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Development of butter fermented with kefir: probiotic properties and sensory acceptance

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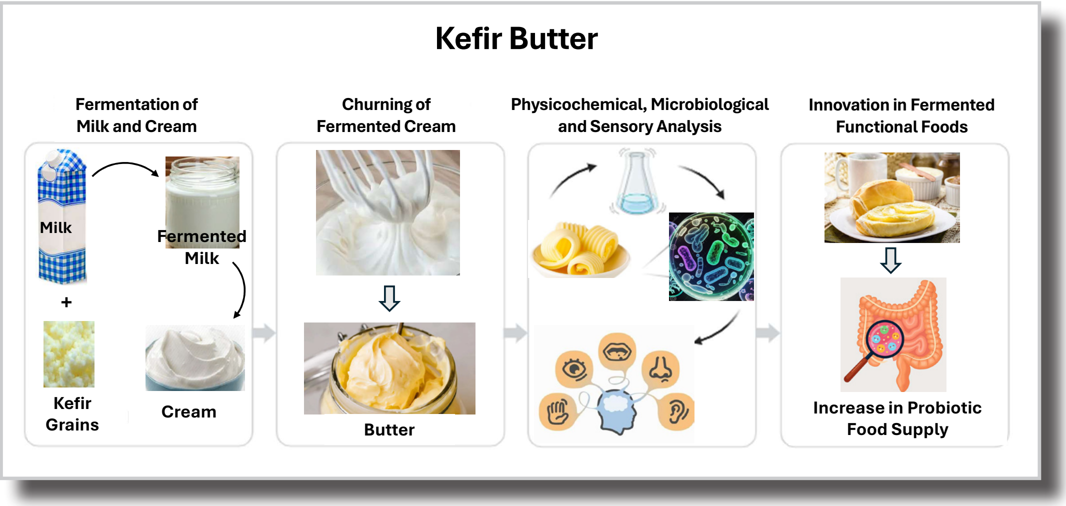
Highlights

- *Kefir butter synthesized after cream fermentation with kefir.*
- *The butter showed good acceptance and high counts of lactic acid bacteria and yeasts.*
- *The use of kefir as a fermenting culture represents a promising innovation.*
- *The technique enables the incorporation of viable probiotics into diverse food matrices.*
- *The use of kefir butter expands probiotic consumption options.*

Graphical abstract



Abstract

Kefir is a fermented milk product obtained through gelatinous grains containing probiotic microorganisms, known for millennia worldwide due to its numerous benefits. Fermented foods, such as kefir, have been widely recognized for their health-promoting properties, including anti-inflammatory, antioxidant, and antimicrobial activities, as well as their ability to modulate the gut microbiota—factors that contribute to the prevention of chronic diseases and the promotion of overall well-being. The aim of this study was to develop a butter with probiotic properties by fermenting cream with kefir and to evaluate its acceptance. After cream fermentation with kefir, butter was obtained through the churning process. Yield, moisture content, titratable acidity, lactic acid bacteria (LAB) count, and yeast count were assessed. Product acceptance was evaluated by sensory analysis using a 9-point hedonic scale and a 5-point purchase intent scale, and results were compared to a commercial butter brand considered as the standard. Sensory analysis was carried out for each butter sample (kefir and standard) on different days. The kefir butter showed higher moisture and acidity levels compared to the standard. The LAB and yeast counts were approximately 10^7 and 10^3 CFU/g, respectively. The kefir butter had good acceptance, achieving an overall score of 7.6 and a purchase intent score of 4.4. The butter obtained from cream fermented with kefir demonstrated good acceptance and a high LAB count, indicating functional and probiotic properties. However, improvements are needed to reduce moisture and acidity, which could enhance both shelf life and consumer acceptance. The use of kefir as a fermenting culture in dairy products, such as butter, represents a promising innovation, allowing the incorporation of viable probiotic microorganisms into diverse food matrices and expanding probiotic consumption options beyond traditional yogurts and fermented beverages.

Keywords: Acidity. Dairy. Fermented Food. Functional Food. Cream.

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INTRODUCTION

Fermented foods are known to enhance the nutritional value of products, mainly through the production of short-chain fatty acids (SCFAs), which have well-established beneficial effects on human health[1]. The modulation of the human immune system by fermented foods is expected to result from the combined effects of compounds present in the initial ingredients and those formed during fermentation, as well as from live and dead or inactivated microorganisms and their cellular components[2].

Kefir is a fermented beverage traditionally made from milk and kefir grains, which contain a symbiotic community of bacteria and yeasts with probiotic[3] and postbiotic properties through SCFA production[4] during the fermentation process. Due to its promising benefits, there is growing global interest in the nutritional potential of kefir, which may lead to increased commercialization and diversification of developed products[5]. The development of dairy products fermented with kefir grains represents an innovation in the market, offering functional options that are well accepted—especially among younger consumers—and that promote a healthy lifestyle[4]. The development of butter fermented with kefir is highly relevant in the current context, expanding market options for probiotic products.

Butter is a product derived from the churning of pasteurized cream, characterized as a water-in-oil emulsion. This process involves transforming the cream into butter grains and buttermilk through agitation and air incorporation, resulting in a structure where fat globules, fat crystals, water droplets, and air bubbles are dispersed[6–8]. There are different types of butter, such as sweet cream butter (with or without salt) and cultured butter (with or without salt), each with distinct sensory properties[7]. The production of butter involves the use of different starter cultures that influence its sensory and nutritional characteristics. Cultures such as *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Lactobacillus acidophilus* are commonly used to ferment cream before butter production, resulting in variations in the aroma profile and nutritional composition of the final product[9,10]. Cream fermentation is a crucial process that affects the acidity and fatty acid profile of butter. During fermentation, lactic acid bacteria such as *Lactobacillus helveticus* and other probiotic cultures are used to increase acidity and modify the fatty acid composition, resulting in butter with improved functional properties[11,12].

Considering the trend toward healthier lifestyles and the development of dairy products fermented with kefir[13] —and that the butter fermentation process uses bacteria present in kefir, such as *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Lactobacillus helveticus*[4] —this project proposes the development of butter obtained from cream fermented with kefir as a functional food and the evaluation of its quality through the determination of physicochemical, microbiological, and sensory characteristics.

MATERIALS AND METHODS

Kefir acquisition and artisanal butter production

The kefir grains used in this research have been cultivated by the research group since 2018. Occasionally, the grains are frozen at -20°C for later use. For the use of frozen grains, they were activated at 5% (w/w) in 1 L of UHT skimmed milk for 24 hours at 25°C ± 2. After this period, the grains were strained through a fine sieve and subjected to a new fermentation under the same conditions.

In previously sterilized glass jars with lids, three batches of butter were produced by fermenting 500 g of pasteurized cream from the brand Paulista®, containing 33% (w/w) fat, to which 10% of the kefir ferment (prepared as described above) was added, based on the weight of the cream. The mixture was fermented at room temperature (25°C ± 2) for 7 days and subsequently refrigerated until it reached 10°C before initiating the butter churning phase. The fermentation conditions were determined empirically, as there were no reports in the scientific literature at the time describing cream fermentation with kefir.

The churning process was carried out using a stand mixer at high speed until the formation of clumps and separation of the whey or buttermilk. A butter washing step followed, using cold water (10°C) in the proportion of 180 mL of water per 500 g of fermented cream. The butter was placed in a container and pressed with a spoon to remove excess water. Then, 2% salt was added relative to the final weight of the obtained butter. The process was artisanal, without a neutralization step and with only one washing step, in order to preserve the microorganisms and fermentation products considered beneficial in the butter composition. The produced butter was stored under refrigeration for the analyses proposed in this study.

Physicochemical and microbiological analyses

Moisture content was assessed using 1 g of the butter sample on a Shimadzu moisture analyzer (MOC 120H). Butter yield was calculated in relation to the mass of cream used, according to the following equation: Yield (%) = (FB × 100) / CC (Equation 1), where FB is the final mass of butter and CC is the mass of cream added. Titratable acidity was determined according to São Paulo[14] and expressed in mL of normal alkaline solution per 100 g of milk fat.

Initially, a small aliquot of the samples was melted in a water bath at 30°C to facilitate the breakdown of fat globules. Then, 50 mg of melted butter was weighed into a sterile plastic microtube using a sterile Pasteur pipette. To this mass, 450 µL of saline solution (0.8%) with 0.1% peptone at 42°C was added. The resulting 10^{-1} dilution was vortexed for 1 minute and serially diluted up to 10^{-4} using the aqueous phase. A 100 µL aliquot of each dilution was inoculated (spread plate technique) onto plates containing culture media.

Lactic acid bacteria (LAB) were counted on MRS agar (De Man, Rogosa, and Sharpe) under microaerophilic conditions (overlay technique) after incubation for 72 hours at 30°C. Yeast counts were conducted on Potato Dextrose Agar (PDA) in acidic medium (approximately 1.5 mL of 10% tartaric acid per 100 mL of medium, adjusted to pH 3.5), after incubation for 7 days at 30°C. Results were expressed in CFU/g.

Sensory analysis

A total of 750 g of kefir butter was produced for the sensory analysis. The fermented butter underwent affective acceptance sensory tests in comparison with a commercial salted butter used as the standard (Itambé®). The tasting panel consisted of 203 untrained individuals over 18 years old, including students and university staff. The volunteers were divided into two groups for conducting the sensory analysis. Evaluations of the kefir butter and the standard butter were conducted on different days with different assessors of similar profiles, so that each evaluation was absolute and free from direct comparison[15]. The sensory analysis of the kefir butter involved 101 assessors; however, two were excluded for reporting a dislike of butter, resulting in 99 effective participants. For the standard butter, 102 assessors participated, with one excluded for the same reason, totaling 101 effective participants. Volunteers who had smoked within the previous hour, had colds or flu symptoms, or had aversions to any ingredients in the formula were excluded, as these conditions could influence the final evaluation of the product[15]. Participants were invited to join the study through internal campus announcements and signed an informed consent form approved by the university's ethics committee (CAAE - 48483015.7.0000.0084).

Tastings were conducted in a designated tasting area with individual separation. Samples were served in clean, odorless, white containers, accompanied by a glass of water, which assessors were instructed to drink before tasting the sample, and a mini toast with a neutral flavor to avoid interference with the butter evaluation. A 9-point hedonic scale acceptance test was applied, anchored with "disliked extremely" and "liked extremely," to evaluate the following attributes: appearance, odor, flavor, texture, acidity, and overall rating. Additionally, participant data such as name, age, and gender were collected.

Results were tabulated using Microsoft Office Excel 2013® and presented using measures of central tendency (mean and mode), dispersion (standard deviation, SD), and frequency distribution charts. The acceptability index was calculated using the formula: mean score divided by the maximum possible score, multiplied by 100. Comparison of the results for kefir and standard butter was performed using the Student's t-test, with a significance level set at $p < 0.05$.

RESULTS

The results of the physicochemical analyses of kefir butter (obtained from the fermentation of cream with kefir) and its comparison with the standard butter are presented in Table 1. Table 2 shows the results of the microbiological analyses conducted for lactic acid bacteria and yeasts.

Table 1 - Results of the physicochemical analyses of kefir and commercial butter, presented as mean ± standard deviation from triplicate measurements, and comparison with the limits established by specific regulations. São Paulo, 2022.

Analyses	Kefir Butter	Commercial Butter	p	Limits[16]
Yield (%)	43.43±0.06	-	-	-
Moisture (%)	26.18±0.09	14.04±1.01	0.17	16
Acidity (mL/100g)	2.99±0.75	1.15±0.35	0.04	3

Table 2 - Microbial counts (lactic acid bacteria and yeasts) in kefir butter. São Paulo, 2022.

Microorganisms	Count (UFC/g)
Yeasts	4.15×10^3
Lactic acid bacteria	5.76×10^7

Table 3 presents the results of the sensory analysis of the butters. The majority of evaluators were women, accounting for 73.7% of the tasters of the kefir butter, with a mean age of 22.5 years. Among the tasters of the standard butter, 71.28% (n=72) were women, with a mean age of 20.8 years. It was found that 25% of the evaluators reported consuming butter daily and 27% reported consuming it three times a week, with an average consumption frequency of 13 times per month. The profile of characteristics among volunteers in both groups was similar.

Figure 1 presents the purchase intent results. Approximately 90% of the tasters indicated a likelihood of purchasing the product, selecting either "would certainly buy" or "would probably buy" as their response.

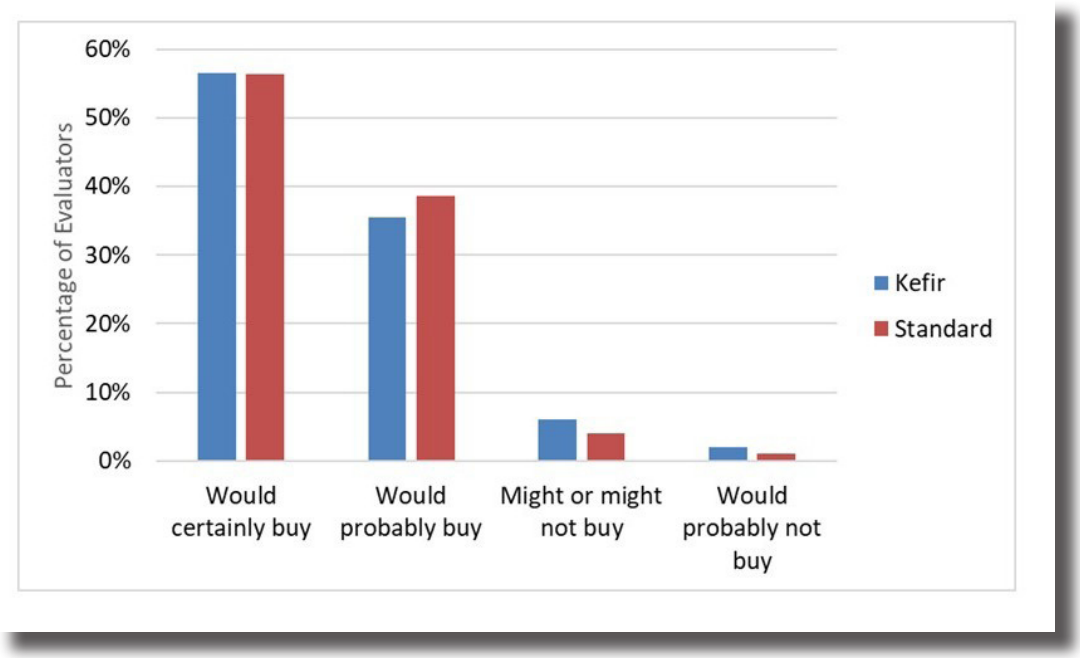

Figure 1 - Purchase Intent Chart for Kefir Butter Compared to Commercial Butter.

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Table 3 - Sensory analysis results for kefir and commercial butter, including mean ± standard deviation, Acceptability Index (AI%), mode, and p-value.

Attribute	Butter	Mean±SD	AI%	Mode	p-value
Color	Kefir	7.7±0.9	85.5	8	0.1327
	Commercial	7.9±1.3	88.1	7	
Shine	Kefir	8.1±4.9	90.5	8	0.0507
	Commercial	7.2±1.1	79.5	7	
Aroma	Kefir	6.9±1.7	75.4	7	0.0049
	Commercial	7.4±1.2*	82.1	7	
Texture	Kefir	7.8±1.2	86.3	8	0.1562
	Commercial	8.0±1.1	88.9	9	
Flavor	Kefir	7.6±1.4	84.4	8	0.005
	Commercial	8.1±1.0*	90.0	9	
Acidity	Kefir	7.1±1.4	79.4	7	0.1204
	Commercial	7.4±1.3	82.8	7	
Overall Acceptance	Kefir	7.6±1.3	84.1	8	0.0109
	Commercial	8.0±1.0*	88.8	8	

$p < 0.05$ indicates a significant difference in the comparison between the two butters.

DISCUSSION

This study aimed to develop butter fermented with kefir, endowed with probiotic and functional properties. The use of kefir as a fermenting culture in butter represents a promising innovation, as it enables the incorporation of viable probiotic microorganisms into diverse food matrices, expanding probiotic consumption options beyond traditional yogurts and fermented beverages[5,13].

The moisture content found in kefir butter was above the legal limit (Table 1). This is justified by the artisanal production process, carried out in small quantities and without specific equipment to improve the separation of fat from buttermilk and wash water. This also explains the high yield observed (Table 1), since the fat content of the cream used was 33%, and the difference can be attributed to the water incorporated into the butter. Elevated moisture promotes rancidity, which not only impairs taste but also affects product stability. Rancidity is a critical concern in butter preservation, as the product should remain stable and compact when refrigerated[8]. However, it is believed that the industrial malaxing process would be sufficient to maintain product moisture within legal limits[13].

The titratable acidity of kefir butter was two to three times higher than that of the standard butter but still within regulatory limits (Table 1). Fernandes[17] analyzed eight samples of commercial common butter and found that at least 50% had moisture and acidity levels above the recommended limits. The higher acidity found in kefir butter can be attributed to the activity of kefir microorganisms, which carry out lactic, acetic, and butyric fermentation[18]. Butter acidity is an important quality indicator and is influenced by the type of culture used and storage conditions. The introduction of fermenting cultures can help maintain acidity within desirable ranges, extending shelf life and improving organoleptic properties[13,19]. Acidity tends to increase during storage, especially in butters fermented with probiotic cultures, due to the continuous activity of lactic acid bacteria[13]. Silva *et al.*[13] developed various types of sheep milk butter fermented by different cultures, including kefir. The kefir-fermented sheep butter initially showed acidity around 4 mL/100 g, which gradually increased over time, reaching 7 mL/100 g. The lower acidity observed in that study is justified by the shorter fermentation time (24 hours), but it also resulted in lower lactic acid bacteria and yeast counts. Adjusting the fermentation time could help control the acidity of the product without compromising the LAB count. Although excessive acidity may negatively affect palatability, it can enhance health benefits through the production of SCFAs[1,6].

The average LAB count found in the kefir-fermented butter was on the order of 10^7 CFU/g, and for yeasts, 10^3 CFU/g (Table 2). According to the Technical Regulation on the Identity and Quality of Fermented Milks[20], kefir should contain LAB and yeast counts of 10^7 and 10^4 CFU/g, respectively. Although there is no legal standard established for kefir butter, the butter developed in this study showed a LAB count consistent with that of kefir-fermented milk. In the study by Silva *et al.*[13] , lower counts were obtained—10^6 for LAB and 10^4 CFU/g for yeasts—in sheep milk butter. However, that study used a pure culture containing kefir microorganisms (CHR Hansen®), rather than a ferment produced directly with kefir grains as in the present study. This demonstrates the greater efficiency of the traditional culture in maintaining higher LAB counts, even with the washing step used in the process, which likely reduced microbial levels.

Studies suggest that kefir may have beneficial effects on oral health, bone metabolism, weight control, lipid profile, gastric protection, glycemic control in type 2 diabetes, and cognitive improvement in Alzheimer's disease. However, the current evidence is still limited, and more high-quality research is needed to confirm these potential benefits[21,22]. The broad range of health benefits associated with regular kefir consumption is attributed to the diversity of microorganisms present in the grains, which include the genera *Lactobacillus (L. brevis*, *L. casei*, *L. kefiri*, *L. acidophilus*, *L. plantarum*, *L. kefiranofaciens subsp. kefiranofaciens*, *L. kefiranofaciens subsp. kefirgranum*, *L. parakefir*), *Lactococcus* (*L. lactis subsp. lactis*), *Leuconostoc (L. mesenteroides)*, *Acetobacter*, *Kluyveromyces (K. marxianus)*, and *Saccharomyces*[3]. The product developed in this study shows high health potential due to its functional characteristics, such as LAB count and acidity (Tables 1 and 2).

Additionally, the kefir butter was well accepted by tasters (Table 3), as evidenced by the Acceptability Index (AI%), which remained above 75%[15]. It is clear that the commercial butter was more favorably rated in terms of aroma, flavor, and overall acceptance (Table 3), although the butter developed in this study received only slightly lower scores than the marketed product. When examining the mode values, only for the flavor attribute did the commercial butter score one point higher than the kefir butter (Table 3). The aroma of the kefir butter was less preferred compared to the commercial butter (Table 3), likely due to the distinctive aroma of kefir resulting from the fermentation process, which imparts a slightly acidic and alcoholic note[23]. This unfamiliar aroma likely influenced tasters' preference toward the commercial butter in this attribute, ultimately affecting overall acceptance.

Some evaluators reported acidic flavor and aroma, as well as rancid odor in the kefir butter (Table 3). These observations may be explained by the high moisture content in the kefir butter (Table 1), which can favor rancidification reactions[8], in addition to the inherently acidic fermentation of kefir. The sensory quality of fermented products is influenced by the profile of SCFAs and free peptides[24]. Despite these remarks, the butter was considered well accepted in the other evaluated attributes. In the study by Clemente and Abreu[25] on butter-from-a-bottle (manteigas de garrafa), 30% of the samples presented oxidative rancidity, which did not alter the appearance of the products, and they were still well accepted by the sensory panel. Texture was a highly rated attribute for kefir butter, with tasters noting good spreadability and creaminess. In the study by Silva *et al.*[13] no significant differences were observed between the non-fermented and kefir-fermented formulations, whether in preference or ranking tests. In general, the products were well accepted by the participants.

Figure 1 presents the purchase intent results, reaffirming the good acceptance of the product developed in this study. More than 50% of the volunteers stated they would certainly buy the product, with no significant difference when compared to the commercial butter. Studies suggest that the most determining factors in food purchasing decisions are taste, price, and health benefits, with the relative importance of these factors varying according to demographic group and socioeconomic context[26–28]. Since no brands or prices were identified in this study, it can be stated that the purchase intent for the kefir butter is a result of the good acceptance demonstrated in Table 3, confirming once again the viability of the product in comparison to the commercial butter.

The artisanally developed kefir butter retained moisture above the recommended limit, but maintained acidity within the expected range (Table 1), showed good sensory acceptance (Table 3 and Figure 1), and potential functional properties, such as high SCFA content[4,13] and a good count of potentially probiotic microorganisms (Table 2). Although the microbiological count of the developed product is satisfactory, it is necessary to more specifically identify which probiotic species are present, assess their stability, and improve the physicochemical quality of the product by reducing moisture and acidity. These improvements could enhance both the shelf life and acceptance of the product.

Lower moisture and acidity contribute to greater oxidative stability, reduced risk of microbiological spoilage, and better sensory acceptance, in addition to extending product shelf life[29–31]. The development of new probiotic foods meets consumer demand for natural, safe products with proven health benefits, while also adding value to dairy by-products and potentially generating income for small-scale rural producers[5,13]. Therefore, research in this area is essential to expand the portfolio of functional foods available on the market and to reinforce the importance of probiotics in modern diets.

CONCLUSIONS

The butter obtained from the fermentation of cream with kefir proved to be a viable product for consumption, with a good count of lactic acid bacteria. Sensory analysis indicated good acceptance, despite slightly elevated acidity. The product still requires improvements to reduce moisture and acidity, as well as identification of probiotic species and bioactive compounds.

The use of kefir as a fermenting culture in dairy products such as butter represents a promising innovation, as it enables the incorporation of viable probiotic microorganisms into diverse food matrices, expanding probiotic consumption options beyond traditional yogurts and fermented beverages.

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CRediT author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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