

## Search for *Staphylococcus* spp. in “Minas Frescal” cheeses made with raw milk and commercialized in the city of Formiga - MG

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### Abstract

“Minas Frescal” cheese is defined by legislation as a fresh cheese obtained by enzymatic coagulation of the milk with rennet and/or other appropriate coagulant enzymes, supplemented or not with the action of specific lactic bacteria. Brazilian law requires cheese to be made from pasteurized milk and prohibits marketing when made with raw milk, considering the risk of being an important vehicle of pathogenic microorganisms. The objective of this work was to analyze the presence of *Staphylococcus* spp. in artisanal “Minas Frescal” type cheeses made with raw milk and sold locally in the municipality of Formiga, Minas Gerais. The microbiological standard for this microorganism is defined in technical regulation. Plating in Mannitol Salt Agar was conducted in order to search for *Staphylococcus* spp. and differentiate *S. aureus* from non *S. aureus*. Results obtained from analyzed samples ( $10^4$  to  $10^6$  CFU/g) suggest the presence of *Staphylococcus aureus* above the maximum established limits ( $10^2$  CFU/g). It can be concluded that sampled cheeses were not suitable for consumption due to high counts of *Staphylococcus* spp., which could be due to the fact of their production with raw milk.

**Keywords:** Contamination. Foodborne illness. Pathogenic Microorganism.

## Pesquisa de *Staphylococcus* spp. em queijos “Minas Frescal” feitos a partir de leite cru e comercializados no município de Formiga-MG

### Resumo

O queijo “Minas Frescal”, de acordo com a legislação, é definido como o queijo fresco obtido por coagulação enzimática do leite com coalho e/ou outras enzimas coagulantes apropriadas, complementada ou não com ação de bactérias lácticas específicas. A legislação brasileira exige que estes sejam elaborados a partir de leites pasteurizados e proíbe a comercialização quando elaborados com leite cru pois, assim, podem ser importantes veículos de micro-organismos patogênicos. O objetivo deste trabalho foi analisar a presença de *Staphylococcus* spp. em queijos do tipo “Minas Frescal” elaborados de forma artesanal (com leite cru) e comercializados em mercados locais do município de Formiga - MG. O padrão microbiológico para este micro-organismo está definido em seu regulamento técnico de identidade e qualidade. O plaqueamento em Ágar Sal Manitol foi realizado em busca de *Staphylococcus* spp. e afim de diferenciar *S. aureus* de *S. não aureus*. Os resultados das amostras analisadas foram sugestivos para a presença de *Staphylococcus aureus* ( $10^4$  to  $10^6$  UFC/g), ultrapassando os limites máximos permitidos estabelecidos ( $10^2$  UFC/g). Pode-se concluir

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que os queijos não estavam aptos para consumo pela elevada contagem de *Staphylococcus* spp., que pode se dar pelo fato destes serem elaborados a partir de leite cru.

**Palavras-chave:** Contaminação. Doenças transmitidas por Alimentos. Micro- organismo Patogênico.

## Introduction

Milk is the ideal growth medium for the development of several microorganisms, especially bacteria, which are the main causes of diseases transmitted by dairy products worldwide (Grace; Haveelar, 2020).

One of the most popular dairy products in Brazil is “Minas Frescal” cheese, being made from enzymatic coagulation of milk (Martins, 2012).

Brazilian law requires that “Minas Frescal” cheeses be produced from pasteurized milk and, in addition, prohibits their legal commercialization when made with raw milk. However, this commercialization has been carried out openly, in several Brazilian states (Lima; Cardoso, 2019; Vinha; Pinto; Chaves, 2018).

“Minas Frescal” cheese flowchart is basically composed of: pasteurization of milk, addition of bacterial culture, calcium chloride and rennet, cutting and stirring the mass, hanging, draining, salting and packaging the cheese. Pasteurization can be done by two different processes, HTST and LTLT. HTST (high temperature, short time) is conducted by heating the milk at 72–75°C for 15 to 20 seconds and the LTLT (low temperature, long time), at 65°C for 30 minutes and, in both cases, the milk must be immediately cooled to 35°C, to add the next ingredients (Santos et al., 2019).

According to Decree-Law Nº 923, of October 10, 1969, the sale of raw milk for direct consumption by the population is prohibited (Brasil, 1969). Although it is prohibited by law to sell it, it still occurs in small towns, where the inhabitants maintain the habit of consuming raw milk due to a belief that this product is purer and healthier. In addition, it has lower cost per liter and its purchase is more comfortable (door to door) (Raymundo; Bersot; Osaki, 2017).

There are many factors that are related to food illness, such as erroneous temperature during cooking, cooling and/or food storage; poor personal hygiene from manufacturers; cross-contamination between raw and processed products; as well as improper monitoring of processes (Lindsay et al., 2021).

One of the major problems with the consumption of these cheeses made with raw milk is the presence of bacteria of the genus *Staphylococcus*. This is due to the fact that these microorganisms are among the most present in artisanal cheeses (especially with regard to the absence of pasteurization) and have high pathogenic potential,

as they produce thermoresistant enterotoxins in foods, which make them important sources of food poisoning (Rosa et al., 2015).

To guarantee food security in the consumption of “Minas Frescal” cheese, Ordinance No. 146 of March 7, 1996, defined the maximum limit of 10<sup>3</sup> CFU/gram of coagulase positive *Staphylococcus* as a microbiological standard for this category of microorganism (Brasil, 1996).

Normative Instruction Nº 57 points out that artisanal cheese that is made with raw milk undergo a maturation period of 60 days or less, when technical-scientific studies prove that the reduced maturation time does not imply in reduced quality and/or no product safety. However, the only cheese made by hand, with raw milk and matured for varying periods, is “Minas Artisanal” cheese, which has guaranteed food security when maturation is carried out for the correct time and form (Brasil, 2013).

Given the illegality of the production of “Minas Frescal” cheese made from raw milk, the importance of the veterinarian role in inspecting this food is highlighted. Therefore, it is worth mentioning that in order to produce a milk or any dairy product with good quality and a high level of safety for human consumption, it is extremely important that the veterinarians involved in the production chain inspection perform their role (Santos; Carvalho, 2013).

The objective of this work was to research, quantify and differentiate bacteria of the genus *Staphylococcus* in “Minas Frescais” cheeses made with raw milk and sold in the municipality of Formiga - Minas Gerais.

## Material and Methods

The present experiment was carried out at the Microbiology Laboratory of the Centro Universitário de Formiga - Minas Gerais. For this, “Minas Frescal” cheeses from five producers, sold in local markets (in Formiga - MG), were used. The samples were purchased and kept under refrigeration until the experiment was carried out.

The methodology used to obtain the results was an adaptation of the technique described by Zurita et al. (2010).

From the samples obtained, one gram of each was weighed, under sterile conditions, for further dilution in sterile saline (0.9% NaCl). Five serial decimal dilutions of each sample were plated in sterile Petri dishes, using the pour plate method, containing mannitol salt agar (Kasvi, São José dos Pinhas, Brasil). This method consists of adding 1 mL of the selected dilutions in Petri dishes, adding the culture medium and homogenizing. After plating, the plates were incubated in temperatures between 35-37°C, for at least 48 hours.

After incubation, the colonies were counted and differentiated. The differentiation was made through the colony staining and they were divided into white and yellow. To differentiate the colonies' morphology, they were visualized in immersion, under optical microscopy, at 100x magnification, after GRAM staining.

The differentiated colonies were later added in test tubes with Brain Heart Infusion Broth (BHI, Himedia, Mumbai, India) and incubated at 35-37°C for a minimum period of 48 hours. After cultivation in tubes containing BHI broth, each tube was plated again, using Mannitol Salt Agar (Kasvi). However, using the striation method, which consists of adding the medium to the Petri dishes and waiting for their solidification and, with the aid of platinum handles, flambéed and submerged in each

tube, the colonies are striated and the plates incubated again, for a period of 48 hours, at temperatures of 35-37°C. Finally, the differentiation of the colonies on the plates was done in *S. aureus* and not *aureus*, observing the fermentation of mannitol present in the medium and a change in color (or not) for this differentiation. The experiment was done in duplicate, with one repetition.

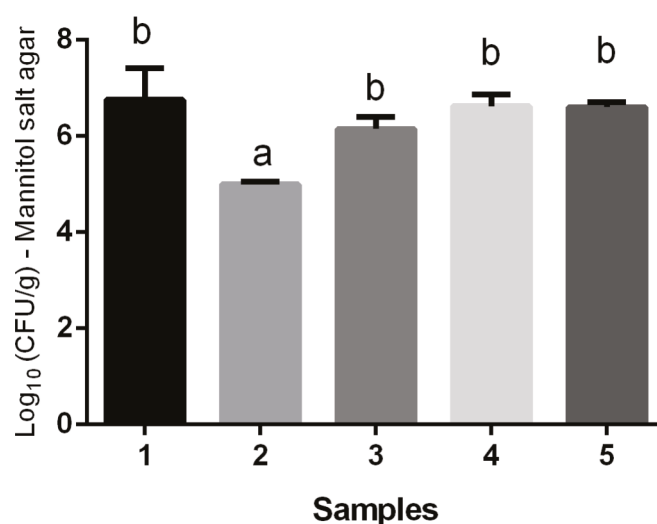
Data comparison was performed with GraphPad Prism 6.0 (GraphPad Software, San Diego, USA) using One-way ANOVA with Tukey post-hoc test, at a 5% significance level ( $p < 0.05$ ).

## Results and discussion

The results of the “Minas Frescal” cheese samples from the Formiga region - Minas Gerais can be seen in Figures 1 and 2.

Figure 1 shows the results of the total count of *Staphylococcus* spp. and it is possible to observe that all samples obtained a very high count of this bacterium, a worrying fact, since this genus of bacteria can be transmitted through the hands of manipulators and become an important source of food contamination. In addition, several species of this genus can cause food related disease in humans (Dittmann *et al.*, 2017).

Figure 1 – Average results of the total counts of *Staphylococcus* spp. in mannitol salt agar of “Minas Frescal” cheeses made from raw milk, sold in Formiga - MG



Caption: different letters represent different results ( $p < 0.05$ ) by One-way ANOVA with Turkey post-hoc test.

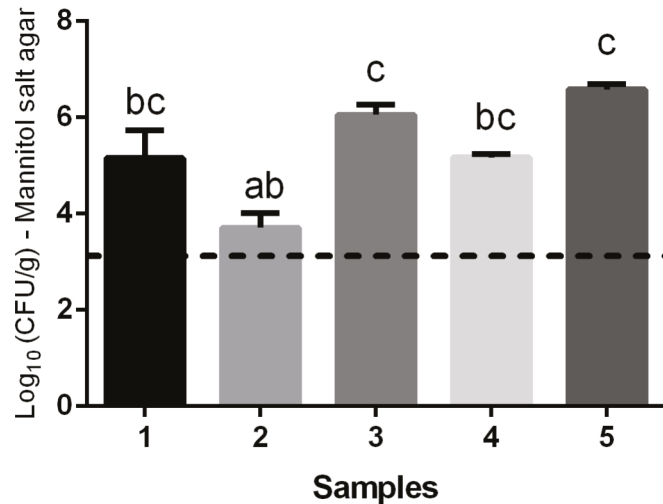
In Figure 2, the results obtained are suggestive of *Staphylococcus aureus*, which are coagulase positive, since there was fermentation of the mannitol from the mannitol salt agar and color change to yellow. It is possible to observe that 100% of the samples, in addition to obtaining a high count of *S. aureus*, do not attend the microbiological standard provided for in Ordinance Nº 146 from 1996, from MAPA (Agricultural and Livestock Ministry and Supply) (Brasil, 1996). This Ordinance

recommends that the samples obtain a maximum count of 1.000 CFU/g (or  $1 \times 10^3$  CFU/g or  $3 \text{ Log}_{10}$  CFU/g) of coagulase positive *Staphylococcus*. A worrying result, since high counts of this microorganism suggest failures in processing, such as absence or deficiency in pasteurization, inadequate hygiene conditions, as well as incorrect storage temperature (Lindsay *et al.*, 2021). Another worrying factor is that this agent can produce staphylococcal toxins, which cause food poisoning. For the toxin to be

able to accumulate at levels that cause food poisoning, approximately  $10^6$  CFU/g of food is needed (Forsythe,

2013). This demonstrates that two of the analyzed samples have the potential to cause food poisoning.

Figure 2 – Average results of total colony counts suggestive of *Staphylococcus aureus* on mannitol salt agar of “Minas Frescal” cheeses made from raw milk, sold in Formiga - MG



Caption: different letters represent different results ( $p < 0.05$ ) by One-way ANOVA with Turkey post-hoc test. Dashed line represents the maximum limit defined by legislation ( $3 \text{ Log}_{10} \text{ CFU/g}$ ).

From the visualization of the Figures 1 and 2, it was possible to observe that sample 2 had a lower ( $p < 0.05$ ) count of microorganisms, when compared to samples 3 and 5, but still not in compliance with the legislation. This result was possibly due to the fact that it was made from a better quality milk, with lower load of microorganisms and/or in better hygiene conditions, which are points closely linked to the quality of the “Minas Frescal” cheese (Filho e Filho, 2000).

The legislation does not have microbiological standards for other species of the *Staphylococcus* genus, such as coagulase negative. This demonstrates that there is no agreement with what has already been reported by Heilmann; Ziebuhr; Becker (2019) about the pathogenicity of coagulase negative *Staphylococcus*, since it is proven it can also cause foodborne poisonings.

According to Ordinance N<sup>o</sup> 451 of 1997 of the National Health Surveillance Agency (ANVISA) (Brasil, 1997), products that present *S. aureus* in a value of up to 10 times the maximum limit established in microbiological standards, are considered “Products in condi-

tions of unsatisfactory health ”and, when they present *S. aureus* in a value greater than 10 times the maximum limit established, they are considered “Products potentially capable of causing foodborne illness”. Therefore, almost all samples approached  $1 \times 10^5$  ( $5 \text{ Log}_{10}$ ) or  $1 \times 10^6$  ( $6 \text{ Log}_{10}$ ) CFU/g, or even passed, except for sample 2, showing that they are products potentially capable of causing diseases transmitted by foods. Sample 2, even though it is of better quality, is considered a product in unsatisfactory hygiene and sanitary conditions, as it is close to  $1 \times 10^4$  ( $4 \text{ Log}_{10}$ ) CFU/g in *Staphylococcus aureus* count.

## Conclusion

According to the results obtained after conducting the research on “Minas Frescal” cheese samples in Formiga - MG, it was found that all samples had high counts of positive coagulase *Staphylococcus (aureus)*, well above that established by therefore, unfit for consumption. This represents a public health concern, as these cheeses are not inspected and their raw material does not undergo heat treatment.

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