# Pequi-flavored yogurt and kefir: development, characterization and sensory evaluation

# Iogurte e Kefir sabor pequi: desenvolvimento, caracterização e avaliação sensorial

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## ABSTRACT

In Brazil, the consumption of milk derivatives, especially fermented milks, have shown steady growth in the last decade, with an increase that can be associated with its high nutritional value and good acceptance by the population in general. This study aimed to develop, characterize, and assess the acceptability and purchase intention of yogurt and Kefir with pequi jam. We characterized the products and the pequi jam regarding the composition and microbiological quality according to the Brazilian legislation. The post-acidification was determined at 1, 7, 14, 28, and 35 days of refrigerated storage. The acceptability and purchase intention of the products were assessed on a structured 9-point hedonic scale and a five-point mixed scale, respectively. Both products met the physicochemical and microbiological standards established by the legislation, except for the viability of BAL, with yogurt counts lower than 107 CFU/g at 35 days of refrigerated storage. The total dry extract content of the Kefir and the yogurt were significantly different, while the other physicochemical characteristics did not differ. The pequi jam showed to preserve the characteristics of the fruit compared with the fruit in natura and met the microbiological standards, thus being harmless to health. During the refrigerated storage, the Kefir differed significantly from the yogurt, presenting lower pH and higher acidity. Over the 35-day refrigerated storage, the acidity of the yogurt did not vary significantly, with an average value of 0.77% of lactic acid, while Kefir tended to a higher acidity, with an increase of 0.29% of lactic acid. The pH of the products did not differ throughout the storage time, with an average value independent of the products of 4.25

pH units. The sensory evaluation pointed out that the products differed significantly concerning the attributes of appearance, flavor, texture, and purchase intention. The pequi candy proved to be an option for flavor diversification of fermented milks, and the pequi fruit can be used to value the Cerrado biome, with products that met the standards established by the Brazilian legislation and showed good acceptability and purchase intention.

**Keywords:** dairy products, fermentation, fruit from the cerrado.

**RESUMO**

No Brasil, o consumo de derivados do leite, em especial os leites fermentados têm apresentado crescimento constante na última década, onde o aumento pode ser associado ao seu alto valor nutritivo e boa aceitação pela população em geral. O presente estudo visou desenvolver, caracterizar e avaliar aceitabilidade e intenção de compra de iogurte e de Kefir adicionados de doce de pequi. Os produtos e o doce de pequi foram caracterizados em relação a composição centesimal e qualidade microbiológica de acordo com a legislação brasileira. A pós-acidificação foi determinada nos tempos 1, 7, 14, 28 e 35 dias de armazenamento refrigerado. A aceitabilidade e intenção de compra dos produtos foi avaliada escala hedônica estruturada de 9 pontos e escala mista de cinco pontos, respectivamente. Ambos os produtos atenderam aos padrões físico-químicos e microbiológicos estabelecidos pela legislação, exceto para viabilidade de BAL, onde o iogurte apresentou contagem inferior 107 UFC/g com 35 dias de armazenamento refrigerado. O teor de extrato seco total do Kefir diferiu significativamente do iogurte e as demais características físico-químicas não diferiram entre si. O doce de pequi mostrou preservar as características do fruto quando comparado ao fruto in natura e atendeu aos padrões microbiológicos, sendo inócuo à saúde. Ao longo do armazenamento refrigerado, o Kefir diferiu significativamente do iogurte apresentando menor pH e maior acidez. Durante os 35 dias de armazenamento refrigerado, a acidez do iogurte não apresentou variação significativa, apresentando valor médio de 0,77% de ácido lático e o Kefir apresentou uma tendência de aumento da acidez, com um aumento de 0,29% de ácido lático. Os pH dos produtos não diferiram entre si ao longo do tempo de armazenamento apresentando valor médio independente dos produtos de 4,25 unidades de pH. Na avaliação sensorial, os produtos diferiram significativamente em relação aos atributos de aparência, sabor, textura e intenção de compra. O doce de pequi mostrou ser uma opção de diversificação de sabor de leites fermentados e alternativa para aplicação deste fruto do cerrado como forma de valorização do bioma, visto que os produtos atenderam os padrões estabelecidos pela legislação brasileira e demonstraram boa aceitabilidade e intenção de compra.

**Palavras-chave:** produtos lácteos, fermentação, fruto do cerrado.

## 1 INTRODUCTION

Throughout history, fermented products have gained ground due to their nutritional properties. Among the fermented products, fermented milk is a food used since the dawn of civilization, with yogurt being the best known and consumed fermented milk. In addition to yogurt, other fermented milks such as Kefir have been gaining attention

due to their potential beneficial effects associated with health (VINDEROLA et al., 2005; MAGALHÃES et al., 2011; CARNEIRO et al., 2012; AZIZI et al., 2021).

According to the Family Budget Survey (FBS) it was revealed that in Brazil the consumption of dairy products has been changing, one of these changes was the consumption of fermented milk, which has shown significant increases in the last decade, with a rise of 28% in the 2008-2009 FBS and a 22% rise in the 2017-2018 FBS. In the first half of 2020, sales of some dairy products grew by 5.3%, with emphasis on fermented milk. In 2021, the yogurt market grew even more when compared to the same period in the previous year (SIQUEIRA; GUIMARAES, 2020; SIQUEIRA et al., 2022).

Due to the good acceptance of yogurt by the population in general, the dairy industries are looking for alternatives to maintain this market niche, making it attractive to the consumer through the addition of ingredients that generate an increase in the benefits of its consumption, such as addition of probiotics, fiber and bioactive compounds (ANTUNES et al., 2007; SILVA et al., 2017; HAMID et al., 2022; MARY et al., 2022).

Another fermented milk that has been gaining more fans in several regions of the country is Kefir, however, its production is still carried out in an artisanal way (GARROTE et al., 1997; BEZERRA et al.,1998; MAGALHÃES et al., 2011; CARNEIRO et al., 2012; FIORDA et al., 2017). Despite being a handmade product, different studies have evaluated its functional potential associated with proteins, vitamins, lipids, minerals, amino acids, and microelement composition, since, in addition to its nutritional effect, it can reduce the risk of some diseases (VINDEROLA et al., 2005; HATMAL et al., 2018; FARAG et al., 2020; AZIZI et al., 2021).

Regardless of the variety of fermented milk, the market has sought to diversify flavors of products that are well accepted and preferred by consumers by inserting ingredients with properties associated with healthiness, thus expanding consumer choice.

Among the fruits of the Cerrado, we have the fruit of the pequi tree, the pequi (*Caryocar brasiliense* Camb.), composed of a very thin exocarp or pericarp with a greenish or greenish brown color, corresponding to the smallest portion of the fruit. The outer mesocarp is a very thick whitish layer, while the inner mesocarp or pulp is characterized by being the edible part of the fruit (ALMEIDA; SILVA, 1994; CARVALHO, 2008; CARVALHO et al., 2015).

Pequi has been the focus of numerous studies, due to countless descriptions of its high percentage of phenolic compounds with antioxidant activity. In addition to being rich in vitamins A, C, $B_2$, $B_3$, it has minerals, such as copper and iron, which are essential

for physiological processes (ROESLER et al., 2007; KHOURI et al., 2007; KERR et al., 2007; GONÇALVES et al., 2011).

Considering consumers' demand for healthier foods, capable of bringing health benefits and with the aim of providing visibility to some fruit from the Cerrado, the present study aimed to develop and characterize two types of fermented milk, yogurt and Kefir, flavor pequi, and evaluate the acceptance and purchase intention of these products.

## 2 OBJECTIVE

Developing, characterizing and sensorially evaluating yogurt and Kefir with pequi jam.

## 3 MATERIAL AND METHODS

### 3.1 MATERIALS

The following ingredients were used: pequi (*Caryocar Brasiliense* Camb.), UHT milk (Piracanjuba, Bela Vista de Goias, GO, Brazil), crystal sugar (União, São Paulo, SP, Brazil), Kefir grain and Harmony yogurt culture (CHR Hansen®, Valinhos, SP, Brazil).

### 3.2 PEQUI JAM MANUFACTURE

The fruits went through a cleaning process, and then they were cooked to remove the pulp. After pulping, it was added to a sugar syrup (40ºBrix), previously prepared and cooked for 50 minutes, then the jam was transferred to a sanitized container and cooled in an ice bath. After cooling, the product was stored under refrigeration (12°C) until use.

### 3.3 YOGURT MANUFACTURE

In the preparation of pequi-flavored yogurt, 8% (m/v) of crystal sugar was added to UHT whole milk and the mixture subjected to heat treatment (90°C/5 min). Subsequently, the mixture was cooled to 43±2°C and 2.5% (v/v) of the yogurt culture was added, homogenized, and incubated at 43±2°C until reaching pH 5.0. After fermentation, the gel was broken, cooled and 8% (w/v) of pequi jam was added. The mixture was homogenized, and the product filled in previously sanitized bottles and stored under refrigeration until the moment of analysis.

3.4 KEFIR MANUFACTURE

In the preparation of pequi-flavored Kefir, 8% (m/v) sugar was added to UHT whole milk and the mixture subjected to heat treatment (90°C/5 min). Then, the mixture was cooled to 25°C and 3% (v/v) of Kefir culture was added, homogenized and incubated at 25°C for 16 hours. After fermentation, the gel was broken and 8% (w/v) of pequi jam was added. The mixture was homogenized and the product filled in previously sanitized bottles and stored under refrigeration until the moment of analysis.

3.5 PHYSICOCHEMICAL COMPOSITION: PEQUI JAM AND FERMENTED MILKS

Jam, milk and fermented milk were characterized for pH (Tecnal pH meter, R-TEC-7-MP), titratable acidity (% lactic acid), total solids (%), ash content (%) and total nitrogen (TN, %) (AOAC, 2006). Total protein content was calculated by multiplying TN by 6.38. Fat content was determined by Bligh-dyer method (Bligh and Dyer, 1959). Analyzes were performed in triplicate.

3.6 POST-ACIDIFICATION

After 1, 7, 14, 28 and 35 days of refrigerated storage, the fermented milks were evaluated for pH (Tecnal pH meter, R-TEC-7-MP) and titratable acidity (% lactic acid). Analyzes were performed in triplicate.

3.7 MICROBIOLOGICAL CHARACTERIZATION OF FERMENTED MILKS AND PEQUI JAM

Yogurt, Kefir and pequi jam were evaluated for thermotolerant coliforms, *Escherichia coli* and total fungal and yeast counts, according to methodologies described by the American Public Health Association (APHA, 2001).

3.8 SENSORIAL EVALUATION

The sensory evaluation of pequi-flavored fermented milks was approved by the Ethics and Research Committee of the Federal University of Mato Grosso, Barra do Garças, MT, Brazil, under registration number CAAE:21825319.9.0000.5587.

In the sensorial analysis, 62 untrained evaluators participated, the criteria used for selection was consumers who liked fermented milk. Before carrying out the sensory tests, the evaluators were asked to sign the Free and Informed Consent Form (TCLE).

The nine-point structured hedonic scale was used for the acceptability test (JONES et al., 1955) based on the attribute's appearance, aroma, color, flavor, texture and overall acceptance (MEILGAARD et al., 1999). Purchase intention was performed using a five-point hedonic scale, ranging from "definitely would buy" to "definitely would not buy".

### 3.9 STATISTICAL ANALYSIS

Physicochemical characteristics and sensory of the fermented milks were assessed by Analysis of Variance (ANOVA) using Minitab 15.0 software (MINITAB, 2006).

Post-acidification was analyzed by regression analysis using Minitab 15.0 statistical software (MINITAB, 2006).

## 4 RESULTS AND DISCUSSION

### 4.1 PHYSICOCHEMICAL CHARACTERIZATION OF MILK

The milk met the standards established by Brazilian legislation, which establishes that the acidity parameters must be 0.14 to 0.18% of lactic acid, minimum fat of 3.0%, total protein of at least 2.9 % and total solids of at least 8.2% (BRASIL, 1997; BRASIL, 2018) (Table 1). The legislation does not establish standards for pH and ash content, however, the data obtained corroborate those described by Tamanini et al. (2011) and Caldeira et al. (2010), respectively.

Table 1. Physicochemical characterization of milk (n=3).

| **Characterization** | **Milk** |
|---|---|
| pH | 6.66 ± 0.02* |
| Acidity (% lactic acid) | 0.18 ± 0.01 |
| Fat (%) | 3.05 ± 0.01 |
| Ash (%) | 0.71 ± 0.02 |
| Total protein (total N x 6.38) (%) | 3.06 ± 0.05 |
| Total solids (%) | 11.47 ± 0.00 |

*Mean ± standard deviation
Source: Author

### 4.2 PHYSICOCHEMICAL CHARACTERIZATION OF PEQUI JAM

Physicochemical characterization of pequi jam is presented in table 2. Physicochemical characterization of pequi jam was not found in the literature. The results obtained were discussed based on the characteristics of the fruit *in natura*, since the Brazilian legislation itself establishes in the development of the jam, this must still carry

characteristics of the fruit used and the elements that were used in the production process (BRASIL, 2005).

With respect to the pH, acidity, and protein content, pequi jam had characteristics similar to those described for pequi pulp. Sousa et al. (2012) describe acidity values of 0.28%. The pH values are like those found by Vera et al. (2005) which ranged from 6.58 to 6.97. Regarding the protein content, the values corroborate those described by Ribeiro (2011) for fruits collected in Mato Grosso, which varied between 2.58% and 2.63%. It should be noted that variations in characteristics between fruit pulps may be associated with factors such as collection time, amount of rain, soil nutrition, which are characteristics that affect plant properties (POTAFOS, 1998).

Table 2. Physicochemical characterization of pequi jam (n=3).

| **Characterization** | **Pequi jam** |
|---|---|
| pH | 6.23 ± 0.06* |
| Acidity (%) | 0.27 ± 0.36 |
| Fat (%) | 7.66 ± 0.00 |
| Ash (%) | 0.18 ± 0.03 |
| Total protein (total N x 6.25) (%) | 2.66 ± 0.31 |
| Total solids (%) | 70.30 ± 0.01 |

*Mean ± standard deviation
Source: Author

## 4.3 PHYSICOCHEMICAL CHARACTERIZATION OF FERMENTED MILKS

Regarding the physicochemical characteristics of the products, a significant difference was observed in the total solids (TS) content, in which Kefir had a higher solid content when compared to yogurt. The other parameters did not differ from each other (Table 3).

Table 3. Physicochemical characterization of fermented milks on day 1 (n=3).

| **Characterization** | **Yogurt** | **Kefir** |
|---|---|---|
| pH | 4.63 ± 0.05[1] | 4.21 ± 0.33 |
| Acidity (% lactic acid) | 0.66 ± 0.01 | 0.84 ± 0.07 |
| Fat (%) | 3.20 ± 0.13 | 3.23 ± 0.20 |
| Ash (%) | 0.72 ± 0.05 | 0.61 ± 0.06 |
| Total protein (total N x 6.25) (%) | 3.35 ± 0.06 | 3.59 ± 0.17 |
| Total solids (%) | 11.96 ± 0.10* | 13.10 ± 0.01 |

[1]Mean ± standard deviation
*Significant in column by F test (P < 0.05).
Source: Author

The acidity, protein and fat content of the products meet the standards established by Normative Instruction No. 46, of October 23rd, 2007, which describes that yogurt-type fermented milk must present a range of 0.6 to 1.5 % lactic acid and Kefir type must have a lactic acid percentage of less than 1%. The protein content must present at least 2.9% and fat greater than 3% when the milk used in the manufacture of fermented milk is whole milk (BRASIL, 2007).

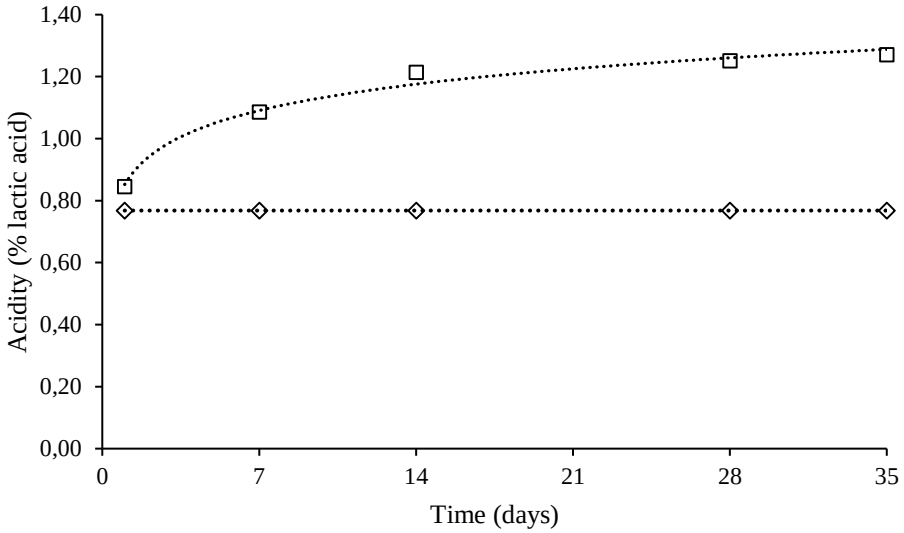
About pH values, Rocha et al. (2008) describes that yogurt added with pequi pulp was 4.32 pH units and Kefir 4.19 pH units, data similar those obtained in this study.

Kefir showed a significant increase of the total solid when compared to yogurt, but the two still presented values close to those found for their main raw material, milk, with a slight increase, due to the 8% of pequi jam incorporated into the formulation. For the ash content, the data obtained are similar to those described in the literature, which present values ranging from 0.47 to 0.79% (SILVA et al., 2018; MASCARENHAS et al., 2012).

## 4.4 POST-ACIDIFICATION

Post-acidification is a measure associated with the sensory quality of yogurts and fermented milks, as it limits the shelf life of these products due to increased acidity and reduced pH. In the acidity analysis of the products during refrigerated storage, the interaction between products and refrigerated storage time was observed (p= 0.016). Figure 1 shows the effect of products and time on yogurt and Kefir acidity. It is observed that with the passage of time, the acidity of the yogurt did not increase, presenting an average value during storage of 0.77% of lactic acid, this stability is associated with the characteristic of the lactic culture used (Harmony, CHR Hansen®), which, according to the manufacturer, features low post-acidification. As for Kefir, there was a tendency towards an increase in acidity, with an increase of 0.29% of lactic acid after 35 days of storage.

Figure 1: Yogurt (◊) and kefir (□) acidity during 35 days of refrigerated storage.




Source: Author

Concerning to the effect of the products on acidity, the products differed from each other ($p < 0.0001$) (Table 4). Kefir showed higher acidity (1.13% lactic acid) when compared to yogurt (0.77% lactic acid), this difference can be associated with the type of fermentation of each product, since Kefir has two types of fermentation: lactic acid and alcoholic, while yogurt only lactic acid fermentation (SILVA; OKURA, 2021).

Regarding pH, yogurt and Kefir differed statistically from each other regardless of the refrigerated storage time ($p < 0.0001$). Kefir had a lower pH when compared to yogurt (Table 4), the difference observed between the two fermented milks can be explained by the low post-acidification of the yogurt culture used, according to the supplier's technical file (Harmony, CHR Hansen®), and due to the fact that Kefir grains present a complex symbiotic culture containing a variety of bacteria and yeasts in different proportions depending on the country/region of production, influencing its physicochemical characteristics (GARROTE et al., 1997; GARROTE et al., 1998; IRIGOYEN et al., 2005; BARUKČIĆ et al., 2017).

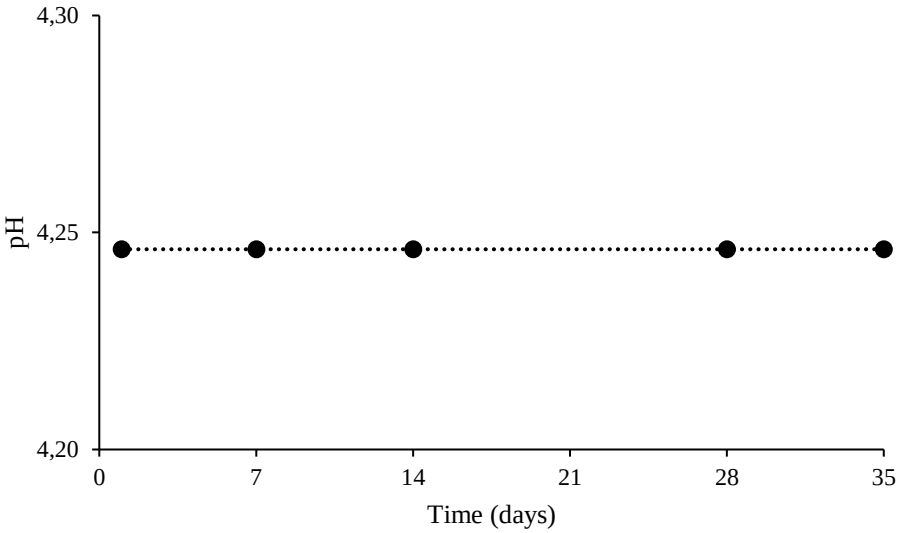
Table 4: Post-acidification of fermented milks (n=3).

| **Products** | **Acidity (%lactic acid)** | **pH** |
|---|---|---|
| Yogurt | 0.77* | 4.57* |
| Kefir | 1.13 | 3.92 |

*Significant in same column by F test ($P < 0.05$).
Source: Author

When analyzing the effect of refrigerated storage time for yogurt and Kefir, there was no significant pH difference since the independent average value of the products was 4.25 pH units (Figure 2).

Figure 2: pH measurement during refrigerated storage time of fermented milks (n=3).




Source: Author

## 4.5 MICROBIOLOGICAL CHARACTERIZATION OF PEQUI JAM AND FERMENTED MILKS

The pequi jam and fermented milks met the standards established by Brazilian legislation. Counting of *Enterobacteriaceae* and fungi and yeasts was less than 10 CFU per gram, absent *Escherichia coli*, indicating satisfactory hygienic-sanitary processing conditions (BRASIL, 2022).

Brazilian legislation states that the total viable lactic bacteria count should be at least $10^7$ CFU/g in the final product during self-life. Yogurt at the end of 35 days of refrigerated storage showed a count of $3.31x10^6$ CFU/g, lower than recommended by legislation, thus inferring that it should have a shelf-life determination lower than the period in which the research was carried out. Kefir met the standard established by legislation, with a count of $1.00\ x10^7$ CFU/g after 35 days of refrigerated storage (BRASIL, 2007).

## 4.6 SENSORY EVALUATION

Sensory acceptance and purchase intention for fermented milks shown table 5. The products did not differ statistically from each other for color, aroma and overall

acceptance attributes, a fact that can be associated with the incorporation of pequi jam, since the fruit gave the products a yellow color and the characteristic and pronounced aroma of pequi. Significant differences were observed regarding to the following attributes: appearance, flavor, texture and purchase intention, and Kefir had higher averages when compared to yogurt.

Table 5. Sensory acceptance and purchase intention for fermented milks.

| Products | Appearance | Color | Aroma | Flavor | Texture | Overall impression | Purchase intention |
|---|---|---|---|---|---|---|---|
| Yogurt | 7.3* | 7.4 | 6.7 | 6.9* | 6.9* | 7.1 | 3.8* |
| Kefir | 7.7 | 7.7 | 7.1 | 7.6 | 7.8 | 7.6 | 4.1 |

*Significant in same column by F test ($P < 0.05$).
Source: Author

The differences observed between the appearance, flavor and texture attributes may be associated with the difference in microorganisms present in yogurt and Kefir, and changes in physicochemical characteristics, which generated rheological changes (IRIGOYEN et al., 2005; TAVARES et al., 2018). Yogurt is characterized by the lactic acid and acetaldehyde flavor from the fermentation of lactic acid bacteria, *Steptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*., whereas Kefir is characterized by its typical specific flavor from both lactic acid bacteria and yeasts, the major products being lactic acid, acetic acid, acetaldehyde, acetoin, diacetyl, ethanol, and $CO_2$ (TAMIME; ROBINSON, 1999; IRIGOYEN et al., 2005; BARUKČIĆ et al., 2017). The difference in fermentation products present in Kefir associated with the addition of pequi jam may have generated a sensation of freshness and lightness in the palate, leading to better acceptance of the product in terms of flavor. Tavares et al. (2018) describe that the incorporation of fruits to Kefir gives better flavor attributes to the product. Regarding to texture, this is a sensory and functional manifestation of the structural, mechanical and surface properties of food perceived through the senses of sight (visual texture), hearing (auditory texture), and touch (tactile texture). Sometimes in some products only one of these senses is used to perceive the texture of the product and in other cases the texture is perceived by a combination of these senses (LAWLESS; HEYMANN, 2010). This sensory characteristic can be related to instrumental measurements, which will indicate food characteristics through physicochemical changes due to manufacturing technology. The stability of a fermented milk is directly related to bound water and/or ability to bind to the casein matrix formed during milk coagulation, as long as pH reduction interferes

with this binding, favoring protein-protein interaction by reducing bound water (DAMODARAN et al., 2010; BARUKČIĆ et al., 2017). Thus, the lower pH and higher acidity observed in Kefir (Table 7) may have influenced its perception of texture, since Irigoyen et al. (2005), describe that the viscosity of Kefir tends to reduce throughout the storage of the product, regardless of the concentration of Kefir grains, differing from the manufacturing process of yogurts that tend to present an increase in viscosity.

Kefir's greater purchase intention can be associated with a better acceptance of all products sensory attributes when compared to yogurt.

## 5 CONCLUSION

The developed products showed good sensory acceptance, met the physical-chemical standards and are innocuous to health, proving to be a good alternative for the dairy product industry, as it has a high nutritional value and generates diversification of flavors with the use of pequi, thus adding, value to this fruit from the Cerrado, which enjoys great appreciation in different regions of the country.

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