been caused by the high amount of soluble solids, which made the culture medium unviable for these microorganisms.

The quantity of colony-forming units of molds and yeasts (Table 4), we observe that all drinks presented between 10^3 and 10^4 UFC. In kefir production, yeasts play an important role in the fermentative process because in addition to providing essential nutrients for growth, such as amino acids and vitamins, changing the pH, and helping to expel ethanol and produce CO_2, produces metabolites, which contribute to improving the flavor of kefir [15]. Nevertheless, to fulfill their role and enrich the fermented beverage, yeasts must be

Table 3. Determination of lactic acid by titratable acidity.

Grain	Nutrient Media	Lactic Acid After Fermentation (g% LA)	pH at Post-Fermentation
Water kefir	Water solution with sucrose	0.16	4.00
Water kefir	Entire grape juice	0.70	3.41
Water kefir	Water-soluble coconut extract	0.41	3.91
Milk kefir	Milk	0.67'	4.71

Table 4. Microbiological analysis of fermented beverages.

Grain	Nutrient Media	Lactic Acid Bacterias (UFC/mL)	Aerobic Mesophiles	Molds	Yeasts
Water kefir	Water solution with sucrose	1.80×10^7	2.00×10^7	6.50×10^3	8.00×10^3
Water kefir	Entire grape juice	1.00 x 10 est.	1.00 x 10 est.	1.00×10^3	2.50×10^3
Water kefir	Water-soluble coconut extract	2.22×10^{10}	1.63×10^{10}	8.60×10^4	4.95×10^4
Milk kefir	Milk	7.74×10^9	1.04×10^{10}	3.30×10^4	9.50×10^4

in controlled quantity. According to the Technical Regulation of Identity and Quality of Fermented Milks, the mold and yeast count should equal or less than 10^4 UFC/mL [4]. Generally, the samples had mold and yeast counts equal to or below the minimum established by the Technical Regulation of Identity and Quality of Fermented Milks.

In the microbiological result of mesophilic aerobic count (Table 4) we note the high value of the same in milk kefir and coconut water-soluble extract kefir. According to Franco (2007) [16], mesophilic bacteria, when present in high counts (more significant than 10^6 UFC/mL), can cause the deterioration of the product and decrease the shelf life of foods [16]. The results obtained in this research exceeded this value, except for the fermented water kefir drink in water with sucrose, which found 2.00 x 10^7 UFC. There was no sign of fermentation and bacterial growth in the whole grape juice due to the high amount of soluble solids.

Conclusion

Observing the behavior of kefir in the different media, we note that all beverages presented pH and lactic acid values within the norms released by the legislation, being suitable for consumption before this requirement. The probiotic potential of the beverage and the yield of grains were favorable in water kefir fermented in water-soluble coconut extract and milk kefir. The beverages had a good yield, except for the water-soluble coconut extract, which lost approximately 15% of the beverage due to the high lipid content present. The grape juice did not obtain satisfactory results, as it was not a suitable medium for kefir fermentation due to its high soluble solids content.

For future research, the behavior and probiotic potential of kefir in coconut milk will be evaluated using milk kefir and diluted grape juice, thus reducing the number of soluble solids fermented with water kefir.

References

1. Silva EO.Kefir: Propriedades Funcionais e Gastronômicas. 1.ed. Cruz das Almas - Bahia: UFRB, 2015. p. 7-123.
2. Borgovoni TF.Biocompostos das polpas de maracujá e de buriti: caracterização e aplicação em leite. São José do Rio Preto: Universidade Estadual Paulista "Júlio de Mesquita Filho". 2018. 90p. (Dissertação, Mestrado em Ciências de Alimentos).
3. Fooks LJ et al. Probiotics as modulators of the gut flora. Br J Nutr 2002:88:S39-S49.
4. Brasil. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Instrução Normativa nº 46, de 23 de outubro de 2007. Adota o Regulamento Técnico de Identidade e Qualidade de Leites Fermentados, anexo à presente Instrução Normativa. Diário Oficial da República Federativa do Brasil, Brasília, 24 out 2007.
5. Chen C et al. Probiotics and prebiotics: role in clinical disease states. Adv Pediatr 2005; 52:77-113.
6. Paiva YF et al. Iogurte adicionado de polpa de abacaxi, base mel: elaboração, perfil microbiológico e físico-químico. Revista Verde de Agroecologia e Desenvolvimento Sustentável 2015;10(5):22-26.
7. Pereira GM et al. Caracterização físico-química e sensorial de kefir tradicional e derivados. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2011;63(2):473-480.
8. Santos FL. Produção artesanal de kefir. Ferlando Lima Santos, 2004.
9. Almeida FA et al. Análise sensorial e microbiológica de kefir artesanal produzido a partir de leite de cabra e de leite de vaca. Rev Inst Latic "Cândido Tostes" 2011;378(66):51-56.
10. Alves V. Desenvolvimento de bebida fermentada com kefir de água em extrato vegetal hidrossolúvel de coco (*Cocos nucifera l.*) com adição de inulina. 2020.
11. Nogueira LK,et al. Milk and açaí berry pulp improve sensorial acceptability of kefir fermented milk beverage, ActaAmazônica 2016;46(4):417-424.
12. Instituto Adolfo Lutz. Métodos Físico-Químicos para Análise de Aimentos.Instituto Adolfo Lutz. 4ª Edição. 2005.
13. Segurança Alimentar. 3M Petrifilm, 2021. Disponível em: <https://www.3m.com/3M/en_US/food-safety-us/support/package-inserts/>.Acesso em: 20, Abril de 2022.
14. Tu C, Azi F, Huang J, Xu X, Xing G, Dong M. Quality and metagenomic evaluation of a novel functional beverage produced from soy whey using water kefir grains. LWT Food Science and Technology 2019;113:108-258.
15. Ghedini TG. Qualidade microbiológica do kefir. Brazilian Journal of Development. Universidade de França. 2020.
16. Franco BDGM, Landgraf M. Microbiologia dos Alimentos. São Paulo: Atheneu, 2007.