

**Kefir-based Petit Suisse: new dessert flavored with nibs and cocoa
honey**

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**Petit Suisse à base de Kefir: nova sobremesa aromatizada com nibs e
mel de cacau**

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Aurélio Santos Agazzi

Mestre

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: aurelioagazzi@gmail.com

Biane Oliveira Philadelpho

Mestre

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: biane_philadelpho@hotmail.com

Clariane Teixeira Pessoa Mamona

Mestre

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: clarianepessoa@hotmail.com

Deise Azevedo Silva

Mestre

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: deiseazevedo.88@gmail.com

Lusiene Lima Rocha

Mestre

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: rocha.lusiene@gmail.com

Mariana Fernandes Almeida

Mestre

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: mari.fernandeas@hotmail.com

Thaís de Souza Santos

Mestre

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: taisinha.02@hotmail.com

Talita Andrade da Anunciação

Mestre

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: tali.anunciacao@hotmail.com

Carolina Oliveira de Souza

Ph.D

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: carolods@ufba.br

Karina Teixeira Magalhães-Guedes

Ph.D

Instituição: Universidade Federal da Bahia (UFBA)

Endereço: Rua Barão de Geremoabo, s/n, Ondina, CEP: 40171-970, Salvador - BA,
Brasil

E-mail: karynamagat@gmail.com

ABSTRACT

Health-conscious consumers are looking for functional foods to improve their body's well-being. Kefir-based foods provide improvements in quality of life. Kefir has a nutritional and probiotic function. This study aimed to elaborate on kefir-based Petit Suisse flavored with nibs and cocoa honey, as well as to evaluate the physicochemical and microbiological parameters and sensorial acceptance. The kefir-based Petit Suisse developed in this study proved to be a viable proposition as a food source. All items listed in the sensory analysis received positive ratings by the majority of the evaluating public, which made the product exceed the value established as a criterion for acceptance. Kefir-based Petit Suisse flavored with nibs and cocoa honey can be considered a promising product for the market with nutritional values and probiotic potential.

Keywords: nutritious dessert, fermented food, healthy food.

RESUMO

Consumidores preocupados com a saúde estão procurando alimentos funcionais para melhorar o bem-estar de seu corpo. Alimentos à base de kefir proporcionam melhorias na qualidade de vida. Kefir tem uma função nutricional e probiótica. O objetivo deste estudo foi elaborar uma Petit Suisse à base de kefir, aromatizado com nibs e mel de cacau, bem como avaliar os parâmetros físico-químicos, microbiológicos e aceitação sensorial. O Petit Suisse à base de kefir desenvolvido neste estudo provou ser uma proposta viável como fonte de alimento. Todos os itens listados na análise sensorial receberam avaliações positivas pela maioria do público avaliador, o que fez com que o produto superasse o valor estabelecido como critério de aceitação. Petit Suisse à base de kefir aromatizado com nibs e mel de cacau pode ser considerado um produto promissor para o mercado com valores nutricionais e potencial probiótico.

Palavras-chave: sobremesa nutritiva, alimentos fermentados, alimentação saudável.

1 INTRODUCTION

Health-conscious consumers are looking for functional foods to improve their body well-being. Kefir-based foods provide improvements in quality of life. Kefir has a nutritional and probiotic function. Kefir is fermented milk, slightly effervescent. Fermented kefir food is easy to prepare and low cost. Kefir has a natural probiotic microbiota present in kefir grains (Magalhães et. al., 2011; Enikeev, 2012; John and Deeseenthum, 2015; Bourrie et al., 2016; Amorim et al., 2019; Magalhães-Guedes et al., 2019; Magalhães-Guedes, 2020,2022). Kefir grains are a symbiotic association of yeasts, lactic acid bacteria, and acetic acid bacteria present in the kefir polysaccharide matrix. Kefir grains have sizes between 0.5-3.5 cm in diameter, irregular shape, yellowish or whitish (Magalhães et. al., 2011; Enikeev, 2012; John and Deeseenthum, 2015; Bourrie et al., 2016; Magalhães-Guedes et al., 2019; Magalhães-Guedes, 2020, 2022).

The microbial composition of kefir grains varies according to the region of origin and the substrate used for the proliferation of grains and the techniques used in their manufacture (Wszolek et al., 2001; Witthuhn et al., 2004). Kefir grains are capable of fermenting on various substrates, such as cow's milk, goat, sheep, buffalo, brown sugar, fruit juices, and soy extract, among others (Magalhães et. al., 2011; Puerari et al., 2012; Magalhães-Guedes et al., 2018; Tavares et al., 2020; Scheffel et al., 2021; Da Silva et al., 2022).

The aim of this study was to elaborate on kefir-based Petit Suisse flavored with nibs and cocoa honey, as well as to evaluate the physicochemical and microbiological parameters and sensorial acceptance. The kefir grains microorganisms used in this study

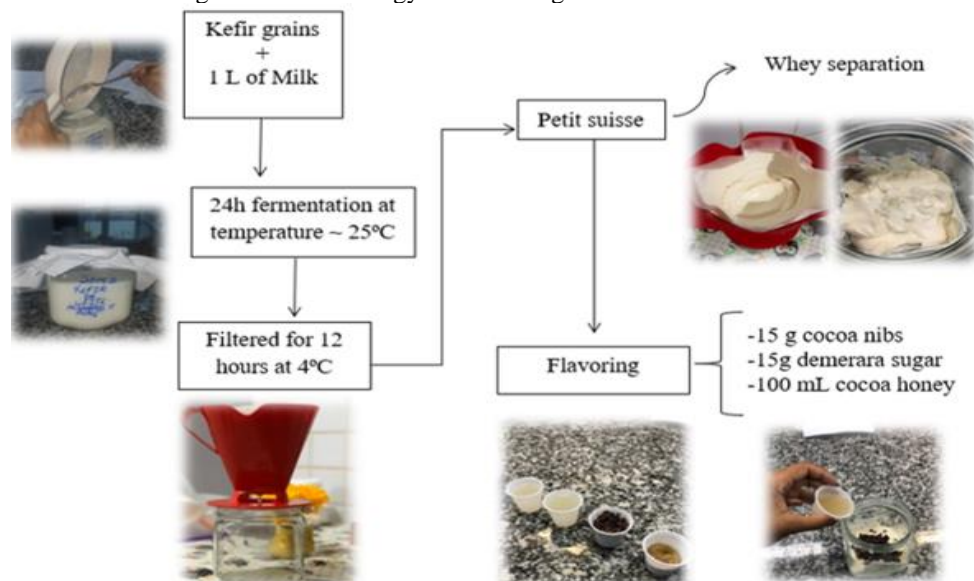
were previously identified according to phenotypic methods by Magalhães-Guedes et al. (2020). The microbial species identified were: *Lactobacillus paracasei* (accession number - AB368902.1), *L. kefir* (accession number - AB3626680.1), *L. parabuchneri* (accession number - AB368914.1), *L. casei* (accession number - EU626005.1), *L. paracasei* subsp. *paracasei* (accession number - NR025880.1), *L. paracasei* subsp. *tolerans* (accession number - AB181950.1), *L. buchneri* (accession number - FJ867641.1), *Lactococcus lactis* (accession number - EU194346.1), *Leuconostoc citreum* (accession number - FJ378896.1), *Acetobacter lovaniensis* (accession number - AB308060.1), *Kluyveromyces lactis* (accession number - AJ229069.1), *Saccharomyces cerevisiae* (accession number - EU649673.1), *Kazachstania aerobia* (accession number - AY582126.1), and *Lachancea meyersii* (accession number - AY645661.1).

2 MATERIAL E MÉTODOS

2.1 KEFIR-BASED PETIT SUISSE PREPARATION

Figure 1 shows the preparation of kefir-based Petit Suisse. Kefir grains (10%) were added to 1 liter of UHT whole milk. The fermentation process at room temperature (~25°C) for 24 h. After this period, the kefir grains were collected, and the fermented product was filtered on filter paper for 12 h at 4°C; the serum obtained from the filtration was discarded and the kefir-based Petit Suisse was homogenized, flavored with 15 g of nibs, 15 g of demerara sugar and 100 mL of cocoa honey. Subsequently, it was molded and stored in refrigeration for conservation of the product until the moment of analysis.

Figure 1. Methodology for obtaining kefir-based Petit Suisse



2.2 CHEMICAL PHYSICAL ANALYSIS

2.2.1 Acidity and pH

The acidity of the samples was obtained by the titrimetric method, which is based on neutralization with 0.1N NaOH solution and 1% phenolphthalein. Approximately 5 g of the Petit Suisse sample produced and industrialized natural yogurt were weighed in an Erlenmeyer flask and diluted with 50 mL of distilled water. Subsequently, 3 drops of phenolphthalein solution (1%) were added and titrated with a 0.1 M sodium hydroxide solution, using a 25 mL burette, until the appearance of pink color (expressed as a percentage of lactic acid). The pH of the samples was obtained using a calibrated digital pH meter (Digimed, DM-23) (IAL, 2008).

2.2.2 °Brix

The °Brix was determined when approximately 5 g of the sample were weighed in an Erlenmeyer flask and diluted with 50 mL of distilled water and then read in the Abbe refractometer (Master-Atago) at room temperature, according to the 932.12 AOAC method (AOAC, 2012).

2.3 MICROBIOLOGICAL EVALUATION

2.3.1 Homogenization and serial dilution

25g of each sample, produced Petit Suisse and industrialized natural yogurt were collected and for the first serial dilution, 25g of the sample were transferred to 225mL of the Peptone Water diluent, at 0.1% and then homogenized (dilution 10^{-1}). From this first dilution, 1 ml was transferred to a test tube containing 9 ml of 0.1% Peptone Water. Dilutions were made from 10^{-1} to 10^{-8} .

a) Molds and yeasts

For each of the samples, a total count of molds and yeasts was performed, according to the methodology described by APHA (1992). 0.1 ml of the 10^{-6} , 10^{-7} , 10^{-8} decimal dilutions of each sample was inoculated in duplicates onto the surface of Petri dishes containing DRBC Agar. The inocula were spread with the aid of a Drigalski loop on the surface. After complete absorption, the plates were incubated in an oven at 22–25°C for five days (Magalhães-Guedes et al., 2020).

a) Lactic acid bacteria

After the decimal dilutions, three dilutions of each sample were selected (10^{-6} , 10^{-7} , 10^{-8}) and a 1mL aliquot of each dilution was poured into a Petri dish containing Mann,

Roggosa and Sharpe agar medium (MRS). The inocula were spread with the aid of a Drigalski loop on the surface. After complete absorption, the plates were incubated in an oven for 48 h at 37 °C under aerobic conditions (Magalhães-Guedes et al., 2020).

a) Acetic acid bacteria

Three dilutions of each sample were selected (10^{-6} , 10^{-7} , 10^{-8}) and a 1mL aliquot of each dilution was poured into a Petri dish containing Glucose Yeast Maltose (GYM) agar. The inocula were spread with the aid of a Drigalski loop on the surface. After complete absorption, the plates were incubated in an oven for 48 h at 37 °C (Magalhães-Guedes et al., 2020).

2.4 SENSORY ANALYSIS

The acceptability analysis was performed with 20 untrained evaluators of both sexes, aged between 18 and 50 years, not allergic to cow's milk protein and/or severe lactose intolerance, randomly and voluntarily recruited from the premises of the Faculty of Pharmacy of the Federal University of Bahia (UFBA), through invitations made through the students themselves.

The minimum number of evaluators was predicted according to sample calculation. The evaluators answered a questionnaire about the frequency of use of yogurts as well as their knowledge about them. The samples were offered in white disposable cups, coded with random numbers 1 (Kefir-based Petit Suisse) and 2 (Natural yogurt). Approximately 10 grams of each product formulation was provided to each evaluator, along with the sensory evaluation sheets and a glass of water for cleaning the taste buds.

The form had a hedonic scale, ranging from 1.0 (“Dislike extremely”) to 9.0 (“Like extremely”), to assess the attributes: appearance, color, texture, taste, odor, and global acceptance. Both commercial natural yogurt and Kefir-based Petit Suisse were also evaluated for the evaluators' purchase intent. Each evaluator received the form with a 5.0-point scale, ranging from 1.0 (“I would certainly not buy it”) to 5.0 (“I would certainly buy it”) to analyze each sample. The sensory analysis project was approved by the Research Ethics Committee of the Nutrition School of the Federal University of Bahia, number 1.759.169.

2.5 STATISTICAL ANALYSIS

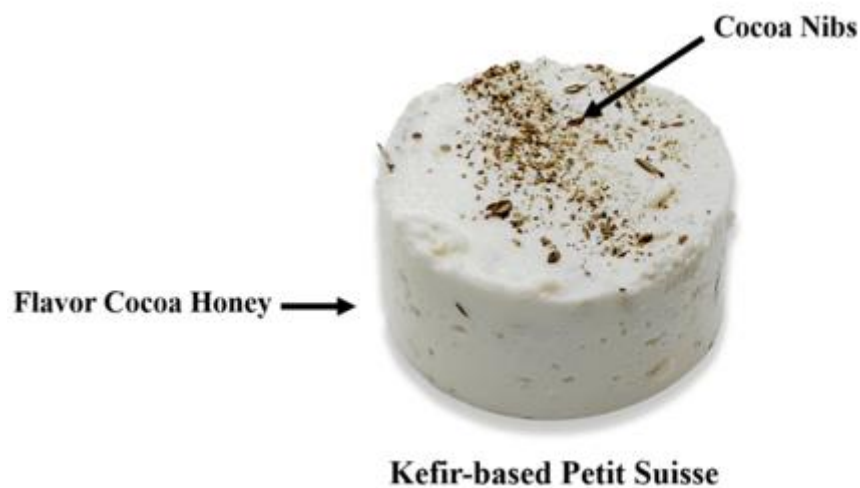
The data obtained were submitted to analysis of variance (ANOVA). To assess the difference between treatments, Tukey's test was used for multiple comparisons, adopting a significance level of 5%, using SigmaStat 3.5 software.

3 RESULTS AND DISCUSSION

3.1 CHEMICAL PHYSICAL ANALYSIS

The kefir-based Petit Suisse flavored with honey and cocoa nibs, produced in this study can be seen in Figure 2.

Figure 2. Kefir-based Petit Suisse flavored with honey and cocoa nibs.



The results of the physical-chemical analysis evaluated in this study of the Kefir-based Petit Suisse samples, industrialized natural yogurt, and cocoa honey, are shown in Table 1. Data were expressed as mean \pm standard deviation.

Table 1. Results of the physical-chemical analysis of Kefir-based Petit Suisse samples and industrialized natural yogurt.

Samples	Acidity	pH	°Brix
Kefir-based Petit Suisse *	2.32 \pm 0.18 ^b	3.78 \pm 0.02 ^b	35+
Natural yogurt *	1.61 \pm 0.06 ^c	4.17 \pm 0.05 ^a	35+
Cocoa honey *	9.75 \pm 0.05 ^a	3.49 \pm 0.01 ^c	19.23 \pm 0,03

*Acidity (% lactic acid); Titratable acidity (%).

The acidity and pH values showed statistical differences between the samples ($p < 0.05$) since they come from different production processes (Table 1). However, for the

acidity analysis, the values varied from 9.75% for the cocoa honey sample and 1.61% for lactic acid for the natural yogurt samples. Acidity is also a determining variable in the time taken until the formation of a stable clot, as well as in the flavor of the final product (Magalhães et. al., 2011; Puerari et al., 2012; Magalhães-Guedes et al., 2018; Magalhães-Guedes et al., 2020).

We observed that the kefir-based Petit Suisse product has high acidity values, however, it is not more acidic than commercially available natural yogurt. This lower value may be related to Petit Suisse-based kefir flavored with cocoa honey, which has more alkaline characteristics (Wang et al., 2017). Possibly the cocoa honey neutralized the content of acids in the product, reducing the percentage of lactic acid when compared to natural yogurt. The Ministry of Agriculture, Livestock and Supply – MAPA, establishes values between acidity for kefir between 0.5 and 1.5% of lactic acid (Brasil, 2000). Therefore, kefir-based Petit Suisse presented acidity slightly higher than the maximum recommended by MAPA. It is worth mentioning that, during the fermentation process, the population of lactic acid bacteria also increases, consequently increasing the acidity content. The presence of lactic acid is of great importance for providing a pleasant and characteristic flavor to kefir-based drinks as well as inhibiting the development of undesirable microorganisms and/or pathogens, as observed in studies by Magalhães et al. (2011).

The pH values of the samples ranged from 4.17 for the natural yogurt sample and 3.78 for the kefir-based Petit Suisse sample, similar results were described by Magalhães et al. (2011) in milk kefir beverage, corroborating the values found for the present study.

The kefir-based Petit Suisse was sweetened with cocoa honey and sugar and the natural yogurt sample was sweetened through industrialization standards (not specified on the packaging). According to the information above, the natural yogurt sample has a higher °Brix than cocoa honey. Even with these differences, it was possible to obtain the same °Brix for the natural yogurt sample and for the Petit Suisse kefir-based sample (Magalhães et. al., 2011; Puerari et al., 2012; Magalhães-Guedes et al., 2018).

It can be concluded that according to the analyses, cocoa honey has 14°Brix and kefir-based Petit Suisse sweetened with honey resulted in a Brix of ~35°Brix. With this, it was possible to observe the difference in taste and sweetness. Suggestions for future studies would be to analyze the °Brix of kefir without cocoa honey and use a sample of skimmed yogurt without added sugar, for further comparisons and results.

3.2 MICROBIOLOGICAL EVALUATION

The results obtained through the microbiological analysis of kefir-based Petit Suisse and industrialized natural yogurt can be seen in Table 2.

Table 2. Results of microbiological analysis of kefir-based Petit Suisse and industrialized natural yogurt.

Analysis	Samples	
	kefir-based Petit Suisse	Natural yogurt
Lactic acid bacteria (CFU/mL)	$>3 \times 10^8$	2.6×10^8
Molds and yeasts (UFC/mL)	$>2.5 \times 10^8$	-
Acetic acid bacteria (CFU/mL)	$>3 \times 10^8$	$>3 \times 10^8$

CFU – colony-forming unit

The two samples analyzed, kefir-based Petit Suisse and natural industrialized yogurt, showed counts of molds and yeasts and lactic acid bacteria according to the values established by the Technical Regulation on Identity and Quality of Fermented Milk (ANVISA), and the values for kefir count they are $\geq 10^7$ and $\geq 10^4$ CFU/mL, respectively, and for yogurt $\geq 10^7$ and zero CFU/mL, respectively. The results obtained in the counting of these microorganisms are similar to the results found in the literature (Leite et al., 2013; Cho et al., 2018). Leite et al. (2013) and Cho et al. (2018) state that the count of lactic acid bacteria and yeast is of great importance in kefir, considering that Brazilian legislation stipulates minimum counts of these microorganisms for this product.

In general, lactic acid bacteria are more numerous (10^8 - 10^9 CFU/g) than yeast (10^5 - 10^6 CFU/g) and acetic acid bacteria (10^5 - 10^6 CFU/g) in kefir grains. However, fermentation conditions can affect this pattern (Magalhães et al., 2011; Leite et al., 2013; Cho et al., 2018; Magalhães-Guedes et al., 2020). The results of the analysis of acetic acid bacteria, both in the kefir-based Petit Suisse sample and in the yogurt sample, showed values above 10^8 CFU/mL, which may indicate the influence of fermentation conditions. Acetic fermentation corresponds to the transformation of alcohol into acetic acid by certain bacteria, giving the characteristic taste of vinegar (Viana et al., 2017). The milk fermentation process takes approximately 24 hours, during which time homofermentative streptococci grow rapidly, initially causing a drop in pH. This low pH favors the growth of lactobacilli but causes a decline in the number of streptococci (Viana et al., 2017). The type of milk substrate together with the fermentation temperature (21-23°C) can promote the growth of aroma-producing heterofermentative streptococci (Magalhães et al., 2011; Magalhães-Guedes et al., 2018). As a result of fermentation, the growth of lactic acid bacteria is favored over the growth of yeast and acetic acid bacteria (Magalhães et al., 2011; Puerari et al., 2012; Magalhães-Guedes et al., 2018; Magalhães-Guedes et al.,

2020). The organic acids, lactic and acetic, resulting from the catabolism of carbohydrates contribute to the decrease in pH, making the environment hostile for most microorganisms. These substances and bacteriocins have been blamed for their antimicrobial properties (Magalhães et. al., 2011; Puerari et al., 2012; Magalhães-Guedes et al., 2018).

The microbiological and chemical compositions indicate that kefir-based Petit Suisse can be considered a product with potential probiotic characteristics, that is, it has in its composition live microorganisms capable of improving the intestinal microbial balance, producing beneficial effects on the health of the individual (Magalhães et. al., 2011; Puerari et al., 2012; Lu et al., 2014; Magalhães-Guedes et al., 2018).

There was no growth of molds and yeasts in commercial yogurt, only in the sample of kefir-based Petit Suisse, e there was the growth of these microorganisms (molds and yeasts).

3.3 SENSORY ANALYSIS

Table 3 shows the results of the sensory analysis of natural yogurt and kefir-based Petit Suisse. For the appearance attribute, there was a statistically significant difference ($p \leq 0.05$) in relation to samples 1 and 2, the lowest grade was assigned to treatment 2. This grade is equivalent to “Like moderately”, while the highest grade was assigned to treatment 2, which means “Like extremely”.

Regarding the texture attribute, natural yogurt had a better score compared to kefir-based Petit Suisse. This is justified by the fact that the kefir-based Petit Suisse resembled a standard Minas cheese. For the attributes of color, flavor, odor, and global acceptance there was no statistically significant difference ($p > 0.05$) between treatments. In this study, the use of cocoa honey and cocoa nibs did not negatively affect the taste of the samples, since in the sensory analysis the flavor criterion obtained scores above 6.0 for all treatments.

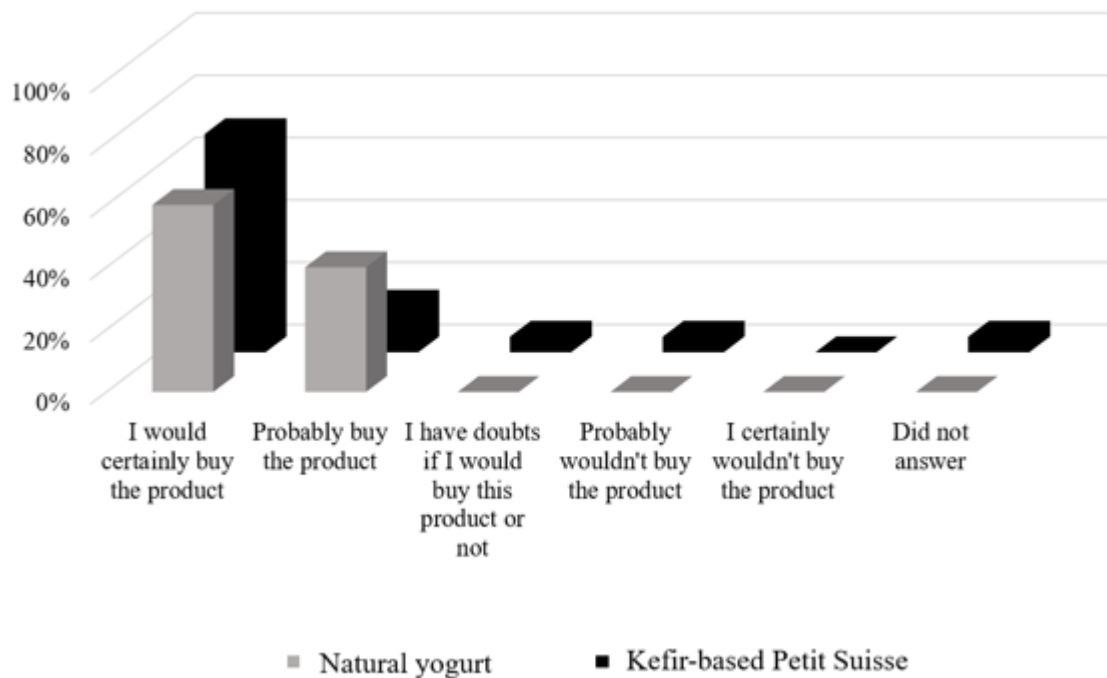
Table 3. Sensory attributes of acceptance of natural yogurt and kefir-based Petit Suisse.

Description of the hedonic scale	Hedonic scale	Frequency of responses									
		Natural yogurt					kefir-based Petit Suisse				
		Appearance	Aroma	Flavor	Texture	Overall impression	Appearance	Aroma	Flavor	Texture	Overall impression
Like extremely	9	11	8	15	11	7	5	5	2	3	1
Like very much	8	7	8	5	9	12	7	4	0	7	10
Like moderately	7	1	2	0	0	1	7	6	9	6	2
Like slightly	6	1	0	0	0	0	1	1	3	2	4
Neither like / nor dislike	5	0	2	0	0	0	0	2	1	2	2
Dislike slightly	4	0	0	0	0	0	0	1	4	0	1
Dislike moderately	3	0	0	0	0	0	0	0	0	0	0
Dislike very much	2	0	0	0	0	0	0	0	0	0	0
Dislike extremely	1	0	0	0	0	0	0	1	1	0	0
Total responses		20	20	20	20	20	20	20	20	20	20
Average		8.4 ^a	8.0 ^a	8.75 ^a	8.55 ^a	8.30 ^a	7.8 ^b	7.0 ^a	6.05 ^b	7.35 ^b	7.05 ^b
Standard deviation		0.82	1.21	0.44	0.51	0.57	0.89	2.03	1.90	1.18	1.36
“Dislike moderately” Responses		0%	0%	0%	0%	0%	0%	10%	25%	0%	5%

Conditions marked with different letters present a statistically significant difference with 95% confidence ($p < 0.05$). Each parameter was compared between samples.

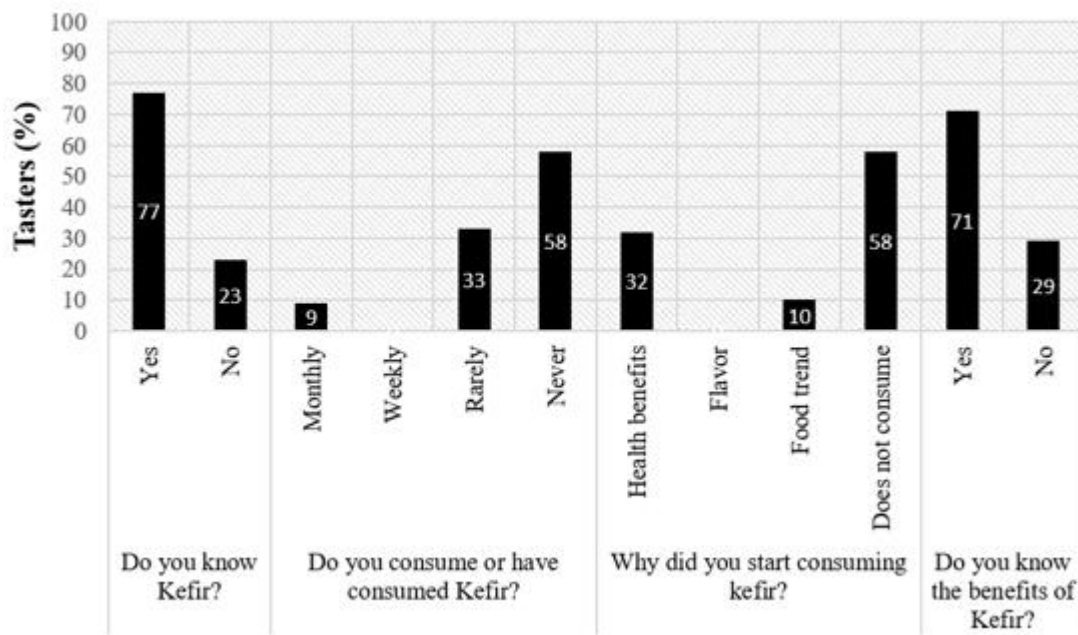
In purchase intent, a significant statistical difference was observed ($p > 0.05$) and the highest score, above 80%, is according to the scale equivalent to “I would certainly buy natural yogurt”, and approximately 50% “I would certainly buy kefir-based Petit Suisse”, despite the relatively high scores in the sensory attributes, the purchase intention was average (Figure 3). According to Puerari et al. (2012) and Magalhães-Guedes et al. (2018), sensory analysis constitutes an important and effective means to better understand the consumer's opinion and purchase intention in relation to a new product. It is of great importance to verify the intention to purchase the product by the panel of evaluators. Scheffel et al. (2021), describe that sensory evaluation and purchase intention can bring more robustness to the data, as well as provide new perspectives of a differentiated product for a traditional market such as dairy products.

Figure 3. Consumer purchase intention of natural yogurt and kefir-based Petit Suisse.



In Figure 4, it is shown that 77% of respondents know Kefir. Among those interviewed, 42% of individuals have already consumed kefir (weekly/rarely), while 58% have never consumed it. The majority of kefir consumers claimed to consume it for health benefits followed by dietary trends. Various authors believe that the increase in kefir consumption seems to be linked to the recognition of its benefits for human health (Magalhães et al., 2011; Enikeev, 2012; Leite et al., 2013; John and Deeseenthum, 2015; Bourrie et al., 2016; Viana et al., 2017; Magalhães-Guedes et al., 2019; Tavares et al., 2020; Magalhães-Guedes, 2020, 2022).

Figure 4. Percentage of respondents who know kefir.



4 CONCLUSION

kefir-based Petit Suisse flavored with nibs and cocoa honey, developed in this study, proved to be a viable proposition as a healthy food source. All items listed in the sensory analysis received positive evaluations by the majority of the evaluating public, which made the product exceed the value established as a criterion for acceptance, classifying the product as acceptable and pleasant. kefir-based Petit Suisse can be considered a promising product for the market with nutritional values and probiotic potential, in addition to being the first scientific study to develop this product.

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