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PRODUCTION OF KEFIR AS AN ALTERNATIVE FOR UTILIZATION OF BUTTERMILK

Produção de kefir como uma alternativa para a utilização de leitelho

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ABSTRACT

Alternatives to buttermilk processing are important for industries and for reducing environmental pollution. This study evaluated the effect of milk substitution for buttermilk on processing and kefir characteristics. Two treatments were used to produce kefir, utilizing milk or buttermilk as the raw material. The products were evaluated over a period of 28 days, where the pH decreased from 6.6 to 4.0 and from 6.5 to 4.1 for the products made with milk and buttermilk, respectively. The final mean yeast and lactic acid bacteria counts were 5.0 log cfu mL^{-1} and 8.0 log cfu mL^{-1}, respectively. Coliforms were not detected. Sensory acceptance of kefir produced with buttermilk was similar to that of the product produced with milk.

Palavras-chave: butter; lactic bacteria; lactic fermentation; probiotic.

RESUMO

Alternativas para o processamento de leitelho são importantes para as indústrias e para a redução da poluição ambiental. Esse estudo avaliou o efeito da substituição de leite por leitelho no processamento e nas características do kefir. Dois tratamentos foram usados para produzir kefir, utilizando leite ou leitelho como matéria-prima. Os produtos foram avaliados em um período de 28 dias, onde o pH diminuiu de 6,0 até 4,0 e de 6,5 até 4,1 para os produtos fabricados com leite e leitelho, respectivamente. As contagens médias finais de leveduras e bactérias láticas foram 5 log UFC mL^{-1} e 8 log UFC mL^{-1}, respectivamente. Coliformes não foram detectados. A aceitação sensorial do kefir produzido com leitelho foi similar à do produto produzido com leite.

Keywords: manteiga; bactéria lática; fermentação lática; probiótico.

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INTRODUCTION

The demand for functional fermented dairy products has increased in recent years as consumers are increasingly interested in higher quality products (NAMBOU et al., 2014). Thus, several fermented dairy products, such as kefir, have been the subject of several studies seeking to evaluate their functional properties, where these are related to the biological activity of microorganisms used in the manufacture of the products. Kefir is considered a functional product and thus, can provide beneficial effects to the health of individuals. The microorganisms present in its composition have the ability to assist in the intestinal microbial balance and have been related to beneficial effects such as antimicrobial, antitumor and anticarcinogenic activity, in addition to improving lactose digestion due to the presence of the enzyme β-galactosidase (LEITE et al., 2013; WENDLING; WESCHENFELDER, 2013; NIELSEN et al., 2014).

The steady growth in demand for dairy products in several countries has led to an increase in the number of dairy industries. This higher production volume has caused greater concern regarding the larger volumes of industrial waste generated during processing. In some cases, these by-products are poorly utilized by industries and are not directed to effluent treatment plants to receive adequate treatment (KUSHWAHA et al., 2011). Thus, in order to avoid environmental damages and also to take advantage of important nutrients, several studies have been carried out to find alternatives for the use of these wastes (BANASZEWSKA et al., 2014; BAHRAMI et al., 2015).

Among the by-products generated in the dairy industry is buttermilk, which is the residue obtained from the manufacture of butter and has a composition similar to skimmed milk (SAFFON et al., 2014). It has become a problem to the dairy industry from an environmental point of view due to its high organic load (BAHRAMI et al., 2015). Therefore, reuse of this by-product by the industry is of great relevance, which can add economic value in the form of several products because of its nutritional value, and also contribute to reduce environmental pollution.

Most buttermilk generated by industries is converted to powder via conventional pasteurization, concentration and drying processes (AUGUSTIN et al., 2015). However, for small industries that do not have the financial resources to purchase drying equipment and for greater investments in effluent treatment systems, simpler technological alternatives for the use of buttermilk are important.

The use of buttermilk by the industry to make products including beverages and cheeses, and as an ingredient of other products is an excellent alternative since it presents a low cost, high availability, good technological properties and desirable sensorial characteristics (FERREIRO et al., 2016). In this context, studies evaluating the use of buttermilk in the production and sensorial acceptance of kefir are scarce. This alternative is important because of the low complexity of the manufacturing process and can be used by small industries as well.

In this study, sought to evaluate the substitution of milk for buttermilk in the manufacture of kefir, proposing a low cost and technically feasible alternative for the use of buttermilk in dairy products.

MATERIAL AND METHODS

The study was carried out at the Food Research Laboratory of Agricultural Sciences Institute of the Federal University of Viçosa – Rio Paranaíba Campus. The physicochemical and microbiological analyses were performed in three replicates over a period of 28 days.

Raw materials and microorganisms

For the elaboration of kefir, skimmed milk and buttermilk were obtained from a local industry and kefir culture was acquired from the Food Processing Laboratory of Agricultural Sciences Institute of the Federal University of Viçosa – Rio Paranaíba Campus.

Activation of the kefir grains

Kefir grains stored in refrigerated milk (4 ºC) were separated from the milk using a sieve and the milk was discarded. The grains were then inoculated in sterile skimmed milk and maintained at 22 ºC in a BOD incubator (Eletrolab, model EL 202/4, São Paulo, Brazil) for 48 h and then used for kefir production.

Kefir production

The cream collected from the mechanical decreaming treatment of pasteurized cow milk was stored at 12 ºC for 15 h. The titratable acidity was 0,20 % expressed as lactic acid. After maturation, the cream was pumped to the butter churner. The obtained butter was washed with cold water and the buttermilk was separated.

Kefir was produced on a laboratory scale in two different treatments, using 200 mL of milk and buttermilk as raw materials according to the methodology described by Kotova et al. (2016), with modifications. The skimmed milk and buttermilk were initially heated to 85 ºC during 30 min and then cooled to 22 ºC and inoculated separately with 1.5 % (w/v) kefir grains previously activated. Thereafter, fermentation was performed at thermostatic bath at 22 ºC for 48 h. The kefir grains were then separated from the product with a sieve and then preserved in sterilized skimmed milk under refrigeration at 4 ºC for future fermentations. The product was cooled and stored at 4 ºC for the final 28 days. The treatments were performed in three replicates.

Physicochemical properties and composition of the milk and buttermilk

Analyses of density, fat, total solids (TS), pH and titratable acidity in milk and buttermilk were performed according to the methodology described by the Zenebon et al. (2008). Protein analysis were performed using the EKOMILK M device (Cap-Lab, model RS232, São Paulo, Brazil), according to the manufacturer's instructions.

Biomass

Mass of the kefir grains (inoculum), expressed in grams, was determined at 0 h and 48 h of fermentation, using an analytical balance.

Yeast and coliform counts at 35 ºC and 45 ºC

The yeast counts and determination of coliforms at 35 ºC and 45 ºC were performed according to the method described by APHA (2001).

Lactic acid bacteria count

The lactic acid bacteria count was performed according to the method described by Tebaldi et al. (2007).

Sensory analysis

The sensory evaluation of kefir was performed at the end of the manufacturing process, after 48 h of fermentation, following the methodology proposed by the Zenebon et al. (2008) and Pereira et al. (2011), with modifications. The test was carried out at the Federal University of Viçosa, Rio Paranaíba

Campus, using a team of 50 untrained evaluators, composed of students, staff, teachers, and campus visitors. The attributes evaluated were flavor, aroma, appearance, texture and overall impression using a 9-point hedonic scale, in which 9 corresponded to “like extremely” and 1 to “dislike extremely”. Purchase intent was evaluated on a 5-point hedonic scale, where 5 corresponded to “would certainly buy” and 1 to “certainly would not buy”. The evaluators received 2 monadic samples, coded with random three-digit numbers. The model form used in the sensory acceptance test is in ANNEX I.

The study was approved by the Human Research Ethics Committee of the Federal University of Viçosa, report nº 1,220,982.

Statistical analysis

The results of the physicochemical, microbiological and sensorial analyses were evaluated via the tests of normality (Kolmogorov-Smirnov) and homogeneity of variance (Barttlett’s), analysis of variance (ANOVA), Tukey test at 5% significance using the Minitab® 17 software and regression analysis using the OriginPro® 8 software.

RESULTS AND DISCUSSION

Physicochemical properties and composition of the milk and buttermilk

The density, protein content and TS of buttermilk were lower than the values found for milk, whereas the fat content of buttermilk was higher (Table 1). The results found for buttermilk are justified by the washing of butter that takes place in order to remove it from the formed mass, provoking its dilution.

Biomass

During fermentation, the mass of the kefir grains of the product obtained from milk increased considerably in relation to the initial mass, while the mass of the kefir produced with buttermilk did not show much increase (Table 2). The microorganisms developed less intensely in buttermilk, probably due to the lower concentration of nutrients in the medium as a result of washing performed during the processing of butter. According to Gauvin et al. (2018), skimmed milk and buttermilk have a similar chemical composition (water, lipids, proteins, lactose and salts), except that buttermilk has a higher proportion of milk fat globule membrane. However, variations in the concentration of constituents can occur due to the procedures adopted in the separation of buttermilk. This occurs mainly when a fraction of the wash water is accidentally incorporated into buttermilk.

The increase in size and number of kefir grains due to the multiplication of microorganisms is influenced by the process conditions, including fermentation time, agitation, temperature and type of raw material (LEITE et al., 2013).

Table 1 – Physicochemical properties and chemical composition of the raw materials used in kefir production

Raw material	Components			
	Density (g mL^{-1})	Fat (% w/w)	Protein (% w/w)	TS* (% w/w)
Milk	1.035	0.05	3.10	9.02
Buttermilk	1.016	0.40	1.76	4.34

*TS = Total Solids

Table 2 – Mean and standard deviation values of the mass (g) of the kefir grains fabricated from milk and buttermilk

Time (h)	Raw material	
	Milk	Buttermilk
0	3.47 ± 0.04^{A}	3.47 ± 0.01^{A}
48	4.03 ± 0.16^{A}	3.52 ± 0.03^{B}

$^{A, B}$ Means on the same line followed by the same letter do not differ at 5% significance by the Tukey test.

Titratable acidity and pH

The milk and buttermilk used for kefir processing presented initial titratable acidity values of 0.18% and 0.09% (w/v) of lactic acid, respectively. After the fermentation period of 48 h at 22 ºC, the products obtained from milk and buttermilk presented acidity of 0.81% and 0.86% (w/v) of lactic acid, respectively. Although buttermilk presented a lower concentration of nutrients, the final acidity values of the products after the initial fermentation were similar. In this case, the nutrient concentration of buttermilk was sufficient to provide final acidity similar to kefir produced with milk. Probably at this point, the microorganisms of the two products were inhibited by the organic acids liberated in the fermentation. The acidity of kefir is an important characteristic that influences the degree of acceptance of the product by consumers.

The milk and buttermilk raw materials and the kefir conditioning time showed significant interaction for the pH parameter, indicating that they were dependent on its variation. At time 0, the kefir made with milk showed a higher pH than that of buttermilk, and at other times the pH of the product with buttermilk was higher (Table 3). Probably, a higher concentration of milk nutrients led to a more pronounced pH reduction. At the beginning of the process the pH reduction was more pronounced and, from the second day, it slowed until stabilizing, probably due to nutrient shortages, the lactic acid inhibitory effect and competition among microorganisms. As can be observed in Figures 2 and 3, the yeast count increased for 21 days, while the lactic acid bacteria count stabilized after this period. The pH reduction occurs due to the production of organic acids, mainly lactic acid produced by lactic acid bacteria during fermentation (LEITE et al., 2013).

According to the results, the pH of the products made with milk and buttermilk showed a significant reduction during the processing and storage periods, reaching similar values (Figure 1).

According to Otles; Cagindi (2003), the pH of kefir usually reaches values between 4.2 and 4.6. In a study conducted by Grønnevik et al. (2011), the pH of kefir was equal to 4.41 in the first week of storage, a result similar to that found in the present study.

Table 3 – Mean and standard deviation values for the pH of kefir produced from milk and buttermilk

Kefir	Time (days)					
	0	2	7	14	21	28
Milk	6.60 ± 0.04^{A}	4.67 ± 0.07^{B}	4.45 ± 0.07^{B}	4.12 ± 0.06^{B}	4.03 ± 0.02^{B}	4.01 ± 0.03^{B}
Buttermilk	6.51 ± 0.01^{B}	4.84 ± 0.05^{A}	4.64 ± 0.05^{A}	4.30 ± 0.05^{A}	4.12 ± 0.05^{A}	4.14 ± 0.05^{A}

$^{A, B}$ Means in the same column followed by the same letter do not differ at 5% significance by the Tukey test.

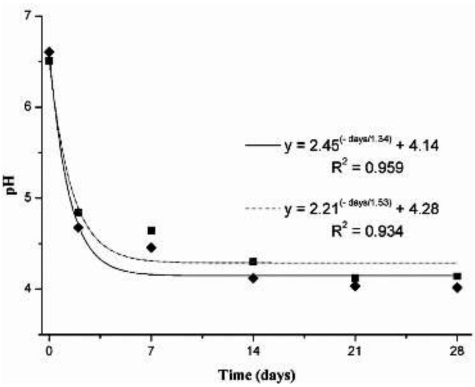

Figure 1 – Evolution of pH during processing and storage of kefir obtained from buttermilk (---■---) and milk (—♦—)

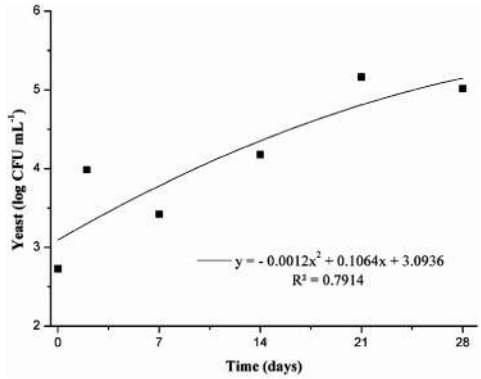

Figure 2 – Mean yeast count (log cfu mL^{-1}) during the storage period

Yeasts

The milk and buttermilk raw materials and the kefir conditioning time did not show significant interaction in relation to the yeast count. Thus, the type of raw material and time acted independently on the development of the microorganisms. The raw materials presented no significant difference, not influencing microorganism growth. The regression equation of the yeast count in function of time is shown in Figure 2.

Ertekin; Guzel-Seydim (2010) encountered yeast counts equal to 5.2 log cfu mL^{-1} on the seventh day after initiating the process, a result similar to that found in this study after 21 days. According to Leite et al. (2013), lactic acid bacteria of the genus *Lactococcus* have a high capacity to ferment lactose and accumulation of lactic acid in the medium favors development of the yeasts.

Coliforms at 35 ºC and 45 ºC

Analysis of the products at the initial and final times did not detect coliforms at 35 ºC and 45 ºC (Table 4), indicating that processing was conducted properly. Furthermore, kefir has an inhibitory effect on the development of several undesirable microorganisms.

In the study conducted by Chifiriuc et al. (2011), which evaluated the antimicrobial activity of kefir against fungi and bacteria, an inhibitory effect of the product was observed against *Bacillus subtilis* spp. *spizizenii* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 8739 e *Salmonella enteritidis* ATCC 13076.

Table 4 – Coliform counts at 35 ºC and 45 ºC during kefir storage

Time (days)	Coliforms at 35 ºC (MPN mL^{-1})		Coliforms at 45 ºC (MPN mL^{-1})	
	Milk	Buttermilk	Milk	Buttermilk
0	< 0.3 ± 0	< 0.3 ± 0	< 0.3 ± 0	< 0.3 ± 0
28	< 0.3 ± 0	< 0.3 ± 0	< 0.3 ± 0	< 0.3 ± 0

The organic acids present in kefir produced from fermentation, mainly lactic acid, have an inhibitory effect on pathogenic and deteriorating microorganisms. Additionally, the composition of kefir includes compounds such as bacteriocins and hydrogen peroxide, which may contribute to the inhibition of microorganism development (LEITE et al., 2013).

Lactic acid bacteria (LAB)

The milk and buttermilk raw materials and the kefir storage time did not show significant interaction in relation to the LAB count, independently influencing the development of the microorganisms. Figure 3 shows the developmental profile of LAB over time, where it can be observed from day 2 that the LAB counts in kefir of the two treatments were superior to 7 log cfu mL^{-1}. Despite the difference in concentration of the substrate constituents, LAB development was not negatively influenced. Furthermore, the counts obtained were similar to other studies performed with kefir.

Guzel-Seydim et al. (2005) obtained LAB counts between 8.00 and 9.00 log cfu mL^{-1} in kefir during 21 days of product storage, while Wróblewska et al. (2009) found LAB counts of 8.20-8.22 log cfu mL^{-1} in 14 days of storage. Similarly, Grønnevik et al. (2011) obtained a LAB count equal to 8.00 log cfu mL^{-1} in freshly fermented kefir.

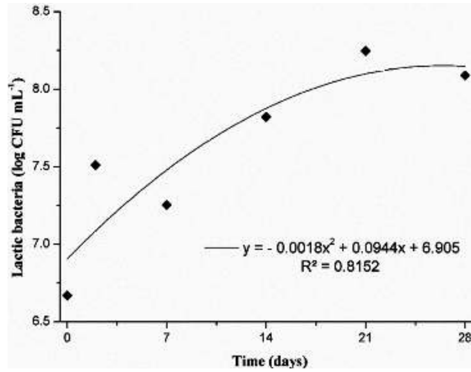

Figure 3 – Mean count of lactic acid bacteria (log cfu mL^{-1}) during the storage of kefir

The final LAB counts were higher than the yeast counts (Figure 2) due to their higher concentration at the beginning of the process and the high capacity of these bacteria to develop during the kefir fermentation process. Lactic acid bacteria present high efficiency for metabolizing lactose, therefore they tend to present faster growth than yeasts present in the medium (LEITE et al., 2013).

Sensory analysis

The two products showed no significant difference with regards to the attributes of flavor, aroma and overall impression. However, they presented a significant difference for the appearance and texture attributes (Table 5). Despite the difference in these

Table 5 – Mean and standard deviation values of the attributes of kefir produced from milk and buttermilk

Kefir	Attributes				
	Taste	Aroma	Appearance	Texture	Overall impression
Milk	5.58 ± 1.72^{A}	6.10 ± 1.58^{A}	7.28 ± 1.21^{A}	7.16 ± 1.36^{A}	6.32 ± 1.55^{A}
Buttermilk	4.94 ± 1.89^{A}	5.44 ± 1.76^{A}	6.46 ± 1.65^{B}	6.18 ± 1.59^{B}	5.64 ± 1.96^{A}

$^{A, B}$ Means in the same column followed by the same letter do not differ at 5% significance by the Tukey test.

two parameters, kefir produced with buttermilk showed good acceptance among the evaluators, being very similar to the product obtained with milk.

Acceptance of the products was classified between "neither like nor dislike" and "like moderately", a result probably influenced by the high acidity characteristic of kefir and the fact that the tasters had little or no habit of consuming the product. Most evaluators had no previous knowledge of kefir. Furthermore, no sugar was used in the technology adopted for product processing, which may contribute to better acceptance of the product.

The difference observed between the two products with respect to the appearance and texture aspects may have occurred due to the higher viscosity visually observed for kefir produced from milk, which probably occurred due to the higher concentration of proteins present in the milk that caused the viscosity increase. The lactic acid produced during the manufacture of fermented milk causes a reduction in the stability of casein micelles, leading to gel formation at pH levels between 4.6 and 4.7.

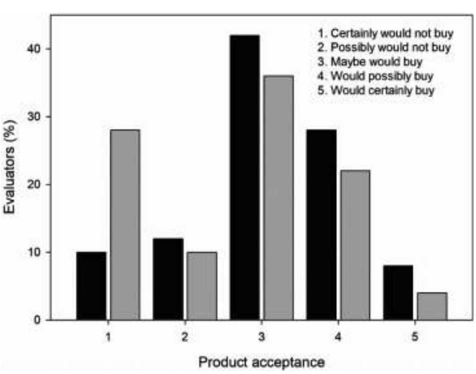

Figure 4 – Purchase intent of kefir produced with milk (■) and buttermilk (■)

Regarding purchase intent, the products obtained similar responses, where 22% and 28% of the tasters "would possibly buy" the kefir produced from buttermilk and milk, respectively (Figure 4).

The relatively low purchase intent the products probably occurred due to the sensorial characteristics of the products and the lack of knowledge and reduced consumption habits of the product by the evaluators.

CONCLUSIONS

The utilization of buttermilk did not result in significant changes to the kefir fermentation process and the product presented similar acceptance to kefir produced with milk, therefore showing that it is possible to use buttermilk to manufacture this product.

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ANNEX I. MODEL OF THE EVALUATION FORM OF THE SENSORY ACCEPTANCE TEST.

Sensory Evaluation Form

Name: ______________________ Sex: Male ( ) Female ( ) Age: ________

You are receiving two coded kefir samples. Please, evaluate for all attributes how much you liked or disliked the product using the scale below.

Code	Flavor	Aroma	Appearance	Texture	Overall impression
______	______	______	______	______	______
______	______	______	______	______	______

(9) Like extremely
(8) Like very much
(7) Like moderately
(6) Like slightly
(5) Neither like nor dislike
(4) Dislike slightly
(3) Dislike moderately
(2) Dislike very much
(1) Dislike extremely

Regarding the purchase intent of these samples, what would be your attitude?

(5) Would certainly buy
(4) Would possibly buy
(3) Maybe would buy
(2) Possibly would not buy
(1) Certainly would not buy

Code	Purchase intent
______	______
______	______

Comments: ____

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