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Physicochemical characterization and antibacterial activity of Brazilian artisanal milk kefir

Caracterização físico-química e atividade antibacteriana do kefir de leite artesanal brasileiro

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Milk kefir has an acidic taste and creamy consistency and results from the fermentation of milk by the microorganisms present in kefir grains. Milk kefir is known worldwide for its health benefits attributed to various bioactive compounds, including organic acids, bioactive peptides, and bacteriocins. This study evaluated the physicochemical composition of Brazilian artisanal milk kefir samples and studied the antibacterial activity of kefir beverage and of microorganisms isolated from kefir against potentially pathogenic bacteria. During the 24 and 48 h kefir fermentation, reducing sugars and pH decreased and lactic acid concentration increased. Kefir fermented in 48 h completely inhibited the bacterial growth of *Staphylococcus* aureus, *Salmonella enterica* and *Escherichia coli*. Of the 29 microorganisms isolated from kefir, 10 strains (34.5%) showed inhibitory activity against *S. aureus*, *Bacillus cereus*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *S. enterica* and *E. coli*. These strains showed inhibition zones of 12-40 mm against potentially pathogenic bacteria tested. Based on the antagonist capacity of the microorganisms isolated from milk kefir, these strains have potential to be used as food preservatives and as probiotic agents.
Keywords: fermented milk, antibacterial activity, antagonist activity.

O kefir de leite tem sabor ácido e consistência cremosa e resulta da fermentação do leite pelos microrganismos presentes nos grãos de kefir. O kefir de leite é conhecido mundialmente por seus benefícios à saúde atribuídos a vários compostos bioativos, incluindo ácidos orgânicos, peptídeos bioativos e bacteriocinas. Este estudo avaliou a composição físico-química de amostras de kefir de leite artesanal brasileiro e estudou a atividade antibacteriana da bebida kefir e dos microrganismos isolados do kefir contra bactérias potencialmente patogênicas. Durante a fermentação do kefir em 24 e 48 h, houve diminuição dos açúcares redutores e do pH, e aumento da concentração de ácido lático. O kefir fermentado em 48 h inibiu completamente o crescimento bacteriano de *Staphylococcus aureus*, *Salmonella enterica* e *Escherichia coli*. Do total de 29 microrganismos isolados do kefir, 10 cepas (34,5%) apresentaram atividade inibitória contra *S. aureus*, *Bacillus cereus*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *S. enterica* e *E. coli*. Estas cepas apresentaram zonas de inibição de 12-40 mm contra as bactérias potencialmente patogênicas testadas. Com base na capacidade antagonista dos microrganismos isolados do kefir de leite, essas cepas têm potencial para serem utilizadas como conservantes de alimentos e como agentes probióticos.
Palavras-chave: leite fermentado, atividade antibacteriana, atividade antagonista.

1. INTRODUCTION

The concern with a healthier lifestyle has been increasing in recent years, causing consumers to change their eating habits, prioritizing consuming foods that confer health benefits. Among these foods, milk kefir (a fermented beverage containing probiotic microorganisms) stands out, due to its effect on the modulation of the intestinal microbiota and other therapeutic effects [1, 2].

Milk kefir has an acidic taste and creamy consistency (like a yogurt), resulting from the fermentation of milk by the microorganisms present in kefir grains. Milk kefir grains are described as small granules (with a diameter of 30 to 40 mm), irregular in shape and with a yellowish-white color. Kefir grains comprise a matrix of polysaccharides combined with casein and a microbial community consisting of lactic acid bacteria, acetic acid bacteria and yeasts. These microorganisms work symbiotically in the biosynthesis of polysaccharides, increasing grains volume [3-5].

Numerous studies have shown that regular consumption of milk kefir is associated with several health benefits (antihypertensive, hypocholesterolemic, antidiabetic, anticarcinogenic, anti-inflammatory, antioxidant, antiallergic and immunomodulatory). These beneficial health properties are attributed to various bioactive compounds produced by microorganisms during kefir fermentation, including organic acids, bioactive peptides, and bacteriocins [1, 2, 5, 6].

Microorganisms isolated from milk kefir demonstrated *in vivo* antimicrobial activity against enteropathogenic bacteria, and among the suggested mechanisms are the ability of probiotic microorganisms to adhere to the intestinal epithelium, preventing the adhesion of pathogens, and their immunomodulatory properties. The antimicrobial activity of milk kefir was also attributed to the presence of exopolysaccharides (especially kefiran), organic acids (mainly lactic acid) and bioactive peptides with antimicrobial activity [1, 6].

The kefir production process is divided into artisanal and industrial methods. In the artisanal approach, kefir grains (5-10%) representing the starter culture are added to the milk and left to ferment for 24-72 hours at room temperature. After incubation, the kefir grains are separated from the fermented product and can be used for the subsequent fermentation. In the industrial method, kefir is made by fermenting milk with pure commercial kefir cultures, which leads to a more standardized product [2, 6, 7].

In Brazil, the production of milk kefir is still predominantly artisanal. Still, in countries such as Russia, the United States, Canada and France, there is already production on an industrial scale. The physicochemical characteristics and microbial composition of artisanal kefir depend on variables such as the geographic origin of the kefir grains, type of milk (cow, sheep, goat), fermentation conditions (time and temperature) and the volume of inoculated grains in milk, which interfere with the concentrations of metabolites produced by microorganisms in the beverage [5, 8].

Therefore, this study aimed to characterize the physicochemical composition of Brazilian artisanal milk kefir and study the antibacterial activity of kefir beverage and microorganisms isolated from kefir against potentially pathogenic bacteria.

2. MATERIALS AND METHODS

2.1 Artisanal milk kefir preparation

The two samples of kefir grains (kefir 1 and kefir 2) were collected from private households in Brazil. For the elaboration of milk kefir, 50 g of kefir grains were inoculated into 1000 mL of sterilized whole cow's milk (5% w/v) and incubated at 28°C for 24 and 48 h. After fermentation, the kefir was separated from the kefir grains with a sterilized stainless-steel sieve. Physicochemical and microbiological analyses were performed on kefir.

2.2 Physicochemical characterization of milk kefir

The lactic acid concentration was determined through the titratable acidity [9]; the pH was measured using a calibrated digital pHmeter Gehaka model PG 1800 [10]. The determination of reducing sugars was performed by the ADNS method [11]. Analyzes were performed in three repetitions, and results were expressed as mean values and standard deviation.

2.3 Bacterial strains used in antimicrobial activity tests

The following bacterial strains were used: Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, *Streptococcus mutans* ATCC 25175); and Gram-negative bacteria (*Klebsiella pneumoniae* ATCC BAA-1706, *Salmonella enterica* ATCC 14028, *Escherichia coli* ATCC 25922). To prepare the bacterial inoculum, a direct suspension of microbial growth in Mueller Hinton broth with turbidity was adjusted between 0.08 and 0.10

optical density in a spectrophotometer at 625 nm, which is equivalent to 0.5 McFarland standard (1.0×10^{8} CFU /mL) [12].

2.4 Antimicrobial activity of milk kefir during the fermentation process

The antimicrobial activity was studied during the kefir fermentation process against the bacteria *S. aureus*, *S. enterica* and *E. coli*, according to the methodology described by Dias et al. (2012) [13]. The bacterial inoculum (1 mL) was incubated with the kefir samples (in autoclaved glass bottles, 100 mL of milk and 5 grams of kefir grains were added, that is, 5% w/v), in triplicate, and incubated at 35°C for 24 and 48 h. After incubation, serial dilutions (10^{-1} to 10^{-4}) were performed, adding 25 mL of kefir in 225 mL of 0.1% (w/v) peptone water solution (10^{-1}). Then, successive serial dilutions were made with 1 mL of the previous dilution in a tube containing 9 mL of 0.1% (w/v) peptone water solution. Then, 0.1 mL of each dilution was inoculated in selective media plates (Salt Mannitol Agar for *S. aureus* and Mac Conkey Agar for *S. enterica* and *E. coli*), to verify the antimicrobial action during the kefir fermentation process against *S. aureus*, *S. enterica* and *E. coli*. The selective media prevent the growth of the kefir microbiota. The bacterial inoculum (1 mL) of *S. aureus, S. enterica* and *E. coli* was incubated for the positive control with 100 mL of milk.

2.5 Enumeration and isolation of microorganisms from milk kefir

Ten mL of each kefir sample were homogenized in 90 mL of 0.1% (w/v) peptone water solution, and then successive serial decimal dilutions were made with 1 mL of the previous dilution in a tube containing 9 mL of 0.1% (w/v) peptone water solution. Then, 0.1 mL of each dilution was inoculated in triplicate by surface spreading on specific solid media. Total number of viable microorganisms was determined on Man Rogosa Sharpe agar (MRS, Merck) by incubation at 30°C for 24 to 48 h. The counts were expressed as the decimal logarithms of the colony-forming units per gram (log CFU/g). For the isolation of microorganisms from kefir, MRS was used for lactic acid bacteria, Hestrin-Schamm agar (HS) (2% glucose, 2% agar, 0.5% yeast extract, 0.5% peptone, 0.115% citric acid, 0.27% dibasic sodium phosphate anhydrous, pH 5.0) for acetic acid bacteria and yeast extract glucose chloramphenicol agar (YE, Merck) for yeast. After incubation at 25°C for 72 h, representative colonies of different morphologies were removed and purified in the same medium by subculturing [14].

2.6 Preservation of microorganisms isolated from milk kefir

Pure colonies of microorganisms isolated from kefir were transferred to tubes containing 20 mL of sterilized whole milk, incubated at 25°C for 72 h and frozen at -80°C. For the activation of pure cultures, MRS broth was used for lactic acid bacteria, HS broth for acetic acid bacteria and YE broth for yeasts. The frozen bacteria tubes were incubated at 30°C for 24 h. To activate the isolated microorganisms, 1 mL of pure cultures were pipetted into tubes with 9 mL of the appropriate broth (MRS, HS or YE). After homogenization, the tubes were incubated for 72 h at 25°C.

2.7 Antimicrobial activity of microorganisms isolated from milk kefir

The antimicrobial activity of microorganisms isolated from kefir samples was performed according to the methodology described by Jacobsen et al. (1999) [15], with adaptations. The microorganisms isolated from kefir samples were inoculated with a sterile calibrated loop of 1 µL in the center of the plate containing the appropriate culture medium (MRS, HS or YE). Plates were incubated at 25°C for 72 h to develop the spots. The volume of 50 µL of each bacterial inoculum (*S. aureus*, *B. cereus*, *S. mutans*, *K. pneumoniae*, *S. enterica*, *E. coli*) was mixed with 7 mL of Brain Heart Infusion (BHI) broth plus 0.7% (w/v) of bacteriological agar, and poured over the plates (MRS, HS or YE) containing the microorganisms isolated from the kefir samples, already grown. The plates were incubated at 37°C for 24 h. After 24 h of incubation, inhibition zones were read. The bacteria *S. aureus*, *B. cereus*, *S. mutans*, *K. pneumoniae*, *S. enterica*, *E. coli* were inoculated into MRS, HS and YE media as a positive control of bacterial growth. Tests were performed in triplicate.

2.8 Statistical Analysis

Data were expressed as mean values ± standard deviation of three independent replicates. The calculated p value was obtained using analysis of variance test (ANOVA) and when the means were significantly different at $p<0.05$, Tukey's test was used to compare the means. Data were analyzed using the STATISTICA software, version 10.0.

3. RESULTS AND DISCUSSION

3.1 Physicochemical characteristics of milk kefir

The results of the physicochemical analyses of the milk and kefir samples are shown in Table 1. During the fermentation process, there was a decrease in the pH of the milk from 6.60 to 4.58-4.38 in 24 h, and to 3.74-3.60 in 48 h. The pH of the milk (6.60) was following the Brazilian legislation for cow's milk (pH from 6.60 to 6.80) [16].

Table 1: Physicochemical analyses of milk and kefir.

	Milk	Kefir 1		Kefir 2	
Analyzes		**24 h**	**48 h**	**24 h**	**48 h**
pH	6.60±0.16^{a}	4.38±0.34^{b}	3.60±0.07^{c}	4.58±0.04^{b}	3.74±0.05^{c}
Acidity (%)	0.16±0.00^{a}	1.03±0.53^{b}	1.71±0.10^{c}	1.10±0.35^{d}	1.70±0.10^{c}
Reducing sugars (%)	5.12±0.07^{a}	4.66±0.08^{b}	2.72±0.15^{c}	4.66±0.21^{b}	2.73±0.12^{c}

Results were expressed as mean ± standard deviation of triplicate measurements. According to Tukey's test, different letters on the same line mean significant differences at $p < 0.05$ (95% confidence level).

Gul et al. (2015) [8] reported similar results for the pH values of kefir (4.26-4.64) in 24 h. Windayani et al. (2019) [17] reported pH values of 3.31 to 3.61 and Weschenfelder et al. (2018) [18] found a pH of 3.97 in 48 h. Al-Mohammadi et al. (2021) [19] observed that kefir presented an initial pH value of 6.50, and the final pH was 3.10 in 24 h and 2.60 in 48 h. Kim et al. (2016) [20] demonstrated that during the fermentation process the pH gradually decreased in kefir samples, and reported pH values of 3.94-4.05 in 24 h and 3.71-3.81 in 48 h.

The milk presented an acidity of 0.16% and followed Brazilian legislation establishing a concentration of lactic acid from 0.14 to 0.18% in cow's milk [16]. The titratable acidity values of the kefir samples were 1.03-1.10% in 24 h and 1.70-1.71% in 48 h. Gul et al. (2015) [8] reported lower acidity values (0.64 to 0.76%) for kefir in 24 h. Windayani et al. (2019) [17] reported higher acidity values (1.96 to 2.64%) for kefir samples in 24 h. Lactic acid is the most abundant organic acid in milk kefir [3]. Ismaiel et al. (2011) [21] reported in cow milk kefir the concentration of lactic acid (7.30 mg/mL), followed by acetic acid (6.50 mg/mL) and malic acid (4.00 mg/mL).

During the kefir fermentation process, reducing sugars decreased from 5.12% in milk to 4.66% in 24 h and 2.72-2.73% in 48 h. The reducing sugar content of 5.12% in milk followed the Brazilian legislation (minimum lactose content of 4.30%) [16]. The initial lactose of milk is an important factor in the quality of kefir, since this sugar is the primary substrate used by the kefir microbiota in the metabolization into lactic acid, formed during fermentation with other metabolites such as acetic acid, vitamins, ethanol, acetaldehyde, diacetyl, carbon dioxide and bacteriocins, sensory and nutritionally enriching the product [1]. During the kefir fermentation process, milk lactose is hydrolyzed by the enzyme β-galactosidase, transforming lactose into glucose and galactose, and subsequently converting it into lactic acid. Thus, kefir is a good option for lactose intolerant individuals [22].

3.2 Antimicrobial activity of milk kefir during the fermentation process

Table 2 shows the antimicrobial activity of kefir during the fermentation process against *S. aureus*, *S. enterica* and *E. coli*. There was a significant reduction in bacterial counts of *S. aureus*, *S. enterica* and *E. coli* between 2.12 and 3.01 log CFU/g in kefir at 24 h compared to the positive control (7.12-8.25 log CFU/g). Kefir in 48 h did not show bacterial growth, with total inhibition of the microorganisms evaluated.

Table 2: Antimicrobial activity of kefir during the fermentation process against Staphylococcus aureus, Salmonella enterica and Escherichia coli.

Samples	***S. enterica* (log CFU/g)**		***S. aureus* (log CFU/g)**		***E. coli* (log CFU/g)**	
	24 h	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**
Kefir 1	2.97±0.10	N	2.12±0.15	N	2.89±0.11	N
Kefir 2	2.90±0.04	N	2.30±0.11	N	3.01±0.06	N
***Control**	7.90±0.11	8.10±0.10	7.12±0.13	7.20±0.16	8.20±0.04	8.25±0.12

N = no bacterial growth. *Control = *S. aureus*, *S. enterica* and *E. coli* incubated with milk. Results were expressed as mean ± standard deviation of triplicate measurements.

Kivanc and Yapici (2019) [23] reported similar results during the kefir fermentation, with inhibition of the growth of *E. coli* and *S. aureus* in 48 h. Al-Mohammadi et al. (2021) [19] observed no growth of *S. aureus* and *S. enterica* in BHI broths inoculated with 4% of milk kefir in 48 h. Kim et al. (2016) [20] compared the antimicrobial activity of four samples of kefirs fermented for 24, 36, 48, and 72 h against *S. aureus*, *S. enterica* and *E. coli*. The highest antimicrobial activity of kefir samples was obtained after 36-48 h of fermentation, although the traditional method of kefir fermentation is 18-24 h.

Tenorio-Salgado et al. (2021) [24] found that cell-free extracts of two kefir samples exhibited antagonism against *S.* Typhimurium, *S. aureus* and *E. coli*. Iraporda et al. (2017) [25] reported that the non-microbial fraction of kefir completely inhibited bacteria growth (*E. coli*, *Salmonella* spp. and *Bacillus cereus*). Zavala et al. (2016) [26] demonstrated that *Lactobacillus* (*L. kefiri* and *L. paracasei*) isolated from milk kefir reduced *in vitro Salmonella* infection in cells.

Al-Mohammadi et al. (2021) [19] reported that both kefir and neutralized kefir (pH 6.0) reduced the growth of *E. coli*, *Listeria monocytogenes*, *B. cereus* and *S. enterica*. Still, the inhibitory activity of acidified kefir was greater than that of neutralized kefir. Some studies have found that kefir's antimicrobial activity is associated with secondary metabolites, such as organic acids and acetaldehydes, and antimicrobial peptides [27, 28].

3.2 Enumeration and isolation of microorganisms from milk kefir

The total count of microorganisms was 8.23 ± 0.12 log CFU/g for kefir 1 and 8.35 ± 0.16 log CFU/g for kefir 2. Zanirati et al. (2015) [29] reported similar results and the bacterial counts of the four milk kefir samples ranged from 8.40 to 8.97 log CFU/g. Garofalo et al. (2015) [14] observed that the counts of six kefir samples ranged from 7.38 to 9.04 log CFU/g. Bourrie et al. (2021) [30] reported a kefir bacterial density of 8.00 log CFU/g. Savastano et al. (2020) [31] observed 7.00-8.00 log CFU/g in the four milk kefir samples.

After screening and purification, twenty-nine strains were isolated from the kefir samples (Table 3). Most strains (58.6%, n = 17) were isolated from MRS, 31.0% of strains (n = 9) were isolated from HS and only 10.4% of strains (n = 3) were isolated from YE.

Table 3: Microorganisms isolated from milk kefir.

Culture medium	Number (%) of isolated strains
MRS	17 (58.6%)
HS	9 (31.0%)
YE	3 (10.4%)
Total	29 (100%)

Lactic acid bacteria are usually the predominant microorganisms in milk kefir [1, 2, 7, 29]. Du et al. (2021) [32] reported that *Lactobacillus kefiranofaciens* represented 99.2% of the Tibetan kefir bacterial community, and after screening and purification, twenty-seven strains were isolated from the kefir. According to Prado et al. (2015) [33], the microbial composition of milk kefir varies according to the geographic origin of the kefir grains, the type of milk used in the fermentation process, and the culture maintenance methods.

Vieira et al. (2021) [6] observed a greater microbial diversity in artisanal kefir than industrialized kefir. Thus, the microbial composition of artisanal kefir is related to producing a greater variety of bioactive compounds, suggesting that artisanal kefir is responsible for providing better functional health benefits than industrial kefir.

3.3 Antimicrobial activity of microorganisms isolated from milk kefir

Of the twenty-nine microorganisms isolated from kefir, ten strains (34.5%) showed inhibitory activity against the bacteria *S. aureus*, *B. cereus*, *S. mutans*, *K.* pneumoniae, *S. enterica* and *E. coli* (Table 4).

Table 4: Evaluation of the antimicrobial activity of microorganisms isolated from milk kefir against several potentially pathogenic bacteria.

Microorganisms isolated from milk kefir	**Culture medium**	**Gram positive bacteria**			**Gram negative bacteria**		
		S. aureus	***B. cereus***	***S. mutans***	***K. pneumoniae***	***S. enterica***	***E. coli***
		Inhibition Halos (mm)					
1	MRS	N	N	N	$12±0.26^a$	$13±0.35^a$	$14±0.70^a$
2	MRS	N	N	N	N	$12±0.07^b$	$19±0.00^b$
3	MRS	$12±0.00^a$	$25±0.00^a$	$12±0.00^a$	$17±0.21^b$	$17±0.45^c$	$16±0.23^c$
4	MRS	$14±0.00^b$	$19±0.00^b$	$14±0.70^b$	$14±0.70^c$	$14±0.70^d$	$19±0.00^b$
5	MRS	$19±0.00^c$	$25±0.00^a$	$19±0.00^c$	$14±0.70^c$	$19±0.00^e$	$19±0.00^b$
6	HS	$25±0.28^d$	$25±0.00^a$	$23±0.15^d$	$23±0.26^d$	$19±0.00^e$	$23±0.20^d$
7	HS	$20±0.10^e$	$40±0.00^c$	$16±0.17^e$	$16±0.40^e$	$18±0.11^f$	$24±0.57^e$
8	HS	$40±0.00^f$	$33±0.21^d$	$25±0.00^f$	$30±0.86^f$	$35±0.86^g$	$33±0.86^f$
9	HS	$19±0.28^g$	$23±0.35^e$	$22±0.89^g$	$16±0.26^e$	$40±0.00^h$	$19±0.17^b$
10	YE	$12±0.70^a$	$19±0.00^b$	$12±0.00^a$	N	N	N

N = no inhibition or inhibition halos <12 mm. Results were expressed as mean ± standard deviation of triplicate measurements. According to Tukey's test, different letters on the same line mean significant differences at $p < 0.05$ (95% confidence level).

The most significant inhibition halos were observed for strains isolated from the HS medium. These strains showed inhibition zones of 19-40 mm for *S. aureus*, 23-40 mm for *B. cereus*, 16-25 mm for *S. mutans*, 16-30 mm for *K. pneumoniae*, 18-40 mm for *S. enterica* and 19-33 mm for *E. coli*. Also noteworthy were strains 3, 4 and 5 isolated from MRS with inhibition halos ranging from 12 to 25 mm for the potentially pathogenic bacteria evaluated. The other strains isolated from MRS and YE showed smaller inhibition halos (12-19 mm) or no inhibition against potentially pathogenic strains assessed (Table 4).

Other studies also reported the antagonist activity of microorganisms isolated from milk kefir. Zanirati et al. (2015) [29] found eleven strains of *Lactobacillus* isolated from Brazilian artisanal milk kefir grains that showed inhibition halos >25 mm against *S. aureus* ATCC 29213, *S. enterica* ATCC 14028, and *E. coli* ATCC 25723. Hurtado-Romero et al. (2021) [34] reported eight strains of *Lactobacillus* and two strains of *Kluyveromyces* isolated from Mexican artisanal milk kefir grains that presented antimicrobial activity (12-19 mm inhibition halos) against *E. coli* ATCC 25922, *S. enterica* and *S. aureus*.

Purutoğlu et al. (2019) [35] evaluated the antibacterial activities of twenty-seven lactic acid bacteria and three acetic acid bacteria isolated from Turkish artisanal milk kefir grains against *S. aureus* ATCC 25923, *Yersinia enterocolitica* ATCC 27729, *E. coli*, *B. cereus* and *S. enterica*. Seven strains of lactic acid bacteria were effective to all tested pathogens. One strain of acetic acid bacteria showed no inhibitory effects to evaluated pathogens, while the other two strains of *Acetobacter* spp. showed inhibitory effects. According to the authors, not many studies in the literature related to the antibacterial activities of acetic acid bacteria. Kim et al. (2019) [36] evaluated the antimicrobial activity of an acetic acid bacterial strain (*Acetobacter fabarum* DH1801) isolated from Korean milk kefir against foodborne pathogens (*B. cereus*, *S. aureus*, *Listeria monocytogenes*, *S. enterica*, enterotoxigenic *E. coli*, and *Shigella flexneri*). The culture filtrate of the acetic acid bacteria inhibited the growth of all pathogenic bacteria.

Thus, studies in the literature show that both lactic bacteria and acetic bacteria in milk kefir can present antagonistic activity against pathogenic bacteria. According to Özdemir et al. (2015) [37], lactic acid and acetic acid bacteria in kefir can contribute positively to the antimicrobial activity and physicochemical characteristics of the beverage. The probiotic species contained in milk kefir produce a variety of antimicrobial compounds, including lactic and acetic acids, carbon dioxide, hydrogen peroxide, ethanol, diacetyl, and peptides (bacteriocins) that help balance and regulate the gut microbiota, as well as help in the prevention and treatment of gastrointestinal infections [38].

4. CONCLUSIONS

The two Brazilian artisanal milk kefir samples showed lower pH and higher acidity in 48 h compared to 24 h of fermentation. The 24 h fermented samples reduced the growth of *S. aureus*, *S. enterica* and *E. coli* compared to the positive control, where milk without kefir grains was inoculated with the pathogenic strains. On the other hand, kefir samples fermented in 48 h completely inhibited bacterial growth, suggesting that the antimicrobial activity of the milk kefir is attributable to higher acidity and antimicrobial substances secreted during the fermentation. Ten microorganisms isolated from milk kefir exhibited antimicrobial capacity against several pathogenic bacteria, which reveals the potential of these strains to be used as preservatives in foods and possibly as probiotics.

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