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# Development and sensory acceptance of *kefir* with guava jelly and evaluation of bioactive compounds

## Desenvolvimento e aceitação sensorial de *kefir* com geleia de goiaba e avaliação de compostos bioativos

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## Abstract

*Introduction:* Bioactive compounds are defined as nutrients or non-nutrients that have specific metabolic or physiological actions in the body such as antitumor, antifungal, antibacterial, immunomodulatory, anti-inflammatory and antioxidant actions. The probiotics in fermented milks and carotenoids in fruits are described in the literature as beneficial to health. *Objective:* To develop, evaluate the sensory acceptance, the antioxidant activity and some bioactive compounds of *kefir* with guava jelly. *Methodology: Kefir* was developed and its acceptance evaluated by the hedonic scale test; the centesimal composition was determined by the official methods; the antioxidant activity was evaluated by the ORAC assay; the total phenolic compounds and the total carotenoids were determined by spectrophotometric method. *Results:* The addition of 30% guava jelly increased the antioxidant activity, phenolic compounds and total *kefir* carotenoids. The product was well accepted by consumers, who would also buy it if it were marketed. *Conclusion:* It was concluded that adding jelly contributed to the increase of the bioactive compounds evaluated, and the fermented beverage could contribute to the health of the consumers.

**Keywords:** *Kefir.* Bioactive compounds. Antioxidant activity. Phenolic compounds. Carotenoids. Sensory acceptance.

## Resumo

*Introdução:* Compostos bioativos são definidos como nutrientes ou não nutrientes que possuem ação metabólica ou fisiológica específica no organismo, como as ações antitumoral, antifúngica, antibacteriana, imunomoduladora, anti- inflamatória e antioxidante. Os probióticos presentes nos leites fermentados e os carotenoides presentes nas frutas são descritos, na literatura, como benéficos à saúde. *Objetivo:* Desenvolver, avaliar a aceitação sensorial, a atividade antioxidante e determinados compostos bioativos de *kefir* com geleia de goiaba. *Metodologia:* O *kefir* foi desenvolvido e sua aceitação avaliada pelo teste de escala hedônica; a composição centesimal foi determinada pelos métodos oficiais; a atividade antioxidante foi avaliada pelo ensaio ORAC; os compostos fenólicos totais e os carotenoides totais foram determinados por método espectrofotométrico. *Resultados:* A adição dos 30% de geleia de goiaba elevou a atividade antioxidante, os compostos fenólicos e os carotenoides totais do *kefir.* O produto elaborado foi bem aceito pelos consumidores, que também comprariam o produto caso este fosse comercializado. *Conclusão:* Concluiu-se que adição de geleia contribuiu para o aumento dos compostos bioativos avaliados e a bebida fermentada poderia contribuir para a saúde dos consumidores.


**Palavras-chave**: *Kefir.* Compostos bioativos. Atividade antioxidante. Compostos fenólicos. Carotenoides. Aceitação sensorial.

## Introduction

Foods that promote positive effects on consumers health are a new market trend and drive success because of the growing concern with nutritional status and well-being, along with increasing scientific evidences on the relationship between diet and health.[1]

The *Regulamento Técnico de Substâncias Bioativas e Probióticos Isolados com Alegação de Propriedades Funcionais e/ou de Saúde* (Technical Regulation for Bioactive Substances and Isolated Probiotics with Functional and/or Health Properties Claims) define that these substances are nutrients or non-nutrients that have a metabolic or specific physiological action on the body. They are classified as carotenoids, phytosterols, flavonoids, phospholipids, organosulfides, polyphenols and probiotics.[2] Probiotics, in turn, can be conceptualized as live microorganisms that may be beneficial to health when administered continuously and at adequate rates.[3,4]

Studies consider cultured milk as the main vehicle for incorporation of probiotic cultures that may have an antioxidant activity and provide health benefits to consumers.[5] Brazilian Normative Instruction nº. 46, of October 23, 2007,[6] defines *kefir* as cultured milk obtained from milk coagulation and pH reduction, and fermentation is achieved by lactic acid cultures produced by microorganisms present in *kefir* grains. The grain is made up of a complex structure of bacteria and yeasts, totalizing about 30 species of microorganisms incorporated into a matrix of polysaccharides and proteins,[7] properties that make *kefir* grains potent probiotics. Their bioactive compounds result from milk cultured with *kefir* grains. In this process, microorganisms produce substances that confer the typical texture and flavor of fermented products. In spite of the small number of studies, scientific researches have demonstrated that the proteolytic enzymes of the microorganisms that produce fermentation may help release bioactive peptides, providing *kefir* with beneficial antioxidant effects on health.[8] During the fermentation process, *kefiran* by *Lactobacillus kefiranofaciens* is also produced, which consists of heteropolysaccharides with equal proportions of glucose and galactose, and is distinguished by its antitumoral, antifungal, antibacterial, immunomodulating, anti-inflammatory and antioxidant action.[9,10]

Studies involving diets containing food products with specific probiotic cultures showed that it reduced lactose intolerance, increased the immune system, antimicrobial, anticarcinogenic and antimutagenic activities, reduced the blood cholesterol level, contributed to the treatment of gastric ulcer infection caused by *Helicobacter pylori* and irritable bowel syndrome and to intestinal detoxication by restoring the mucosal balance.[1,11]

Consumption of fermented milks began to grow in 1960, when the addition of fruits contributed to soften the acidic flavor of this beverage, resulting in a better sensory acceptance by consumers who dislike the taste of fermented milk in its natural form.[12]

Guava *(Psidium guajava L)* is a fruit native to South America, mainly cultivated in tropical countries, which has a high acceptance for consumption either *in natura* or processed. Studies show that this fruit has nutritional and biofunctional properties due to the contents of phenolic compounds, carotenoids, essential oils, triterpenoid acids, vitamin C and minerals such as calcium, phosphorus and iron, thus being considered one of the fruits with higher antioxidant potential.[13,14]

Studies of bioactive compounds are important to broaden knowledge on food properties and encourage their introduction into people's diets. Taking into account the above considerations, this study aimed to develop *kefir* with guava jelly, assess its sensory acceptance, the antioxidant activity, total phenolic compounds and total carotenoids present in the food product.

# Methodology

## Material

The study was carried out at the *Laboratório de Alimentos e Dietética* (Foods and Dietetics Laborarory), *Laboratório de Análise Sensorial* (Sensory Analysis Laboratory), *Faculdade de Nutrição* (Nutrition Faculty) and *Laboratório de Biotecnologia de Alimentos da Faculdade de Farmácia* (Foods Biotechnology Laboratory, Faculty of Pharmacy) of the Universidade Federal Fluminense. *Kefir* was prepared using viable, sterilized whole fluid milk and 5% active *kefir* grains, in order to yield a minimum of $10^7$ total lactic bacteria (CFU/g) and $10^4$ specific yeasts (CFU/g) to the end product, as prescribed by Brasil[6] and Nielsen et al.[7] The inoculum was incubated to ferment at room temperature (25±1°C) for 24 hours and maturate under refrigeration (4±1°C) for 24 hours.

Afterwards, the cultured milk product was strained to remove the *kefir* grains, which were incubated in a new substrate to maintain their viability. The cultured product was separated from the whey portion during four hours under refrigeration for final finishing and stored in glass jars at 4±1°C. To prepare the guava jelly, the fruits were bought from local stores in Niterói-RJ, and after being sanitized and peeled, they were cooked at a ratio of four parts of fruit to one part of sucrose. Half percent of vanilla essence (Dr. Oetker, BRA) and 30% of guava jelly were added to *kefir* portions for the next experiments.

## Methods

The project was submitted to the Ethics Committee of the Medical School of the Federal Fluminense University and approved with number 45481815.7.0000.5243. Consumers who agreed to participate in the sensory test signed the informed consent form. Acceptance of *kefir* with guava jelly was determined by 92 consumers, who received monadic, randomized samples.[15]

A structured hedonic, hybrid scale of nine points, in decreasing order, with the ends anchored on the terms "liked it very much" = 1; "disliked it very much" = 9, and "neither liked nor disliked it" = 5 at the middle of the scale .[15] The sensory evaluation form included the question about which sensory quality the consumer liked or disliked most. To determine purchase intention, a structured hybrid scale of seven points, with the ends anchored on the terms "would always buy it" = 1, "would not buy it" = 7 and "would buy it occasionally" = 4 at the middle of the scale, was evaluated by 91 consumers.[16] Data were input in a Microsoft *Excel* spreadsheet and then plotted on a graph.

For characterization of *kefir* mixed with guava jelly, a centesimal composition analysis was carried out in triplicate, according to the methods of the Association of Official Analytical Chemists.[17]

Moisture was determined by the gravimetric method after heating to 105°C. Total ashes were determined by the gravimetric method after incineration in a muffle furnace at 550°C. Total nitrogen was obtained by the Kjeldahl method using factor 6.25 to determine the proteins value. The ethereal extract was obtained by the gravimetric method after extraction of fat by the Soxhlet method with petroleum ether. Total carbohydrates were obtained by calculating the difference, on a wet basis, thus obtaining the NIFEXT (nitrogen free extract) fraction.

*Samples of Kefir,* guava jelly and *kefir* with jelly were frozen and lyophilized in a Liotop® lyophilizer (Liobras, BRA). Extracts from *kefir,* guava jelly and *kefir* with guava jelly samples were obtained according to Ribeiro et al.,[18] using five grams of the lyophilized sample, 50% (v/v) methanol solvent and 70% (v/v) acetone. The antioxidant activity was determined by the ORAC *(Oxygen Radical Antioxidant Capacity)* assay, according to the methodology described by Dávalos et al.,[19] using phosphate buffered saline (PBS) (at 7.4 pH value), Trolox and fluorescein. Readings were made by FLUOstar Optima® microplate reader (BMG Labtech, ENG) with fluorescence detector. The analysis was conducted in triplicate, and the results were expressed in $\mu$mol of Trolox equivalents per gram of sample.

For determination of total phenolic compounds, sample extracts with 50% (v/v) Folin-Ciocalteu reagent and about 4 ml of 7.5% (w/v) aqueous sodium carbonate solution[20] were used, and the samples absorbance was measured by a UV-2600 spectrophotometer (Shimadzu, BRA) at 750nm. The analyses were carried out in triplicate, and the results were expressed in milligrams of gallic acid equivalents per gram of sample. Extraction and determination of total carotenoids were made according to the method described by Rodriguez-Amaya & Kimura[21] in triplicate, and reading was made at the spectrophotometer at 450nm. Results were expressed in milligrams of carotenoids per 100 g of sample.

The results of the tests of hedonic scale, antioxidant activity, phenolics and total carotenoids were subjected to analysis of variance (ANOVA) and Tukey test. The *Statgraphics* software was used in the statistical analyses, and $p<0.05$ values were considered significant.

## Results and Discussion

The results obtained in the hedonic scale test show that *kefir* mixed with guava jelly obtained mean scores between “2” and “3”, which correspond to descriptors “liked it very much” and “moderately liked it” according to the decreasing 9-point scale used in the study (Fig. 1). Consumers, therefore, accepted the cultured drink and indicated flavor as the attribute that they liked most (37%), followed by aroma (25%), overall acceptance (24%) and consistency (18.5%). As mentioned by Garcia[12] about fruits addition, the addition of guava jelly softened the typical sour taste of *kefir*, conferring sweetness and balance to the product, leading to a better sensory acceptance by consumers who do not appreciate the taste of sour milk in its natural form. Regarding the purchase intention test (Fig. 2), *kefir* mixed with guava jelly received a weighted average of 3.03, corresponding to the category "would buy it often". This result indicates good prospects for consumption of this fermented beverage, if marketed and presented to consumers as a potential food product with functional properties.

The characteristics of *kefir* with guava jelly (moisture, proteins, lipids, ashes and total carbohydrates) and the energy value in 100 grams and per portion of the product are shown on Table 1. From the nutritional point of view, 100 grams of this dairy product contain approximately 75kcal, whereas a 170-gram portion provides about 127.9kcal. These values are slightly higher than those of natural fruit yogurt, which has 70kcal in 100 grams and 119kcal in a 170-gram portion.[22]

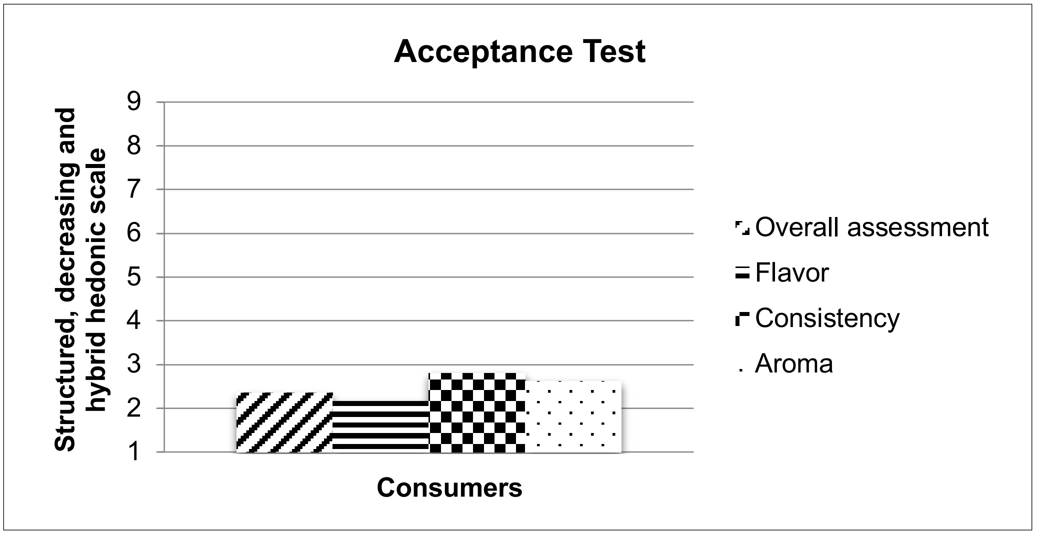


**Figure 1.** Weighted mean values of the scores given to each attribute assessed in the acceptance test of *kefir* with guava jelly.

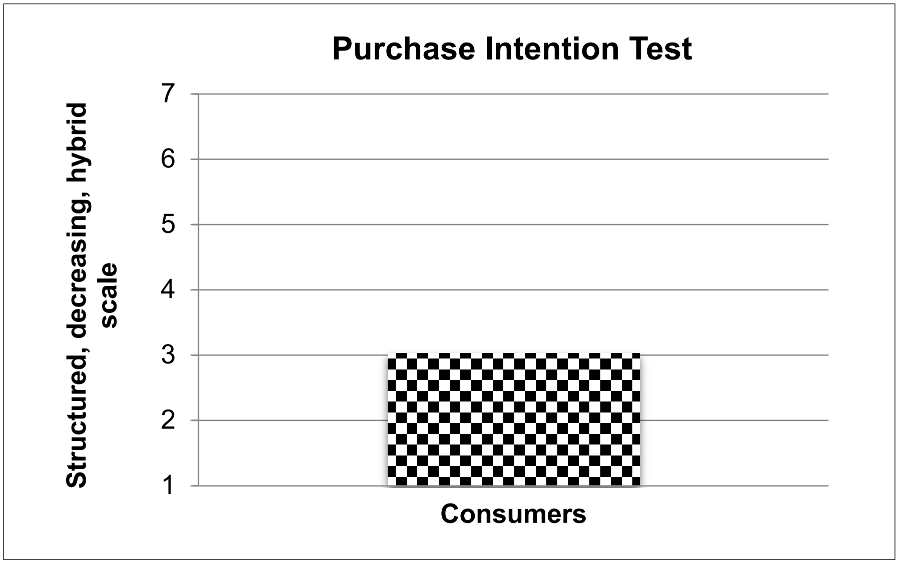


**Figure 2.** Weighted mean values of the scores given on the purchase intention test of *kefir* plus guava jelly.

**Table 1.** Composition and energy value of 100 g and portion of *kefir* with guava jelly. Niterói-RJ, 2017.

| Composition | Centesimal (g/100g) | Portion (g/170g) |
|---|---|---|
| Moisture | 80.87±0.02 | 137.48 |
| Proteins | 3.22±0.08 | 5.47 |
| Lipids | 0.25±0.01 | 0.43 |
| Ash | 0.63±0.01 | 1.07 |
| Carbohydrates[1] | 15.03 | 25.55 |
| TEV[2] | 75.25 | 127.95 |

Mean values ± standard deviation (SD); n=3 (analysis triplicate); [1]by difference, TEV [2]= total energy value in kcal/100g

The assessment of the antioxidant activity of *kefir,* guava jelly and *kefir* with guava jelly presented values of, respectively, 38.9; 138.4 and 165.5 μmol of Trolox equivalents per gram of sample (Fig. 3). So, it was observed that the 30% addition of guava jelly to *kefir* increased the antioxidant activity by 74.5% and 16.5%, when compared to unmixed *kefir* and unmixed jelly, respectively. The study conducted by Melo et al.[23] shows that acetone extract from guava exhibited a high antioxidant activity due to the high scavenging ability (≥ 70%) of DPPH-inducing oxidation radicals.

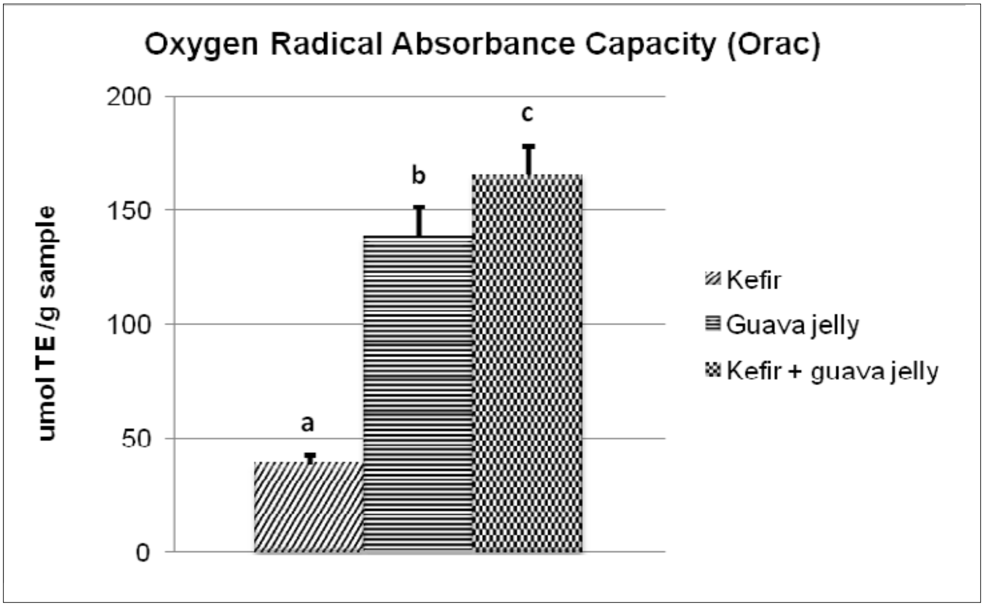


**Figure 3.** Antioxidant values in extracts from *kefir,* guava jelly and *kefir* + guava jelly by ORAC assay. Different lowercase letters indicate significant difference (p<0.05).

Total phenolic contents in the extracts of *kefir,* guava jelly and *kefir* with guava jelly can be seen on Fig. 4. It is known that fruits, especially those which are red and blue in color,[24] have a high content and different classes of phenolic compounds, mainly due to the presence of anthocyanin pigments, which account for the red, purple and violet color of the fruits. Therefore, they are important sources of phenolic compounds, potentializing the beneficial biological effects that fruits provided to the body. Guava is one of the fruits with this property.[14]

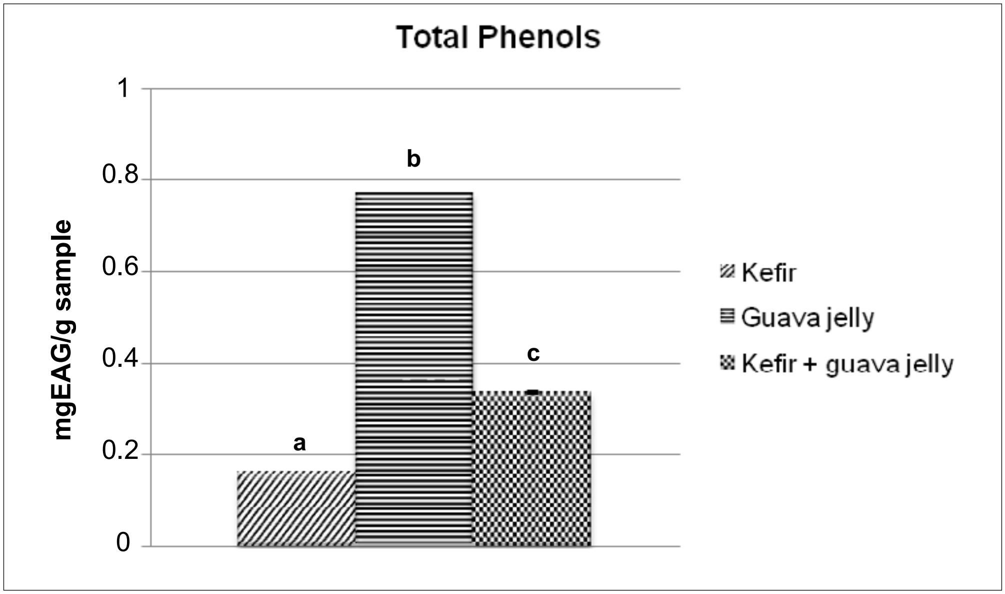


**Figure 4.** Total phenols concentration in extracts of *kefir,* guava jelly and *kefir* + guava jelly determined by spectrophotometry. Different capital letters indicate significant difference ($p$<0.05).

In this study, the highest content of total phenols was found in *kefir* with guava jelly (0.33 mgEAG.g$^{-1}$) than in plain *kefir* (0.16 mgEAG.g$^{-1}$). Cheuczuk & Rocha[25] found similar result in a study with cultured milk whey and pulp of *cajá-manga (Spondias dulci).* However, plain guava jelly obtained a higher value than *kefir* mixed with guava jelly. Guava jelly, even though it had been cooked and added with sucrose during its preparation, still exhibited higher contents of total phenols (0.77 mgEAG.g$^{-1}$) than plain *kefir* and *kefir* with guava jelly.

This result can be explained by a possible interaction of peptides and milk proteins with phenolic compounds, causing difficulties to quantify these compounds through the method of analysis used in this study.[26] There are few studies explaining this possible influence on the method of analysis, which requires more scientific studies.

The results obtained in the analysis of total carotenoids in the extracts of *kefir,* guava jelly and *kefir* with guava jelly via spectrophotometry can be seen on Fig. 5. There is a similarity between total phenolic compounds because the value obtained for guava jelly (12.69mg/100g) was higher than the values obtained for *kefir* (6.75 mg/100g) and *kefir* with guava jelly (10.13mg/100g). The result of the present study was similar to the one obtained by Moura Filho,[27] where a preparation of the fermented milk drink with fruit resulted in a product with lower carotenoid content than the fruit alone.

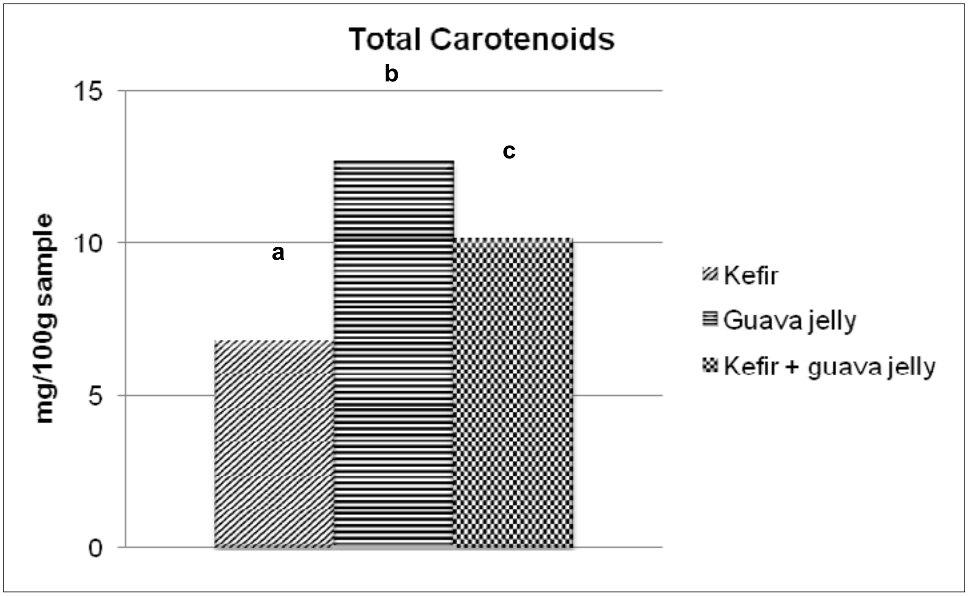


**Figure 5**. Total carotenoid concentrations in extracts of *kefir,* guava jelly and *kefir* + guava jelly determined by spectrophotometry. Different lowercase letters indicate significant difference $p<0.05$).

These results are likely related to the interaction of peptides and milk proteins with carotenoids.[26] However, a comparison between *kefir* and *kefir* with guava jelly shows that the fruit, in the form of jelly, added bioactive compounds to plain *kefir*. It should also be considered that proteins and polysaccharides produced by *kefir probiotic bacteria*[7] are added to the fruit's bioactive compounds, resulting in a food product that can contribute to consumers health.

## Conclusion

*Kefir* with guava jelly was accepted by consumers, who would buy the new product if it were marketed. *Kefir* added with 30% guava jelly exhibited a higher antioxidant activity than *kefir* and guava jelly alone, and thus it is a good source of antioxidant compounds. The addition of guava jelly also contributed to increase the phenolic compounds and carotenoids in *kefir*.

Thus, we can conclude that this fermented drink would contribute to consumers health. We recommend further studies addressing optimal packaging and storage conditions of this dairy product, aiming to hygienic-sanitary safety and preservation of its nutritional value.

## Collaborators

CS Cavararo participated in the study design, execution and interpretation of results of analyses, and in writing the manuscript up to its final version; CCJ Chiappini worked in the study conception and design, supervision and interpretation of the sensory analysis results and in the manuscript revision up to its final version; JR Domingues participated in the study design, supervision and interpretation of the results of analyses of antioxidant activity, total phenols and total carotenoids, revision of the manuscript up to its final version: JS Pinho Jr contributed to the conduction of analyses of antioxidant activity, total phenols and total carotenoids and interpretation of results.

Conflicts of interest: The authors declare that there are no conflicts of interest whatsoever.

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