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Kefir fermented milk as starter culture in Minas Frescal cheese production

Leite fermentado com kefir como cultura lática para a produção de queijo Minas Frescal

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The present work aimed to produce Minas Frescal cheese with the replacement of the commercial lactic culture by fermented kefir milk with kefir (FKM) and verify some of its physicochemical characteristics, aiming mainly at the development of a new commercial probiotic dairy product with low production cost as compared to the use of commercial probiotic cultures. Kefir grains were added to whole UHT milk and fermented at 22ºC/24 hours in an incubator, after fermentation the grains were separated and the FKM was used in the manufacture of cheeses with the addition of 1, 3 or 5% of the total volume of milk. They were analysed the concentrations of fat and protein in the milk used in cheese manufacturing; the acidity of FKM, milk, FKM-added milk, whey and cheeses; in those ones their total solids (TS), fat and fat in the total solids (FTS) and manufacturing yields were evaluated. The results obtained indicated that it was possible to produce Minas cheeses using KFM in all of the tested concentrations because the results for all treatments were close to those found in the literature with the use of commercial lactic cultures and among the treatments only the acidity had significant differences, however more studies should be carried out mainly aiming at the standardization of kefir cultures for the industrial process of manufacturing these cheeses.
Keywords: probiotics, dairy products, cheese.

O presente trabalho teve como objetivo produzir queijo Minas Frescal com a substituição da cultura lática comercial por leite fermentado com kefir (LFK) e estudar algumas de suas característica físico-químicas visando principalmente o desenvolvimento de um novo produto lático probiótico comercial com baixo custo de produção em comparação ao uso de culturas probióticas comerciais. Os grãos de kefir foram adicionados em leite UHT integral e fermentados a 22ºC/24 horas em estufa, após a fermentação os grãos foram separados e o LFK empregado na fabricação dos queijos com a adição de 1, 3 ou 5% sobre o volume total de leite. Foram analisadas as concentrações de gordura e proteína no leite usado nas fabricações; a acidez do LFK, leite, leite adicionado de LFK, soro e queijos; nesses últimos foram avaliados seus sólidos totais (ST), gordura e gordura no extrato seco (GES) e os rendimentos das fabricações. Os resultados obtidos indicaram ser possível produzir queijos Minas empregando o LFK em qualquer uma das concentrações testadas, uma vez que os resultados para todos os tratamentos ficaram próximos aos encontrados na literatura com o emprego de culturas láticas comerciais e entre os tratamentos somente a acidez dos apresentou diferenças significativas, contudo mais estudos devem ser realizados visando principalmente a padronização das culturas de kefir para o processo industrial de fabricação desses queijos.
Palavras-chave: probióticos, produtos lácteos, queijo.

1. INTRODUCTION

The Minas Frescal cheese is one of the most popular cheeses in Brazil, it is consumed by a large part of its population. It is a raw, unmatured, not standardized cheese and its composition varies from 12% to 18% of proteins and from 20.5% to 29.22% of fat, nowadays there is a light version with reduced fat content [1, 2].

The Minas cheese is a fresh cheese obtained by enzymatic coagulation of milk by rennet and/or other appropriate coagulating enzymes sometimes complemented by the addition of starter cultures [3, 4]. There are two production models: industrial, mandatorily processed with pasteurized milk and "colonial" (farm cheese). It is considered farm cheese when its production

is made with raw milk, less technology and generally sold without a label, it has been produced as a traditional subsistence dairy product on small farms for many years in the countryside of the country [4, 5].

Fermented dairies are products of the fermentation of milk by different lactic cultures. In that group of foods are the yoghurt, acidophilus milk, curd, kumis and kefir. That last one is a fermented, sour, slightly alcoholic milk made from grains that have a relatively stable population of microorganisms, some of them are scientifically recognized as probiotics and during the fermentation process those microorganisms synthesize some compounds that give a characteristic flavour and aroma to the product and bioactive substances that are responsible for its nutraceutical properties [6-8].

Kefir grains are irregular gelatinous structures where lactic acid bacteria, acetic acid bacteria and yeasts are fixed in a matrix of proteins and polysaccharides, the main polysaccharide found in the grains is the kefiran. The microorganisms most commonly isolated from kefir grains are of 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*) e *Saccharomyces* [7, 9].

Kefir's popularity is related to its long history of health benefits (antimicrobial, anticancer, hypocholesterolemic, reduction of lactose intolerance, improving the immune system and intestinal conditions), the interest in that beverage has considerably increased in recent years when more consumer demand for kefir products took place, therefore, various research efforts have been made to improve the traditional kefir drink and to develop a range of value-added products incorporating the biomass of kefir cultures in different products, such as Feta-type cheese, Ricotta, whey-based beverages and baking yeast [10].

In this context, the main objective of this work was to study some physicochemical characteristics and the yield of Minas Frescal cheese using different concentrations of kefir fermented milk as a starter culture.

2. MATERIAL AND METHODS

2.1 Preparation of kefir starter culture

Kefir grains were added to whole UHT milk in conical flasks (500 mL) and fermented at a temperature of 22ºC/24h in incubator. The Kefir fermentation typically occurs at temperatures ranging from 8 to 25°C, in a partially closed container, at a variable time from 10 to 40 h, however, the most common incubation time is 24 h, due to the fact that there is not a time/temperature binomial standard, researchers can choose the binomial they want to produce Kefir according their objectives [11].

After fermentation, the grains were separated from the fermented milk and used for new fermentations and the fermented kefir milk (FKM) was used as a starter culture in the manufacture of cheeses according to the following treatments: T1 addition of 1.0% FKM; T2 addition of 3.0% FKM and T3 addition of 5.0% FKM.

2.2 Cheese manufacturing

Pasteurized homogenized milk with 3.0% of fat was heated until 36ºC followed by the addition of calcium chloride 40% (0,5 mL/L), FKM according to the treatment and the rennet at the recommended volume by the manufacturer, the mix was kept at rest for 40 minutes when the curd was cut in cubes and stirred for 60 minutes, the whey was removed and the curd put in moulds for 24 hours for draining and fermentation. The cheeses were salted in brine with 30% NaCl for 2 hours and dried at room temperature and they were vacuum-packed in plastic bags and frozen.

2.3 Physicochemical analysis

The analyses carried out were: In the milk the protein content was determined according to Wolfschoon and Vargas (1977) [12], in 10 ml of diluted milk (1:1) with deionized water, 1.0 ml of 1% phenolphthalein, and 0.4 ml of 28% potassium oxalate were added, it was titrated with 0.1M NaOH and 2 ml of 35% formaldehyde was added and the sample was titrated again with the same solution, the volume of NaOH spent in the second titration was multiplied by the factor 1.747 to obtain the protein concentration in milk, and the results were expressed in percentage. Acidity was analyzed before and after the addition of FKM in milk, whey and cheeses by titration with NaOH 0.1 M, the concentration of fat by the butirometric method, Total Solids by drying at 105ºC/8h [13]. All analyses were performed in triplicate.

2.4 Determination of cheeses yield

The determination of cheeses yield was carried out considering the volume of milk used in the fabrication plus the volume of milk fermented by kefir (starter culture) divided by the weight of cheese obtained after salting and drying. The yield was expressed in the volume of milk used per kg of cheese obtained (L/kg).

2.5 Statistical analysis

The results were submitted for Analysis of Variance and the Tukey Test when there were significant differences using the BioEstat software [14].

3. RESULTS AND DISCUSSION

The acidity in dairy products is the total amount of acids in the samples and the lactic acid is the main organic acid found in those products. The acidity in cheeses is directly related to their population of microorganisms, mainly lactic acid bacteria, which are the main agents in the conversion of lactose into lactic acid, the acidity tends to increase due to the multiplication of the microorganisms in the storage period that benefits the product by inhibiting pathogenic microbiota [1, 15].

There are three distinct microbial populations in Brazilian kefir beverage: lactic acid bacteria are the predominant, followed by the yeasts and gram-negative bacteria from the *Acetobacter* genus. *Lactobacillus paracasei* is the most abundant bacterium, while *Saccharomyces cerevisiae* is the predominant yeast strain and the different microorganism groups identified in this beverage perform three different kinds of fermentations such as lactic, alcoholic and acetic [16].

The results found for acidities are shown in Table 1, concentrations from 0.140 to 0.180 g of lactic acid/100 mL of milk are considered normal, as those found in this work [17].

Alves et al. (2021) [18] have produced kefir with semi-skimmed UHT milk (1.8% of fat) and fermentation temperature of 20ºC, they found an acidity value of 0.65 g/100mL of lactic acid after 24h, results lower than the ones found in this work. Kok-Tas et al. (2013) [19] produced milk kefir at a temperature of 25ºC and found acidity values between 0.810 to 0.890 g/100mL. Paredes et al. (2022) [20] found acidity values in kefir that varied according to the percentage of inoculum used (1 to 4 %) and those values ranged from 0.515 to 2.042 g/100 mL of milk and the fermentation conditions were 26ºC/24h, those results can demonstrate that there is a relationship between the fermentation temperature and the percentage of inoculum with the production of lactic acid by kefir bacteria, but it should consider that different Kefir grains have different microbiological compositions and the results can be very heterogeneous and the microorganisms present in kefir grains vary according to where they were produced, so the final drink does not always have the same standard [21].

As shown in table 1 when the concentration of FMK used in the cheese productions has enhanced, the same effect was seen in cheeses, it has demonstrated that the curd can retain part of the lactic acid added into the milk and the same effect could be verified in the whey samples,

however, the results showed that this higher lactic acid retention in cheeses started with the addition of 5% of FKM where there are statistically significant differences among the treatments.

Table 1: Titratable acidity of different products used or produced in this experiment (g lactic acid/100g).

Treatment	Milk	Kefir	Milk with kefir	Whey	Cheese
T1	0.179 ±0.006 a	1.029 ±0.131a	0.187±0.008 a	0.117±0.004a	0.402 ±0.039a
T2	0.179 ±0.007 a	1.030 ±0.131a	0.203±0.010 a	0.140±0.006b	0.447 ±0.032a
T3	0.177 ±0.002 a	1.194 ±0.000a	0.255±0.002b	0.144±0.003b	0.599 ±0.054b

T1 addition of 1.0% kefir; T2 addition of 3.0% kefir and T3 addition of 5.0% kefir. Values with different letters in the same column are significantly different ($p < 0.05$).

The levels of protein and fat in milk are seasonal, so their values vary throughout the year, but in general, the protein levels are between 2.5 and 3.5% and the fat from 3.0 to 4.0%, depending on the climate, diet and the dairy herd management [22]. In this work, standardized milk at 3.0% fat was used, which is the standard for whole processed milk in Brazil [23].

The cheese composition and yield (Table 2) are directly related to the fat and protein contents in milk then it is often to use the casein/fat ratio (C/F) or protein/fat ratio (P/F) in the standardization of milk for cheese manufacture allowing an increase in the yield and producing cheeses with uniform quality at any time of year, reducing differences due to seasonal variations in milk components [24].

Researchers used kefir in fresh cheese traditionally produced in Jordan to replace the starter culture normally used and found acidity values ranging from 0.254 to 0.455 g/100g over 14 days of storage under refrigeration and they suggested that soft cheeses are potential vehicles for kefir cultures to human, as the counts of kefir cultures remained above the minimum probiotic therapeutic counts during processing and cold storage, particularly for lactic acids bacteria [10].

Andrade et al. (2020) [25] found values for titratable acidity ranged from 0.77 to 1.73% in samples of Minas Frescal cheese, similar results were found by Oliveira et al. (2016) [26] with values for titratable acidity from 0.67 to 1.77%, those results are higher than all found in the present work but in those works the authors have not used kefir as starter culture.

Table 2: Protein contents and protein/fat ratio found in milk used in cheese processing.

Treatment	Protein (g/100 mL)	Protein/fat ratio
T1	3.200 ±0.093 a	1.067 ±0.031 a
T2	3.230 ±0.099 a	1.077 ±0.033 a
T3	3.260 ±0.080 a	1.087 ±0.027 a

T1 addition of 1.0% KFM; T2 addition of 3.0% KFM and T3 addition of 5.0% KFM. There were no significant differences among the treatments.

The manufacturing yield and the proximate composition of the cheese are determined by the properties of the milk, especially the composition and the steps of the manufacturing process [27]. Soares et al. (2018) [28] in a study with Minas colonial cheese in the state of Minas Gerais found the following average percentage indices of the physicochemical parameters for the cheeses: 30.10± 3.67g/100g fat and 35.39±8.11g/100g moisture. The fat in total solids (FTS) was 47.15±4.63g/100g and the acidity was 1.97±1.42 grams of lactic acid/100g. The differences found among the results are probably due to the ripening process in the Minas colonial cheese that reduces the humidity and enhances the other compound concentrations.

Table 3: Physicochemical composition of the cheeses obtained in this work.

Treatment	Fat (g/100g)	TS (g/100g)	FTS (g/100g)	Yield (L/kg)
T1	19.33 ±0.816 a	44.297 ±0.366 a	43.651 ±1.948 a	6.535 ±0.446 a
T2	19.68 ±0.816 a	43.992 ±1.008 a	44.723 ±2.116 a	6.662 ±0.247 a
T3	19.50 ±0.500 a	44.571 ±0.513 a	43.766 ±1.490 a	6.601 ±0.253 a

T1 addition of 1.0% KFM; T2 addition of 3.0% KFM and T3 addition of 5.0% KFM. There were no significant differences among the treatments.

Saboya et al. (1998) [29] produced Minas Frescal cheese with milk whose centesimal composition was close to the one used in this present work (Table 3) and they found the following composition: 21.59 g/100g of fat, 42.69 g/100g of TS and 50.59 g/ 100g of FTS, their cheese was produced with a commercial mesophilic culture daily used in dairy industries based on *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris*, these results indicate that the use of KFM in different proportions as a starter culture does not change the composition of the cheeses obtained and it can used in commercial productions of cheese.

4. CONCLUSION

The results obtained in this work sustain that the introduction of kefir fermented milk as a starter culture in the manufacture of Minas Frescal cheese did not change its physicochemical composition because the values found are close to those described in the literature, however, kefir is produced traditionally for homemade it does not have a process of standardization of its counts of microorganisms, which can make its use on an industrial scale difficult so that more studies must be carried out aiming at its standardization as well as the production process of cheeses.

5. BIBLIOGRAPHICAL REFERENCES

1. Sangaletti MV, Porto E, Brazaca SGC, Yagasaki CA, Dalla Dea RC, Silva MV. Estudo da vida útil de queijo Minas. Ciênc Tecnol Aliment. 2009 Apr; 29(2):262-69. doi: 10.1590/S0101-20612009000200004
2. Visotto RG, Oliveira MA, Prado SPT, Bergamini AMN. Queijo Minas Frescal: perfil higiênico-sanitário e avaliação da rotulagem. Rev Inst Adolfo Lutz. 2011;70(1):8-15.
3. Brasil. Diário Oficial da União. 05 Sep 1997. Brasil. Ministério da Agricultura e do Abastecimento. Portaria nº 352, de 04 de setembro de 1997. Regulamento Técnico para Fixação de Identidade e Qualidade de Queijo Minas Frescal. Diário Oficial [da República Federativa do Brasil]. 8 set 1997;172(Seção 1):19684-7. Available from: https://wp.ufpel.edu.br/inspleite/files/2016/03/Portaria-n%C2%B0-352-de-4-de-setembro-de-1997.pdf
4. Sobral D, Costa RGB, Paula JCK, Teodoro VAM, Moreira GMM, Pinto MS. Principais defeitos em queijo Monas artesanal: uma revisão. Rev Inst de Laticínios Cândido Tostes. 2017;72(2):108-20. doi:10.14295/2238-6416.v72i2.600
5. Ausani TC, Lopes GV, Costa EF, Corbellini LG, Cardoso M. Microbiological quality of colonial cheese sold in Porto Alegre – RS. Semina: Ciência Agrárias. 2019 Mar;40(2):639-50. doi: 10.5433/1679-0359.2019v40n2p639
6. Ahmed Z, Wang Y, Ahmad A, Khan ST, Nisa M, Ahmad H, et al. Kefir and health: A contemporary perspective. Critical Rev Food Sci Nutrition. 2013 Feb;53(5):422-34. doi: 10.5433/1679-0359.2019v40n2p639
7. Prado MR, Blandón LM, Vandenberghe LPS, Rodrigues C, Castro GR, Thomaz-Soccol V, et al. Milk kefir: composition, microbial cultures, biological activities, and related products. Front Microbiol. 2015 Oct;6:1-10. doi: 10.3389/fmicb.2015.01177
8. Rosa DD, Dias MMS, Grzeskowiak LM, Reis AS, Conceição LL, Peluzio MCG. Milk kefir: nutritional, microbiological and health benefits. Nutrition Res Rev. 2017;30:82-96. doi: 10.1017/S0954422416000275
9. Chen TH, Wang SY, Chen KN, Liu JR, Chen MJ. Microbiological and chemical properties of kefir manufactured by entrapped microorganisms isolated from kefir grains. J Dairy Sci. 2009 Mar;92:3002-13. doi: 10.3168/jds.2008-1669

10. Awaisheh S, Rababah TM, Rahahleh RJ, Haddad MA, Al-Groom R, Ibrahim SA. Development of a novel white soft cheese using kefir starter cultures: Microbiological, physicochemical and sensory properties. Milk Sci Intern. 2016;69:18-22.
11. Rosa DM, Dias MMS, Grzeskowiak LM, Reis SA, Conceição LL, Peluzio, MCG. Milk kefir: nutritional, microbiological and health benefits. Nutrition Res Rev. 2017;30:82-96. doi: 10.1017/S0954422416000275
12. Wolfschoon AF, Vargas OL. Aplicação do método de formol para a determinação do teor de proteína no leite cru e pasteurizado. Rev ILCT. 1977;32(192):3-13.
13. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis of AOAC International. 17th ed. Arlington (US): AOAC; 2005.
14. Ayres M, Ayres Jr M, Ayres DL, Santos, AAS. Bioestat 5.0 aplicações estatísticas nas áreas das ciências biológicas e médicas. Belém (PA): IDSM; 2007.
15. Wolfschoon-Pombo AF, Lima A. Extensão e profundidade de proteólise em Queijo Minas Frescal. Rev Inst Latic "Cândido Tostes". 1989;44(261-266):50-4.
16. Magalhães KT, Pereira1 GVM, Campos CR, Dragone G, Freitas Schwan RF. Brazilian kefir: Structure, microbial communities and chemical composition. Braz J Microbiol. 2011;42:693-702. doi: 10.1590/S1517-83822011000200034
17. Rocha KL, Oliveira AP, Carvalho JWP. Avaliação da qualidade do leite "in natura", pasteurizado e esterilizado (UHT), comercializado em Barra do Bugres-MT. Enciclopédia Biosfera. 2016 Jun;13(23):114-26. doi: 10.18677/Enciclopedia_Biosfera_2016_010
18. Alves E, Ntungwe EM, Gregório J, Rodrigues, LM, Pereira-Leite C, Caleja C, et al. Characterization of kefir produced in household conditions: Physicochemical and nutritional profile, and storage stability. Foods. 2021 May;10(5):1050. doi: 10.3390/foods10051057
19. Kök-Taş T, Seydim AC, Özer B, Guzel-Seydim ZB. Effects of different fermentation parameters on quality characteristics of kefir. J Dairy Sci. 2013 Feb;96(2):780-9. doi: 10.3168/jds.2012-5753
20. Paredes JL, Escudero-Gilete ML, Vicario IM. A new functional kefir fermented beverage obtained from fruit and vegetable juice: Development and characterization. LWT – Food Sci Technol. 2022 Jan;154:112728. doi: 10.1016/j.lwt.2021.112728
21. Dias PA, Silva DT, Timm CD. Atividade antimicrobiana de microrganismos isolados de grãos de Kefir. Cienc Anim Bras. 2018;19:1-8. doi: 10.1590/1809-6891v19e-40548
22. Frigeri KDM, Frigeri KDM, Santin TP, Kalles NZ, Agostini A. Variação da composição, qualidade, produção e preço do litro do leite nas estações do ano em uma propriedade leiteira no norte do estado do Rio Grande do Sul - Brasil. Agrar Acad. 2020 Jun;7(13):65-74. doi: 10.18677/Agrarian_Academy_2020a7
23. Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Regulamentos Técnicos que fixam a identidade e as características de qualidade que devem apresentar o leite cru refrigerado, o leite pasteurizado e o leite pasteurizado tipo A. Diário Oficial da União. 30 nov 2018;230(Seção 1):9-10. Available from: https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=30/11/2018&jornal=515&pagina=9
24. El-Gawad MAM, Ahmed NS. Cheese yield as affected by some parameters. Review. Acta Sci Pol Technol Aliment. 2011;10(2):131-53.
25. Andrade APC, Quirino MF, Silva TL, Carvalho JDG. Avaliação dos parâmetros físico-químicos de queijos Minas Frescal e Ricota comercializados em Fortaleza Ceará. Rev Ciência Agron. 2020;51(2):e20186414. doi: 10.5935/1806-6690.20200022
26. Oliveira KAM, Jardim DM, Chaves KS, Oliveira GV, Vidigal MCTR. Avaliação físico-química, microbiológica e sensorial de queijo minas frescal de leite de cabra desenvolvido por acidificação direta e fermentação lática. Rev Inst Latic "Cândido Tostes". 2016;71(3):166-78. doi: 10.14295/2238-6416.v71i3.533
27. Paula JCJ, Carvalho AF, Furtado MM. Princípios básicos de fabricação de queijo: do histórico à salga. Rev Inst Latic "Cândido Tostes". 2009 Mar/Jun;64(367/368):19-25.
28. Soares DB, Monteiro GP, Fonseca BB, Freitas EA, Mendonça EP, Melo RT, et al. Análise sanitária e físico-química e adequação bacteriológica do queijo Minas artesanal produzido em duas propriedades. Cienc Anim Bras. 2018;19:1-13. doi: 10.1590/1809-6891v19e-36499
29. Saboya LV, Oliveira AJ, Furtado MM, Spadoti LM. Efeitos físico-químicos da adição de leite reconstituído na fabricação de queijo Minas Frescal. Food Sci Technol. 1998;18(4):368-78. doi: 10.1590/S0101-20611998000400002