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Probiotification of Whole Grape Juice by Water Kefir Microorganisms

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HIGHLIGHTS

- All the proposed formulations favored the growth of lactic bacteria.
- Grape juice proved to be a suitable medium for the development of probiotics.
- Viability of probiotic strains for 14 days of storage.
- The recommendation of daily intake of the probiotic drink is 100 mL per day.

Abstract: Probiotics are live microorganisms that when ingested in adequate amounts help in the balance of the intestinal microbiota. Kefir is considered a probiotic beverage associated with the improvement of several pathologies. Fruit juices are considered an excellent culture medium for these microorganisms, presenting nutrients that favor microbial growth. This work aimed to prepare a probiotic drink from the fermentation of whole grape juice by microorganisms from the water kefir. Different amounts of kefir grains were tested (5, 10, 20, 30, 40, 50, and 60 g) in 500 mL of grape juice. The fermentation processes were developed under static conditions at room temperature for 24 h. Seven formulations were tested for physical-chemical and microbiological analyses. A questionnaire on the knowledge and consumption of probiotics was applied to the academic community. Three formulations were selected for sensory analysis and stored under refrigeration. All formulations showed growth of lactic acid bacteria and no reduction in storage viability. A significant reduction in soluble solids was observed in all formulations, and an increase in acidity and pH formulations with 5 and 20 g of kefir, respectively. About 64% of the participants reported knowing the definition of probiotics. In addition, a low intake of this product was observed. The formulation with 5 grams of water kefir grains was better accepted by the consumers when compared to the others. Thus, grape juice can be a great alternative to consume probiotic microorganisms, especially for those who do not consume dairy products.

Keywords: Fruit juices; Probiotics; Questionnaire; Sensory analysis; Viability.

INTRODUCTION

Kefir is a fermented drink with a characteristic aroma and flavor resulted from the symbiotic activity of microorganisms present in its grains [1]. Traditionally, milk was used to produce kefir. Currently, other matrices have been employed, such as vegetable juices [2], soy milk [3], cheese whey [4], fruit juice [5], and honey [6].

Kefir grains are a consortium of bacteria and yeasts, incorporated into a complex matrix of polysaccharides and proteins [1]. There are two types of kefir: milk and water kefir. Both drinks are considered probiotics [7], however, the culture medium is different/. Water kefir grows in a solution of water and sucrose [1].

The microbiota found in kefir grains depends on the collection site of the grains. The microbiota consists mainly in the genera *Lactobacillus, Lactococcus, Leuconostoc, Bifidobacterium, Acetobacter, Enterococcus, Pediococcus, Streptococcus, Saccharomyces, Kluyveromyces, Candida*, and *Pichia* [4]. These microorganisms are responsible for the production of metabolites that contribute to human health. These benefits include reduced serum cholesterol level [8], improved gastrointestinal function [9], and improved immune system [10]. For this, the microorganisms must be ingested in adequate amounts, so that the microorganism arrives viable in its active site to perform its function properly [10].

Probiotic drinks are available on the market mainly in milk form, which makes it necessary to seek new alternatives for vegan or intolerant consumers [11]. The global probiotic market has grown considerably recently and non-dairy food products have been gaining popularity [12].

An option already highlighted and of interest to researchers is the production of probiotic drinks from fruit juices. Fruit juices offer natural nutrients and sugars that favor the growth of microorganisms. In addition, the digestion of these products is facilitated, which results in less permanence in the stomach, resulting in a greater number of viable cells of microorganisms in the intestine [12]. Fruit juices are consumed and enjoyed worldwide, not only for their taste but also because they are nutritionally important in the human diet. They contain water, sugar, proteins, amino acids, vitamins, and minerals and are an appropriate medium for microbial growth [13]. Grape juice, specifically, is an energy drink, unfermented, with a characteristic color, aroma, and flavor. It has phenolic compounds that act in the body with antioxidant action [14].

Given the above and considering that the development of health-promoting foods is one of the priorities of the food industry, the use of whole grape juice (no added preservatives, chemical aromatics, and/or sugars) may be a viable alternative as a culture medium for the growth of water kefir microorganisms. The objective of this research was to develop a probiotic fermented drink with whole grape juice and to evaluate the microbiological, physical-chemical, and sensory aspects, as well as the probiotic viability during cold storage.

MATERIAL AND METHODS

Kefir grains

Brazilian water kefir grains were obtained from a donor and used in the experiments. The samples of Kefir grains were preserved by freezing.

The inoculum was prepared by cultivating kefir grains in a solution with 5% w/v of brown sugar at room temperature for 24 h. After this time, the grains were recovered and washed with distilled water, and inoculated into whole grape juice.

Production of kefir-like beverage

The whole grape juice was used as a base beverage to produce a kefir-like beverage. The juice selected was an industrial product to assure a standard quality. The bottles were purchased from local supermarkets in Alegre, Espírito Santo, Brazil.

Seven formulations were made: 5, 10, 20, 30, 40, 50, and 60 g of water Kefir grains in 500 ml of juice. The fermentation processes were developed under static conditions at room temperature for 24 h. After this period (end of fermentation), the kefir grains were recovered and washed with distilled water, and stored under freezing. Physical-chemical and microbiological analyzes were performed on all beverages produced.

Physico-chemical and microbiological determinations

The chemical composition and microbiological analysis of the whole juice and beverage made with kefir were analyzed.

Soluble solids (SS) (°Brix) were measured in a digital refractometer (Milwaukee, USA) by the direct reading method and pH was determined at room temperature in a bench pHmeter (Lucadema, Brazil) [15].

The total titratable acidity (TTA) (expressed as a percent of tartaric acid) was measured by titrating the samples with 0.1 M NaOH [16]. The color analysis was performed in a colorimeter (Konica Minolta, Singapore) recording CIElab chromaticity coordinates (L*, a*, b*) [17]. All analyzes were carried out in triplicate.

Kefir-like beverages were microbiologically evaluated according to Silva and coauthors [18]. Decimal dilutions of samples were prepared in peptonized water (Himedia, India). Total Aerobic Mesophilic Bacteria (MB) were enumerated by spread plating and incubated aerobically at 35°C for 48 h, while lactic acid bacteria (LAB) by pour plating on Man-Rogosa-Sharpe (MRS) agar and incubated anaerobically at 30°C for 48 h. Molds and yeasts (MY) were spread plated on potato dextrose agar (PDA), incubated aerobically at 25°C for 5 days. The results were expressed in CFU.mL^{-1}. Total and thermotolerant coliforms were estimated using the most probable number technique using lauryl sulfate tryptose broth (LST). To confirm the presence of total coliforms, aliquots from the LST tubes exhibiting gas production were transferred to tubes containing brilliant green broth (BGB) (Difco, Brazil) and EC broth (Difco, Brazil) for thermotolerant coliforms. The tubes with BGB broth were incubated at 35°C for 48 h, while the tubes containing EC broth were incubated for 24 h at 45°C. The results were calculated in the NMP of coliforms.ml^{-1}. All microbiological analyses were done in triplicate.

Effect of storage conditions on probiotic cell viability in kefir-like beverages

Three formulations with kefir were selected based on the results of the microbiological and physicochemical analysis. These beverages were stored in sterile glass bottles under refrigeration for 14 days.

MB, LAB, and MY counts were conducted immediately after the 24 h of fermentation (0 day - storage start) and at 14 days of storage (storage end). Likewise, pH, SS, TTA, and color were measured. The analyses were conducted as previously described.

Assessment of knowledge and consumption of probiotics

To evaluate the consumption of probiotic products, a questionnaire was applied to 200 people from the academic community at the Federal University of Espirito Santo, campus Alegre. The questionnaire comprised questions about the consumption of whole grape juice, knowledge about probiotic products, the acceptability of a probiotic grape juice, the price that consumers would be willing to pay, and if the consumers presented gastrointestinal diseases. All participants signed the Informed Consent Form. Ethics Committee for Research with Humans of the Federal University of Espirito Santo granted ethical approval for this study (CAAE nº. 14103419.5.0000.8151).

Sensory evaluation

Three formulations of whole grape juice fermented by kefir were selected for acceptability by 100 untrained tasters over 18 years old who enjoyed whole grape juice and who did not have gastrointestinal diseases. In the sensory evaluation, the tasters had to drink the sample and answer to a nine-point hedonic scale varying between “liked extremely” (Score 9) and “disliked extremely” (Score 1) for each one of the attributes: flavor, color, aroma, acidity, and overall impression. The attitude scale was also used to assess the possible frequency of consumption of probiotic beverages by the tasters [19]. The samples (40 ml) were evaluated in individual booths with white light and served chilled in plastic cups, randomly encoded with three-digit numbers.

The project was approved by the Committee of Ethics in Research with Humans of the Federal University of Espirito Santo, with the emission of a Presentation Certificate of Ethical Appreciation (CAAE nº. 14103419.5.0000.8151).

Statistical analysis

The main experiment consisted of seven proportions of kefir grains (5, 10, 20, 30, 40, 50, and 60 g) in 500 ml of whole grape juice. It was outlined using a completely randomized design (CRD), with three repetitions. Regression analysis was used for describing the correlation between the independent factors (amount of kefir) and the dependent factors (pH, SS, color, TTA, LAB, MB, FY).

Another experiment consisted of evaluating three formulations of whole grape juice fermented with kefir at two different times: time 0, which corresponds to the day of storage and the 14th day. It was outlined using a completely randomized design (CRD), with a 3x2 factorial scheme and three repetitions. The data were submitted to analysis of variance (ANOVA) and the media were compared using the Tukey test with a 5% probability.

For the sensory evaluation data was used as a random block design (RBD), in which the treatments were the formulations and blocks the judges. The results were evaluated by an analysis of variance and the means were compared using the Tukey test at 5% probability. All the analyses were conducted using the software GENES – Computational Application for Data Analysis in Experimental Statistics and Quantitative Genetics [20].

RESULTS

Characterization of whole grape juice

The whole grape juice presented the following characteristics: 14.73 ± 0.05 °Brix, pH value of 4.15 ± 0.03, TTA of 0.52 ± 0.01 g of tartaric acid per 100 ml and values for L^*, a^* and b^* of 23.07 ± 0.84; 1.70 ± 0.14 and 0.12 ± 0.12, respectively.

Physico-chemical and microbiological characteristics of the beverages

Regression analysis was used for describing the correlation between the independent factors (amount of kefir) and the dependent factors (pH, SS, color, TTA, LAB, MB, FY).

The model that best adjusted to the behavior of the experimental data of the growth ratio of LAB as a function of adding different amounts of kefir to grape juice, was the first-degree polynomial equation ($p \leq 0.05$). Significant variation in the growth of fungi and yeasts was observed in all proposed formulations, and the model that best fitted this behavior was the second-degree polynomial equation ($p \leq 0.05$). Regarding the growth of total aerobic mesophilic bacteria, none of the tested statistical models were adjusted to the behavior of the experimental data ($p > 0.05$) (Table 1).

Table 1. Adjusted regression models for microbiological analyzes of probiotic drinks after 24 hours of fermentation as a function of different concentrations of kefir.

Equation obtained	p-value	R^2
LAB = -0.0056 CONC + 7.7279	0.0060	0.8144
FY = 0.0008 $CONC^2$ - 0.0516 CONC + 7.9549	0.0100	0.8539
MB = -	0.0678	-

Where: LAB: Lactic Acid Bacteria; MB: Total Aerobic Mesophilic Bacteria; FY: Fungi and Yeasts; CONC: Concentration; p-value: ≤ 0.05.

The model that best fitted the behavior of the experimental data for the pH variable was the second-degree polynomial equation ($p \leq 0.05$). Regarding SS and TTA, the models that best fit were the first and third-degree polynomial equations, respectively ($p \leq 0.05$). None of the tested statistical models adjusted to the behavior of the L* and b* data, after 24 h of fermentation of the drinks. However, a variation in the color of the drink was observed with the addition of 5 g of kefir (-a) concerning the other formulations (+a), and the experimental model that best fitted these data was the first-degree polynomial equation (Table 2).

Table 2. Adjusted regression models for the physical-chemical analysis of probiotic drinks after 24 hours of fermentation as a function of the different concentrations of kefir.

Equation obtained	p-value	R^2
pH = 4E - 05 $CONC^2$ - 0.0034 CONC + 2.9543	0.0269	0.8974
SS = -0.0816 CONC + 14.027	0.0006	0.9383
TTA = 7E - 06 $CONC^3$ – 0.0006 $CONC^2$ + 0.0156 CONC + 0.7542	0.0067	0.9526
L* = -	0.2045	-
a* = 0.0226 CONC + 0.0532	0.0700	0.6441
b* = -	0.0369	-

Where: SS: Soluble Solids; TTA: Total Titratable Acidity; L*: Brightness; a *: Red (+ a) to green (-a); b*: Yellow (+ b) to blue (-b); CONC: Concentration; p-value: ≤ 0.05.

With the increase in the proportions of kefir added to grape juice, a pH variation from 2.94 to 2.89 was observed. A reduction in the values of SS was observed as more grain mass was added to the culture medium, varying from 14.23% to 9.40%. Regarding the TTA of probiotic drinks, a variation in the results was observed, comprising values from 0.83 g to 0.92 g of tartaric acid.100 mL^{-1} of the sample.

Effect of storage conditions on LAB population in kefir-like beverages

As all tested formulations had a LAB population greater than 10^7 $CFU.ml^{-1}$. Considering the consumption of this probiotic drink, the criterion for choosing the formulations to be stored was based on the values obtained from SS and mainly from the TTA of the drinks. Taking these parameters into account, the selected formulations were those containing 5, 20, and 60 g of kefir. They were stored for 14 days under refrigeration.

All drinks were evaluated for their microbiological quality, and all formulations showed the absence of coliforms, which indicates good product quality and greater safety for consumption. All stored formulations did not differ statistically concerning the count of LAB in any evaluated time, remaining with a count greater than 10^7 $CFU.ml^{-1}$ during the entire storage period (Table 3).

Table 3. Count of microorganisms in probiotic juices during the period of storage of the drink.

Time (days)	Formulations								
	5 g of Kefir			20 g of Kefir			60 g of Kefir		
	LAB	MB	FY	LAB	MB	FY	LAB	MB	FY
0	7.5 A	7.3 A	4.4 B	7.6 A	7.2 B	7.3 A	7.3 A	7.9 A	7.9 A
14	7.5 A	7.3 A	7.5 A	7.5 A	8.0 A	7.4 A	7.5 A	8.2 A	7.3 B

Where: LAB: Lactic Acid Bacteria; MB: Total Aerobic Mesophilic Bacteria; FY: Fungi and Yeasts; Results expressed in log $UFC.mL^{-1}$; Means followed by the same letter in the column, do not differ by Tukey's test at 5% significance.

Regarding the count of FY, a significant increase was observed in the formulation with 5 g of Kefir on the 14th day of storage, whereas in the drink with the addition of 60 g of Kefir there was a reduction of these microorganisms (Table 3). The only formulation that obtained a significant variation in the count of MB during storage was that with 20 g of kefir, with an increase from 7.2 to 8.0 log $CFU.mL^{-1}$ ($p \leq 0.05$).

With storage, a significant increase in TTA was observed in the formulation with the addition of 5 g of kefir, varying from 0.83 to 0.89 g of tartaric acid.100 mL^{-1} of the sample. Alternatively, acidity did not vary when the other formulations were analyzed (Table 4). All probiotic juices had a significant reduction in SS, concerning pH, only the formulation with 20 g of Kefir had a significant increase in this variable, from 2.90 to 3.12 on the 14th day of storage (Table 4).

Table 4. Physicochemical analysis of probiotic juices during the storage of the drink.

Time (days)	Formulations								
	5 g of Kefir			20 g of Kefir			60 g of Kefir		
	pH	SS	TTA	pH	SS	TTA	pH	SS	TTA
0	2.94 A	14.2 A	0.83 B	2.90 B	11.80 A	0.87 A	2.89 A	9.4 A	0.92 A
14	2.97 A	11.8 B	0.89 A	3.12 A	10.00 B	0.83 A	2.94 A	8.2 B	0.86 A

Where: SS: Soluble Solids expressed as %; TTA: Total Titratable Acidity expressed in g of tartaric acid.100 mL^{-1} the sample; Means followed by the same letter in the column, do not differ by Tukey's test at 5% significance.

Regarding the color of the probiotic juice, none of the formulations differed statistically concerning the values of L*, which represents the product's luminosity. However, concerning the other parameters evaluated, a* and b*, all drinks obtained significant variations between analyzed times, with an increase in the red and yellow colors of the product (Table 5).

Table 5. Color analysis of probiotic juices during storage.

Time (days)	Formulations								
	5 g of Kefir			20 g of Kefir			60 g of Kefir		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	25.02 A	-0.32 B	-1.00 B	25.54 A	0.68 B	-1.13 B	25.64 A	1.12 B	-0.52 B
14	25.23 A	0.96 A	-0.41 A	25.61 A	2.23 A	0.02 A	26.08 A	1.95 A	-0.31 A

Means followed by the same letter in the column, do not differ by Tukey's test at 5% significance.

Assessment of knowledge and consumption of probiotics

Consumers are increasingly concerned about their health and have been looking for healthier food alternatives for consumption. Probiotic foods are one of the main products purchased for this purpose, as their consumption brings health benefits. However, there is a need to assess consumer knowledge about these products. For this purpose, there are numerous alternatives available on the market. However, not all fermented products can be classified as probiotics, considering that not all fermenting microorganisms have the capacity to survive stomach acidity and to remain viable in the intestinal mucosa in sufficient numbers to exert their action [10,12]. Given this impasse, a questionnaire was applied to the academic community at the Federal University of Espirito Santo to assess consumption and knowledge of these products. Two hundred individuals participated in the study, among them undergraduate, master, and doctoral students, and professors, with ages ranging from 18 to 45 years old ($\bar{X} = 23.4$), the majority being female (72.5%).

When asked about the definition of probiotic products, 64% of the participants reported knowing their definition, however, only 85% of them, defined it correctly. About 61% of individuals do not consume probiotic products, and only 39% reported consuming this type of product. The consumed products were: fermented milk (22%); probiotic yogurts (18%); and products fermented with kefir (4%). Regarding the consumption of probiotic products during the week, about 25% of the participants who reported consuming this product consume 1 to 2 times a week, 10% 3 to 4 times, 1% 5 to 6 times, and only 3% reported consumption diary.

Only 49% of the participants in this study reported knowing any benefit from the consumption of probiotics, which are: modulation of intestinal microbiota (49%); improvement in intestinal transit (36%); improvement of the immune system (10%); and improved digestion (5%).

Regarding the presence of gastrointestinal diseases and disorders, it was possible to observe a predominance of gastritis (59%), constipation (14%), lactose intolerance (8%), reflux (5%) and irritable bowel syndrome (3%), affecting about 19% of the total participants.

Most individuals reported liking whole grape juice (73%) and about 12% maybe. Sixty-four percent of these participants reported the habit of consuming this drink 1 to 2 times a week and only 4% consume 5 or more times (Figure 1). About 57% of the participants stated that they would consume a fermented drink of whole grape juice with kefir, 32% maybe and only 11% report they would not consume.

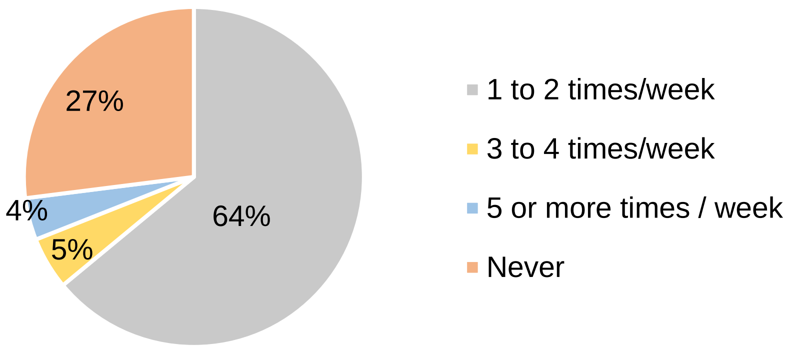

Figure 1. Consumption of whole grape juice by the academic community.

The suggested retail price of 100 mL of probiotic drink (recommended portion per day) was R$ 3.00 with approximately 35% of responses. Some participants reported that they would also be willing to pay amounts such as R$ 4.00 (23%), R$ 2.00 (19%), and R$ 5.00 (12%), indicating good profitability with the production and commercialization of a probiotic grape juice.

Sensory evaluation

In comparison to the other formulations, the drink with the addition of 5 g of Kefir obtained a better acceptance by the tasters concerning color, acidity, aroma, and overall impression ($p \leq 0.05$) (Table 6). Regarding flavor, all formulations differed statistically ($p \leq 0,05$). However, the formulation that obtained the best average concerning this attribute was the one with 5 g of kefir, followed by the formulations with 20 and 60 g.

Table 6. Sensory analysis of probiotic juices.

	Formulations		
Attributes	**5 g of Kefir**	**20 g of Kefir**	**60 g of Kefir**
Color	8.35 A	7.92 B	7.78 B
Flavor	6.76 A	5.66 B	4.87 C
Acidity	6.77 A	5.79 B	5.42 B
Aroma	7.24 A	5.48 B	5.02 B
Global impression	6.91 A	6.13 B	5.69 B

Means followed by the same letter on the line, do not differ by Tukey's test at 5% significance.

Grape juice with the addition of 5 g of kefir was considered the best formulation among the others since it obtained mean scores for all evaluated attributes ranging from 6.76 to 8.35, these values being within the region of acceptance of a product (Table 6).

When evaluating the frequency of consumption of probiotic juices, only the formulation with the addition of 5 g of Kefir differed statistically from the other drinks ($p \leq 0.05$), obtaining an average score of 6.57, which in the attitude scale indicates the option “I like this and would drink from time to time”. For the other formulations, the averages varied from 4.14 for the juice with the addition of 60 g and 4.63 for the formulation with 20 g of Kefir, with no significant difference between the taster's evaluations for these two formulations.

DISCUSSION

The parameters found for whole grape juice analysis may differ from other studies, as they can be affected by species and maturation of grape used for juice production.

The *p*-value and coefficient of determination were calculated to check the adequacy of the models (Table 1 and 2) that described a correlation between the amount of kefir and pH, SS, color, TTA, LAB, MB, and FY of the beverage.

All the formulations proposed had a LAB count above 10^7 CFU.mL^{-1} (Table 1), indicating that grape juice is a good growth matrix. For to be classified as probiotic, the minimum number of viable cells ingested from the microorganism must be 10^8 to 10^9 CFU in the daily product recommendation [21]. Thus, for the individual to meet the daily intake recommended by legislation [21], the consumption of probiotic grape juice must be at least 100 mL per day.

Juices produced from vegetables [2], Cantaloupe melon [22], and different Mediterranean fruit juices [5] obtained satisfactory results in the growth of probiotic microorganisms, showing that the juices of fruits are favorable means for the growth of these microorganisms.

Foods with high acidities, such as grape juice, provide a favorable medium for the growth of fungi and yeasts. Fermented products naturally present a high load of MB [18]. In addition, the Brazilian legislation that establishes microbiological standards for nonalcoholic beverages does not define the maximum values allowed for these microbial groups [23].

With the increase in the proportions of kefir added to grape juice, a pH variation from 2.94 to 2.89 was observed. This may be related to the production of lactic acid from the fermentation process, resulting in a significant reduction in this variable [5, 2].

A reduction in the values of SS was observed as more grain mass was added to the culture medium (Table 2). Studies report that fermentation decreases the content of SS since the microorganism uses the sugars naturally present in the medium to grow and multiply [22, 5, 2].

Brazilian law does not define the maximum and minimum permitted values of acidity for beverages fermented by water kefir, it only defines the value of acidity for beverages fermented by milk kefir, this value is less than 1 g of lactic acid.100 g $^{-1}$ sample [24]. However, this value cannot be used as a parameter for a drink fermented by water kefir, since this microorganism can be grown in different culture media of non-dairy origin, and these have different physical-chemical characteristics from milk, which directly influences the value of that variable. The acidity of a drink is essential for acceptance by the consumer, being a fundamental criterion for consumption and acceptance of the product [25].

The color of the probiotic juice change may be related to the fermentation process, since there is the production of acids in the product and this can affect the pH of the drink, resulting in the color change of anthocyanins [5, 2, 25].

All stored formulations had probiotic counts above 10^7 CFU.mL^{-1} during the entire storage period (Table 3). It was observed that at the end of storage (14 days) all beverages had a LAB count of 7.5 log CFU/mL. Even with the addition of larger amounts of kefir in the drink, the growth of LAB was similar. Despite the greater number of microorganisms with the increase in kefir mass, these microorganisms do not grow indiscriminately. Some factors limit this growth and with that, the number of cells after 14 days is the same. It can be mentioned as a limiting factor the amount of nutrients.

Some studies report the good viability of probiotic microorganisms during the storage of the drink [26, 27]. There was a reduction in the cells of the microorganism after 35 days of storage of a fermented apple juice drink without sucrose, however, this reduction did not interfere with the viability of the product [26]. Other studies report a reduction in the viability of probiotic strains as a function of the storage time of the drink [28, 25]. In this study, 14 days is the maximum time for which probiotic drinks can be stored under refrigeration.

Some studies report that the fermentation process reduces the SS and the pH of the product, due to the production of lactic acid in the medium [5, 2, 25]. However, the pH of the formulation with 20 g of kefir had a significant increase with storage (Table 4), differing from these results. Other studies have reported an increase in pH during the storage of probiotic drinks [26, 28]. However, Furtado and coauthors [25] reported non-significant differences in pH and SS during the storage of probiotic mango juices.

It is important to emphasize that for developing a new functional product, the selection of the culture medium and the appropriate probiotic strain is essential since the interaction of the strain and the medium must be viable for the growth and resistance of the microorganism [29]. In addition, the number of viable cells of the probiotic microorganism must be high during the entire storage of the drink, so that the microorganism arrives in adequate quantities at its place of action and performs its function properly [10]. From this work, it is possible to produce a probiotic drink with whole grape juice and water kefir with the permanence of viability after 14 days under refrigeration.

Santos and Varavallo [30] also assessed the knowledge of university students about probiotic products and reported that about 63.1% of the 348 participants correctly defined the product. Alternatively, Holanda and coauthors [31] found that only 8% of university students knew the correct definition of these foods. Santos and Varavalho [30] also reported that only 21.7% of students in the Health Area and 2.8% in the Humanities Area stated the daily consumption of these products. Holanda and coauthors [31] found that about 93% of students reported consuming yogurts and fermented milk, and of these, about 16% reported daily consumption, 34% weekly consumption, and the majority reported consuming rarely. The results of these studies differ from each other since the knowledge about probiotic products, as well as their consumption, depends a lot on the population evaluated and the place where the research was conducted.

The region of acceptance of a product according to Minim [19] is found between the average scores of 6 to 9, while the region of rejection is determined by scores from 1 to 4, with score 5 representing the region of indifference to the product. Based on this context, grape juice with the addition of 5 g of kefir obtained a better acceptance by the tasters concerning color, acidity, aroma, and overall impression, obtained mean scores for all evaluated attributes ranging from 6.76 to 8.35 (Table 6).

CONCLUSION

All the proposed formulations of grape juice and kefir have obtained good results concerning the count of LAB, all of which are classified as probiotic drinks. Grape juice proved to be an adequate matrix for the growth of probiotic microorganisms and enabled the viability of the strains for 14 days of storage. The recommended portion of the probiotic drink is at least 100 mL per day, and this intake must be associated with healthy lifestyle habits.

The probiotic juice production technology is relatively simple, requiring only the grape juice and water kefir grains that are usually obtained by donation. This technology can be conducted at home and by small grape producers, who can produce a new type of drink, add value to the product and generate income for the family.

However, further studies are needed to improve the product made with whole grape juice and water kefir grains, since the development of probiotic products of non-dairy origin is essential for the increase and expansion of the consumer market.

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