

Article **Artisanal Cream Cheese Fermented with Kefir Grains**

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Abstract: This is the first study that investigates the effect of kefir with an emphasis on the production of short-chain fatty acids (SCFAs) during the fermentation process in food products. The products developed and characterized were an artisanal cream cheese without cream and one with added cream, and for the analysis of the fatty acid profile, both cream cheeses were compared with commercial cream cheese. The artisanal cream cheese had a high amount of lactic acid bacteria characterizing the product formed by Lactobacilli and a low concentration of lactose due to the fermentation process. Compared to commercial cream cheese, our products without and with added cream had a higher concentration of short-chain fatty acids (SCFAs), especially butyric acid, which is important for the health of the gastrointestinal tract, omega 3, and oleic fatty acid, which has been associated with the prevention and control of some diseases. Overall, the artisanal cream cheese cream with fermented cream with kefir grains is a functional product with an innovative character compared to current products on the market and was well accepted by the younger public. This new product comes as an option for those who need to change their eating habits and maintain a healthy lifestyle.

Keywords: cream cheese; kefir; probiotics; short-chain fatty acids

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1. Introduction

Kefir grains vary in size from 1 to 4 cm in length; they and resemble small cauliflower florets with an irregular shape and a color ranging from white to light yellow, and are made up of lactose-fermenting and non-lactose-fermenting yeasts [\[1\]](#page-8-0). Kefir is a homemade viscous and slightly effervescent beverage obtained through milk fermentation with kefir grains, which are built up by a complex community of lactic acid and acetic acid bacteria and yeasts confined in a matrix of proteins and polysaccharides. Its consumption has been associated with a wide range of functional and probiotic properties that could be attributed to the microorganisms present in kefir and/or to the metabolites synthesized by them during milk fermentation [\[2\]](#page-8-1).

Kefir can be produced through artisanal or traditional methods, as well as on an industrial scale. In the artisanal method, raw materials are sourced directly from the production site, and the techniques and tools used are predominantly manual. Traditionally, kefir grains are not sold but rather donated to those interested in consuming them. To ensure product safety, good manufacturing practices must be followed, and the process must adhere to traditional recipes with minimal additives, prohibiting the use of colorings and flavorings [\[3\]](#page-8-2). Typically, kefir grains are inoculated into pasteurized milk, and the fermentation process occurs at room temperature. After fermentation, the grains are separated from the fermented product by means of filtration through a sieve and then inoculated into dairy products. In Brazilian artisanal cheese, rennet is added instead of kefir grains, and due to the different methods of incorporating dairy yeast and varying maturation periods, unique characteristics are imparted to the product [\[4\]](#page-8-3).

On an industrial scale, in addition to the inoculation and fermentation processes, there is also a maturation phase. This phase occurs under controlled temperature and time, facilitating the growth of microorganisms and allowing the food product, generally beverage and yogurts, to develop the desired flavor.

The regular consumption of kefir is important for the health of the gastrointestinal tract and has been associated with better digestion, as kefir-fermented dairy products generally have β-galactosidase activity and a reduced lactose content compared to milk, making them ideal for those suffering from lactose intolerance [\[5,](#page-8-4)[6\]](#page-8-5). In addition, kefir has been associated with many other health benefits, including cardiovascular, immune, and nervous system protection, cancer prevention, and therapeutic activity in clinical trials [\[7\]](#page-8-6). Its importance in cancer prevention is due to the antimutagenic action and antitumor properties of the lactic acid bacteria in kefir [\[8\]](#page-8-7).

Fermented foods with probiotic properties have the ability to modulate the intestinal microbiota, improve the control of intestinal permeability, increase its barrier function, and assist in the production of short-chain fatty acids (SCFAs), which have an anti-inflammatory and protective effect on the intestinal mucosa [\[9](#page-8-8)[,10\]](#page-8-9). In this regard, probiotics can improve the immune function of the mucosa of the digestive tract and consequently of the body through the anti-inflammatory effect related to the probiotic microbiota and its own substances metabolized in the intestine, such as SCFAs [\[11\]](#page-9-0). In neurological disorders and other diseases associated with intestinal bacterial dysbiosis, microbiota-derived SCFAs, together with dietary modulation, probiotics, prebiotics, and fecal microbiota transplantation, are potentially capable of changing inflammatory nervous diseases [\[12\]](#page-9-1).

The production of SCFAs, such as butyrate, produced by fermentation in the large intestine by the intestinal microbiota, has been shown to be protective against insulin resistance and fatty liver [\[13,](#page-9-2)[14\]](#page-9-3). A normally low production of butyrate has an effect on the autoimmunity associated with type I diabetes [\[15\]](#page-9-4) and may decrease the intestinal microbiota of patients with type II diabetes, compared to healthy individuals [\[16\]](#page-9-5). An imbalance of the intestinal microbiome that affects SCFA-producing bacteria often occurs in patients with inflammatory bowel diseases, irritable bowel syndrome, type II diabetes, obesity, and autoimmune diseases or in patients with cancer [\[17–](#page-9-6)[21\]](#page-9-7).

Most studies on kefir consumption only focus on the production of short-chain fatty acids present in the intestine. However, there is also a need to investigate the effect of kefir with an emphasis on the production of short-chain fatty acids and also other important fatty acids, such as omega 3 and oleic acid during the fermentation process in food products.

The probiotics available on the market are fermented beverages made with milk, because dairy products serve as an ideal vehicle for the survival of probiotics in gastric juice, as they have a buffering and protective effect. Nevertheless, several challenges remain regarding the stability and functionality of probiotics in dairy products. Cheeses are equally efficient as a vehicle compared to fermented milks and yogurts. Consequently, a new challenge arises in designing and monitoring probiotic foods that can preserve probiotic function until the moment of consumption [\[22,](#page-9-8)[23\]](#page-9-9). The determination of the fatty acid profile during the fermentation process using kefir in the production of cream cheese directs future research and possible applications in dairy products richer in fatty acids beneficial to human health.

Our artisanal cream cheese, with added cream and fermented with kefir grains, is a functional product rich in oleic fatty acid, with an innovative character compared to current products on the market, and was well received by the younger public. In this context, the objective of the present study was to develop and characterize cream cheese fermented with kefir grains; determine its fatty acid profile with an emphasis on short-chain fatty acids; and carry out a sensory analysis of the product's global acceptance.

2. Materials and Methods 2. Materials and Methods

2.1. Artisanal Cheese Cream Fermented with Kefir Grains 2.1. Artisanal Cheese Cream Fermented with Kefir Grains

Whole raw milk was pasteurized and 50 g of milk kefir grains was added to 500 mL Whole raw milk was pasteurized and 50 g of milk kefir grains was added to 500 mL of milk at room temperature. The fermentation period was 30 h at a temperature of 20 ◦C. of milk at room temperature. The fermentation period was 30 h at a temperature of 20 °C. Subsequently, the sample was filtered through a sieve to remove the kefir grains, and then Subsequently, the sample was filtered through a sieve to remove the kefir grains, and then a cloth filter was used to separate the serum and obtain a paste mass (50 g) to which 2 g of a cloth filter was used to separate the serum and obtain a paste mass (50 g) to which 2 g cream was added to obtain cream cheese (Figure [1\)](#page-2-0). of cream was added to obtain cream cheese (Figure 1).

Figure 1. Flowchart of artisanal production of fermented kefir grains to obtain cream cheese.

Figure 1. Flowchart of artisanal production of fermented kefir grains to obtain cream cheese. *2.2. Lactic Acid Bacteria Count*

2.2. Lactic Acid Bacteria Count The total count of viable lactic bacteria was performed using serial dilutions (between The total counteria count of via ble the performed water, and I file and does were sabsed quently plated at depth using Man, Rogosa and Sharpe (MRS) agar plates and incubated at of actiny plated at depth daily in 0.1% steps and 5 and 1 methods and 1 methods are subsected at 37 °C for 48 h. The number of viable lactic cells was obtained by direct plate count and was σ is the number of via the depth cannot was obtained by ancel plate count and was expressed in colony forming units (CFU) per mL [\[24\]](#page-9-10). 10−⁶ and 10−⁸) carried out in 0.1% sterile peptone water, and 1 mL aliquots were subse-

2.3. Soluble Solids, pH, Acidity Measurement, and Centesimal Composition

The concentration of soluble solids for measuring the Brix scale was analyzed with a portable analog refractometer (Bel RTS, Piracicaba, SP, Brazil). The pH was measured with a digital pH meter (Mettler Toledo, Barueri, SP, Brazil), and the acidity was determined by

titration using a concentrated NaOH solution and phenolphthalein as an acid-base indicator. The determination of the centesimal composition was based on the method in [\[25\]](#page-9-11); the moisture content was measured using the oven drying method (Marconi, Piracicaba, São Paulo, SP, Brazil) at a temperature of 100–105 °C for 12 h; the ash content was determined by carbonizing the sample in a muffle furnace (JUNG, São Paulo, SP, Brazil) at 550 ◦C for 16 h; fat was determined using the Soxhlet method using hexane as a solvent in a Soxhlet extractor (Marconi, Piracicaba, São Paulo, SP, Brazil); and protein was determined using the Kjeldahl method, which consisted of 3 stages: digestion of the organic matter, distillation of the ammonia resulting from the digestion in Kjeldahl equipment (Tecnal, Piracicaba, São Paulo, SP, Brazil), and titration after distillation.

2.4. Glucose-Reducing and Non-Sucrose-Reducing Carbohydrates

The determination of reducing carbohydrates in glucose (glucose, maltose, and lactose) and non-reducing carbohydrates (sucrose) was carried out using titration with Fehling's solution [\[26\]](#page-9-12): reducing sugars react with copper II ions (Cu^{2+}) from the Fehling solution, reducing to copper I ions $(Cu⁺)$ under the action of heat in an alkaline medium. When reacting with Cu^{2+} ions, the sugars undergo oxidation, while Cu^{2+} is reduced to Cu⁺, making a red precipitate (reactions: RCHO + $2Cu^{2+}$ + $5OH^-$ (blue) to RCOO⁻ + $Cu₂O$ + $3H₂O$ (red). Non-reducing sugars are hydrolyzed with hydrochloric acid, dissociating the disaccharide into its monosaccharides. The results are calculated using factors, and, generally, the determinations of reducing carbohydrates are calculated: Lactose: the GRC value \times 1.39 (hexose to lactose conversion factor);

Total carbohydrates in glucose (TCG);

Sucrose: $TCG - GRC \times 0.95$ (hexose to sucrose conversion factor);

Total carbohydrates: sucrose + GRC.

2.5. Fatty Acid Profile

To analyze the fatty acid profile, artisanal cream cheese fermented with kefir grains with the addition of cream and without the addition of cream was compared with a commercial brand of cream cheese. The sample preparation was based on the method described by [\[27](#page-9-13)[,28\]](#page-9-14). Description of fatty acid extraction: a sample of 0.25 g was weighed into a screw-cap test tube, and 20 mL of 15% KOH in absolute ethanol was added. The tubes were then agitated for 12 h at room temperature. After this, 10 mL of a saturated NaCl solution was added to the sample. To extract the unsaponifiable matter, 5 mL of hexane was added and vortexed for 5 min. The hexane phase was then transferred to another screw-cap tube. This extraction process was repeated two more times. Finally, the entire hexane phase was evaporated using nitrogen gas. The methylation of samples was performed according to the methodology in [\[29\]](#page-9-15). The quantification and identification of short chain fatty acids was performed using undecanonical acid (Chem Service Inc., West Chester, PA, USA) as internal standard and fatty acid methyl ester (FAME, Sigma Aldrich, St. Louis, MO, USA), and a gas chromatograph with a Flame Ionization Detector (FID) detector, according to the methodology described by [\[30\]](#page-9-16). Chromatographic conditions were as follows: column: J&W DB-FastFAME GC Column, 30 m, 0.25 mm, 0.25 µm, 7 inch cage; column temperature: 250 ◦C; runtime: 19 min; injection volume (loop): 1.0 µL; split: 50:1; temperature: 250 ◦C and 250 ◦C.

2.6. Sensory Analysis

A total of 120 people received two samples of the artisanal cream cheese with cream to assess the overall acceptance of the product. The cream cheese was served on toast and people were given a 180 mL glass of water to cleanse their taste buds between one sample and the next. The acceptance test with the Hedonic Scale (1-hated to 9-loved it) was applied, and, in addition, the following questionnaire: (1) Would you buy this product? () yes, () no, () maybe; (2) For you, this product resembles: () Cottage cheese; () Cream cheese; () Yogurt; () None of the above.

2.7. Statistical Analysis

The treatments were repeated three times and all the analyses were carried out in triplicate. The results were tabulated and expressed as mean \pm standard deviation. Analysis of variance (ANOVA) and comparison of means were carried out using Tukey's test at a 95% confidence level (*p* < 0.05). Statistical analysis was performed using Minitab statistical software, version 16.

3. Results and Discussion

3.1. Lactic Acid Bacteria Count

The samples of cream cheese fermented with kefir grains showed a high amount of lactic bacteria, with an average of colony forming units (CFU) of 7.7 Log CFU/mL, characterizing a product formed by Lactobacilli. To ensure a continuous effect on the human body, Normative Instruction 46/2007 [\[31\]](#page-9-17) of the Ministry of Agriculture, Livestock and Supply (Brazil) establishes the minimum count of total lactic bacteria in kefir-based foods at 7 Log CFU/mL. Fermented milk contains 9 to 10 Log CFU/mL of viable lactic acid bacteria [\[32\]](#page-9-18). The kefir microbial community comprises a complex mixture of lactic acid bacteria, acetic acid bacteria, and yeasts. Strains of the genus Lactobacillus are recognized for their therapeutic capacity, highlighting their anti-inflammatory and anticarcinogenic activities [\[33](#page-9-19)[,34\]](#page-9-20). Probiotics are the target of studies due to their functional capacity that covers endocrine, immunological, and gastrointestinal aspects. The consumer's search for foods that offer additional health benefits has grown considerably and, in this sense, industries have sought innovation to meet these expectations.

3.2. Soluble Solids, pH, Acidity Measurement and Determination of Carbohydrate Reduction in Lactose

The values of Brix-grade soluble solids in samples of whole milk and whole milk fermented with kefir before and 30 h after fermentation were 9.2 and 6, respectively. Whole milk fermented with kefir presented significantly a lower Brix level ($p < 0.05$) compared to the initial sample without fermentation. Whole milk fermented with kefir presented a lower soluble solids content. This is because during the fermentation process, kefir consumes milk soluble solids for its growth and converts them into other products and various important metabolites such as peptides, amino acids, vitamins, ethanol, and CO2 that contribute to the flavor and aroma of the product [\[35](#page-9-21)[,36\]](#page-9-22).

The pH value decreased and the percentage of lactic acid increased $(p < 0.05)$ in the samples after 30 h of fermentation (Table [1\)](#page-4-0).

Table 1. Values of pH and percentage of lactic acid in samples of pasteurized whole milk and whole milk fermented with kefir before and after fermentation.

 $n = 3$. Different lowercase letters on the same line indicate differences between values of $p < 0.05$ within the group, according to Tukey's test.

The pH value decreased and the percentage of lactic acid increased. In the literature, similar pH values were found ranging from 3 to 4 in fermented milk [\[37,](#page-10-0)[38\]](#page-10-1). The pH can also reduce the level of alcohol produced during the process; for example, using kefir grains to 1% at a pH of 4.5 led to a decrease in alcohol level to 0.3% in goat milk [\[39\]](#page-10-2). Lactic acid is produced from lactose degradation, while diacetyl and acetoin are formed from citric acids and lactose. While acetaldehyde is produced from the degradation of proteins, it is used as a source of growth for microorganisms. Kefir fermentation must contain an acidity of less than 1 g of lactic acid/100 g. According to the quality standards of fermented milk, the acidity was within the values determined by the legislation [\[40\]](#page-10-3). Studies found higher acidity values of 0.88 to 1.17 g/100 g in fermented products sold in the country and similar values with average values of 0.87 to 1.20 $g/100 g$ [\[37,](#page-10-0)[38\]](#page-10-1). A lactic acid value of 1.89 is considered ideal for a kefir grain concentration of 5% [\[41\]](#page-10-4).

Table [2](#page-5-0) describes the analysis of the reduction in carbohydrates in lactose. A significant reduction in lactose was observed after the fermentation process of fermented cream cheese with cream and without cream compared to pasteurized whole milk.

Table 2. Analysis of the reduction in reducing carbohydrates in lactose.

 $n = 3$ Different lowercase letters on the same line indicate differences between values of $p < 0.05$ within the group, according to Tukey's test.

Lactose originally present in fermented beverages undergoes lactic acid fermentation, so its concentration is lower than in processed milk [\[42\]](#page-10-5). This process has already been observed with hard cheeses that have proven to be the most suitable for consumption by people with lactose intolerance. Milk-based probiotic products for vegan and lactoseintolerant populations provide therapeutic and dietary support for the gastrointestinal tract [\[5\]](#page-8-4). The challenge of the dairy industry is to develop new products with reduced lactose content, but what one sees is a range of plant products that do not have the same concentration of vitamins and bioavailable calcium as those found in milk and its derivatives.

3.3. Centesimal Composition

Table [3](#page-5-1) describes the centesimal composition of fermented cream cheese without and with added cream.

 $n = 3$ Different lowercase letters on the same line indicate differences between values of $p < 0.05$ within the group, according to Tukey's test.

According to the USDA food composition table [\[43\]](#page-10-6), when fermented cream cheese without added cream is compared with some commercially available dairy products, it resembles the composition of the whole yogurt sample in (g) (moisture: 85.3; carbohydrates: 5.57; protein: 3.82; lipids: 4.48; and ash: 0.85), but with higher carbohydrate and protein content, and compared to ricotta cheese in (g) (moisture: 72.9; carbohydrates: 6.86; protein: 7.81; lipids: 11; and ash: 1.36), it has lower protein content, lipids, and ash. In our study, 2 g of cream at 50 g of cheese fermented with kefir was added to obtain the cream cheese, which adds 4% fat to the product, which is even lower compared to extra-fat cheese with a fat content equivalent to 60%; fat cheese with a content between 45 and 59.9%; semi-fat cheese with a content of 25 to 44.9%; low-fat cheese with a content of 10 to 24.9%; and skimmed cheese with a content of less than 10%. Commercial cream cheese is characterized by a minimum fat content of $25 g/100 g$ in the dry extract and a maximum moisture content of 78 g/100 g [\[31\]](#page-9-17).

3.4. Fatty Acid Profile

In the analysis of the fatty acid profiles of commercial cream cheese, fermented cream cheese without cream, and fermented cream cheese with cream (Table [4\)](#page-7-0), the concentrations of butyric fatty acid (C4:0) to capric fatty acid (C10:0) stood out in the fermented cream cheese with cream. Probably, the addition of cream and/or the fermentation process itself contributed to the increase in short-chain fatty acids in the product. These findings may enhance the understanding of SCFA production in foods, thereby adding value to the product and defining its functional properties. SCFAs have the ability to modulate the intestinal microbiota, improve the control of intestinal permeability, and increase its barrier function, anti-inflammatory action, and the activation of digestive enzymes [\[9](#page-8-8)[,10](#page-8-9)[,44\]](#page-10-7).

Table 4. Fatty acid profile in commercial cream cheese brand, fermented cream cheese without cream and fermented cream cheese with cream.

Table 4. *Cont.*

 $n = 3$. Different lowercase letters on the same line indicate differences between values of $p < 0.05$ within the group, according to Tukey's test.

Saturated fatty acids with the potential to develop cardiovascular diseases, such as lauric, myristic, palmitic, and stearic fatty acids, were higher in fermented cream cheese with cream than in commercial cream cheese. The amount of saturated fatty acids in dairy fat can vary depending on the type of milk and the production process, but in general, dairy fat contains about 60% of saturated fatty acids that are mainly long-chain, including lauric acid, palmitic acid, and stearic acid [\[45\]](#page-10-8). Previously, all saturated fatty acids such as myristic, palmitic, and stearic were related to the increase in the concentrations of LDL cholesterol and apolipoprotein B [\[46\]](#page-10-9), but stearic fatty acid reduced LDL cholesterol, was neutral in relation to HDL cholesterol, and directionally reduced the ratio between total cholesterol and HDL cholesterol [\[47\]](#page-10-10). On the other hand, palmitic acid did not present associations with cardiovascular risk factors or with inflammatory markers, and stearic acid was neutral on blood lipids but was associated with biomarkers of inflammatory and endothelial dysfunction in individuals with cardiovascular risk [\[48\]](#page-10-11).

Although saturated fatty acids were found in higher amounts, in ascending order, in fermented cream cheese with cream, fermented cream cheese without cream, and commercial cream cheese, it was also observed that the concentration of unsaturated fatty acids, such as omega-3, was higher. Specifically, monounsaturated fatty acids, such as oleic acid ($C18:1 \omega$), were also found at elevated levels. In this sense, fermented cream cheeses with and without cream showed a profile of fatty acids that contribute to a healthier diet. The review by [\[49\]](#page-10-12) reports that oleic fatty acid is an antitumor agent in different types of cancer and mainly has antiproliferative action including the suppression of the migration and proliferation of breast cancer cells, as well as the stimulation of tumor suppressor genes. In some studies, a diet rich in oleic fatty acid has positive results in disorders related to inflammation and protects against metabolic syndrome and risk factors for cardiovascular diseases [\[50–](#page-10-13)[52\]](#page-10-14). Replacing 1% of lauric and palmitic acids with mono- or polyunsaturated fatty acids reduces the risk of coronary heart disease. This effect is associated with the impact of unsaturated plasma lipids that reduce the concentrations of total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) [\[53\]](#page-10-15). Omega 3 has been known to reduce the risk of a number of chronic diseases and provide potential health benefits to humans. Omega-3 fatty acids are associated with blood–brain barrier integrity in a healthy aging population [\[54\]](#page-10-16) and the treatment of depression [\[55\]](#page-10-17). Additionally, they have been shown to improve clinical symptoms in patients with COVID-19 [\[56\]](#page-10-18). Alpha linoleic fatty acid has anticancer, anti-inflammatory, and antioxidant properties; protects the nervous system; and improves memory and learning [\[57\]](#page-10-19).

3.5. Sensory Analysis

In the overall evaluation of product acceptance, the majority of the participants in the research, 71.7%, were female. The age group from 21 to 25 years (37%) was predominant, followed by the group under 20 years (36%). On average, 14% of the survey participants answered that they liked it slightly, 24% liked it moderately, 24% liked it very much, 18% loved it, and only 1 to 3% disliked the artisanal cream cheese with cream that was fermented with kefir grains. Regarding the questionnaire question "Would you buy this product?", 60% answered yes, 30% answered maybe, and 10% answered no. As for the question "For you this product resembles: () Cottage cheese; () Cream cheese; () Yogurt; ()", 44.17% answered that the product resembles yogurt, due to the acidity present in the product; 27.5% that it resembles a cream cheese, due to its firmer, less moist consistency; 25% found it similar to curd; and 3.33% chose none of the alternatives. In the overall evaluation of product acceptance, generally speaking, the artisanal cream cheese with cream that was fermented with kefir grains was well accepted by the young public, and 60% responded that they would buy the product.

4. Conclusions

Artisanal cream cheese, due to its high content of short-chain fatty acids, omega-3, and particularly oleic fatty acid, as well as its abundance of Lactobacilli and low lactose concentration, presents itself as a viable dietary choice for those seeking to improve their eating habits and maintain a healthy lifestyle.

The artisanal cream cheese fermented with kefir grains is a functional product with an innovative character compared to current products on the market and was well accepted by the younger audience.

Author Contributions: The authors' responsibilities were as follows: M.R.M. conceived the idea for the manuscript; E.S.K. collaborated with laboratory analyses; D.R.F. analyzed the data; M.R.M. and D.R.F. wrote the manuscript; M.R.M. supervised and revised the manuscript; M.R.M. had primary responsibility for the final content and approved subsequent drafts of the manuscript; and all authors read and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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