

MICROBIOLOGICAL AND PHYSICAL-CHEMICAL QUALITY OF MINAS FRESCAL CHEESES COMMERCIALIZED IN A MUNICIPAL FAIR IN JI-PARANÁ – RONDÔNIA

QUALIDADE MICROBIOLÓGICA E FÍSICO-QUÍMICA DE QUEIJOS MINAS FRESCAL COMERCIALIZADOS EM FEIRA MUNICIPAL DE JI-PARANÁ - RONDÔNIA

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RESUMO: A demanda da produção de queijo tem aumentado de forma demasiada, fazendo-se necessária uma busca pela qualidade do produto comercializado. Por isso, objetivou-se verificar a qualidade microbiológica e físico-química de queijos minas frescal comercializados em feira municipal de Ji-Paraná-RO. Foi realizado um estudo experimental de finalidade explicativa com a abordagem quali-quantitativa por meio de coletas de amostras de queijo minas frescal comercializados em feira municipal de Ji-Paraná-RO. Foram adquiridas três amostras em dias distintos de três comerciantes diferentes. As amostras foram nomeadas como Q1, Q2 e Q3 e foram acondicionadas em sacos estéreis como também em bolsa isotérmica e foram encaminhadas para análises no Laboratório de Microbiologia do Centro Universitário São Lucas, Câmpus de Ji-Paraná-RO. O estudo mostrou que 100% das amostras do queijo minas frescal estavam contaminadas coliformes totais, termotolerantes e *Salmonella sp*, inclusive houve detecção de *Escherichia coli* nas amostras de queijo. Os resultados encontrados para pH e umidade indicaram alterações de qualidade. Esses resultados são indicativos da má qualidade das condições de produção do queijo, sobretudo no armazenamento e na manipulação. Dessa forma, demanda-se uma fiscalização mais rígida na região de produção, como melhor monitoramento dos materiais utilizados, sanidade do rebanho e a metodologia de armazenamento. Além do mais, deve-se salientar a importância de os produtores serem capacitados e conscientizados sobre a implantação das boas práticas de fabricação, bem como a inserção de selos municipais para venda, para que se possa assegurar a saúde do consumidor.

Palavras-chave: coliformes totais, queijos de feira, *Salmonella sp*, saúde pública, termotolerantes

ABSTRACT: The demand for cheese production has increased a lot, making a search for the quality of the marketed product necessary. Therefore, the objective was to verify the microbiological and physical-chemical quality of minas frescal cheeses marketed at a municipal fair in Ji-Paraná-RO. An experimental study with an explanatory purpose was carried out with a qualitative and quantitative approach through the collection of samples of minas frescal cheese

sold at a municipal fair in Ji-Paraná-RO. Three samples were purchased on different days from three different traders. The samples were named Q1, Q2 and Q3 and were packed in sterile bags, as well as in an isothermal bag, and were sent for analysis at the Microbiology Laboratory of Centro Universitário São Lucas, *Campus* de Ji-Paraná-RO. The study showed that 100 % of the Minas Frescal cheese samples were contaminated with total coliforms, thermotolerants and *Salmonella sp*, including the detection of *Escherichia coli* in the cheese samples. The results found for pH and humidity indicated changes in quality. These results are indicative of the poor quality of cheese production conditions, especially in storage and handling. Thus, a stricter inspection is required in the region of production, such as better monitoring of the materials used, herd health and the storage methodology. In addition, it is important to emphasize the importance of producers being trained and aware of the implementation of good manufacturing practices, as well as the insertion of municipal seals for sale, in order to ensure consumer health.

Keywords: fair's cheeses, public health, *Salmonella sp*, thermotolerant microorganisms, total coliforms

INTRODUCTION

With an important role played in the Brazilian market, milk influences the social and economic order, with an important participation in the Gross Domestic Product (GDP), with 172 million bovine heads and with an annual production reaching 30 billion liters of milk. Thus, the derivatives market has considerable growth, with cheese as its main product, the same having 316 thousand tons of annual production (IBGE, 2017; MORAES; BREMER FILHO, 2017).

The municipality of Ji-Paraná, located in the state of Rondônia (RO), in the central region, has a herd of 35,980 heads of milking cows distributed in 2,229 milk properties (IBGE, 2017). With the excessive growth of the sector and in view of a larger organization, it is possible to apply some public policies that can leverage milk production. As it is characterized as a chain that is constantly evolving, a perspective was implemented for Brazil and expects that, in 2023, there will be a production of 46.7 billion liters of milk, considering an annual growth of 3.2 %, which would be 42 % higher than the records of 2012 (IBGE, 2017).

From the analysis of the industrial production of dairy derivatives regarding the destination of the milk collected, it appears that most dairy products produce mozzarella cheese, followed by industry cream, common butter with salt and pasteurized milk (SEBRAE, 2015). Having a mixed composition of proteins, which in turn have a high biological value, as well as lipids, minerals and vitamins, both in Brazil and in the rest of the world, cheese is one of the most widespread and also the most suffered products adaptation in their production techniques, giving rise to new production techniques (ABIQ, 2019).

Given the factors that can directly influence the quality of the cheese, as well as the nutritional value, flavor and aroma, there is: the nutrition in the lactation period, the origin of the milk, the breed and the region in which the dairy animal is located, their food nutrition, which is differentiated in some periods of the year, as well as the production process (PEREIRA, 2001; SILVEIRA JUNIOR et al., 2012). The differentiation of the types of cheese is based on some characteristics resulting from the type of milk used, type of coagulation, consistency of the paste, fat content, maturation time, type of skin, among other factors that directly influence its production (PERRY, 2004). Artisanal cheeses can be characterized by their production method. This method can be traditional, produced in a rudimentary way, outside the legislation and with low quality control, and in most cases it is sold informally (NHUCH, 2004; SEBRAE, 2015).

Among the various types of artisanal cheese sold, the one that stands out the most is minas frescal, being a product that emerged to add value to milk production, complementing the income of small producers. According to the Brazilian Micro and Small Business Support Service (SEBRAE, 2015), this cheese is originally from Brazil and has a maturation period of about 30 days (SILVA et al., 2015; ECKERT and WEBBER, 2016). Considering its rudimentary production, minas frescal cheese can be characterized as a frequent vehicle of foodborne microorganisms and pathogens, focusing on its artisanal production, which in short is made with raw milk, which for the most part is consumed without proper maturation. The microbiological contamination of artisanal cheeses assumes an outstanding relevance, as much for the industry as for the economic loss and for the population, considering the risk of being able to cause alimentary diseases (HARDY et al., 2003).

For the production of cheese, pasteurized milk must be used. However, the use without processed milk is allowed for cheeses that will go through the maturation process, as long as the maturation time is respected, and if high quality raw materials are used and comply with what is foreseen in the Good Practices (PERRY, 2004). Milk destined for the production of cheeses submitted to a maturation process at a temperature of more than 5°C for a period of not less than 60 days is excluded from the obligation of pasteurization or other heat treatment (BRASIL, 2017). There are no standardized techniques for the production of artisanal cheeses, as well as technical regulations on identity (RTIQ). Considering the absence of data such as its chemistry composition, production, technology employed and its commercialization, it can be emphasized that its clandestine manufacture is assiduous (SILVA et al., 2015).

It is worth mentioning that the quality of minas frescal cheese is strongly linked to the quality of the raw material and the hygiene of equipment, heat treatment, handling and storage, as well as its conservation and distribution (ROOS, 2005). Thus, the importance of searching for a product that has good origins and meets quality standards is emphasized. The present work is justified by the studies developed on the quality of cheeses produced in the municipality of Ji-Paraná-RO, since changes in quality, both microbiological and physical-chemical, are evidenced (ANTONELLO et al., 2013; SEBRAE, 2015).

Based on this context, the objective was to verify the microbiological and physical-chemical quality of minas frescal cheeses marketed at a municipal fair in Ji-Paraná-RO.

MATERIAL AND METHODS

For microbiological analysis, serial dilutions of the samples were performed in 1.0 % peptone water. 25.0 g of each sample were weighed, aseptically, and placed in sterile bags with 225.0 ml of peptone water, previously identified (SILVA et al., 2010; SOARES et al., 2018). The methodology used to analyze total coliforms and thermotolerant coliforms was the Most Probable Number (MPN), being a methodology performed in two phases: presumptive test and confirmatory test. Therefore, the methodology mentioned is described by Silva et al. (2010) and Soares et al. (2018).

The presumptive test for total coliforms consists of the use of a selective enrichment medium for coliforms, thus recovering the injured microorganisms, with an increase in the population and gas production. To carry out the presumptive test, 1.0 mL of the serial dilutions of the samples were inoculated in the Broth Lauryl Sulfate Tryptose (LST), in tubes containing Durham tube. The tubes were incubated for 24 hours at 35 to 37°C. After 24 hours, gas formation was observed, which indicated positivity (DIAS et al., 2016).

In the confirmatory test for total coliforms, aliquots of the positive tests were inoculated in tubes containing Durham tube in Bright Green Bile Lactated Broth, and the presence of bile salts in this medium inhibits the growth of gram-positive microorganisms. The

tubes were incubated for 24 to 48 hours, at 35 to 37°C, so that the formation of gas would confirm the presence of total coliforms (DIAS et al., 2019).

To confirm the presence of thermotolerant coliforms, an aliquot of each tube that tested positive for the presumptive test was inoculated into the *Escherichia coli* (EC) broth with a Durham tube. The inoculated tubes were incubated in a water bath at 45.5°C for 24 hours, with the objective of confirming the presence of thermotolerant coliforms, as they have the capacity to ferment lactose at higher temperatures (PINTO et al., 2011). For confirmation of *Escherichia coli*, EMB agar (Methylene Blue Eosin) was used, where positive samples were replanted in the EC broth and depleted in the EMB broth, and incubated for 48 hours at a temperature of 35 to 37 °C.

For the analysis of *Salmonella sp.*, 25.0 g of each sample were weighed and placed in separate sterile bags containing 225.0 mL of peptoned water and vigorously homogenized, after which were diluted for 5 minutes and then incubated at 35°C for 24 hours. After that, dilutions of up to 10^{-3} were performed in tetrathionate broth, and incubated again for 24 hours at 35°C. After the incubation period, streaks were performed on the *Salmonella - Shigella* (SS) agar and incubated for 24 hours at 35°C. After, it was observed whether there was formation of black colonies and an analysis of the colony's morphology was performed (SILVA et al., 2010; CAVADO, 2010).

To perform the moisture analysis, the methodology of the Adolfo Lutz Institute (2008) was adopted, where 10.0 g of each sample were weighed and placed in previously prepared porcelain crucibles, which were subjected to incubation (105.0°C) and dried in a desiccator with silica gel until they reached the appropriate temperature to store the samples. Soon after, the samples were incubated for 3 hours, at 105.0 °C, and posteriorly cooled in a desiccator and weighed. This process was repeated until the weight of the samples was constant.

For the analysis of the hydrogen potential (pH), 10.0 g of the sample were weighed, and with the aid of a beaker and a beaker the sample was diluted until the particles were uniformly suspended. Soon after, with the pH meter previously calibrated, the pH of the sample was determined. It should be noted that all physical-chemical tests were carried out in triplicate.

RESULTS

In 100 % of the samples of minas frescal cheese from the municipal fair of Ji-Paraná-RO, total coliforms, thermotolerants and *Salmonella sp.*, also including *Escherichia coli*, were detected. The values obtained for total coliforms were 4.6×10^5 , on average. However, this group of microorganisms does not yet have a detection limit established by national Brazilian legislation (**Table 1**).

Table 1. Results of the analysis of thermotolerant coliforms, *Escherichia coli* and *Salmonella sp.* in samples of minas frescal cheese sold at a municipal fair in Ji-Paraná-RO.

Cheese samples	Thermotolerant coliforms (NMP/g x 10^2)	<i>Escherichia coli</i>	<i>Salmonella sp.</i>
Q1	75	Presence	Presence
Q2	64	Presence	Presence
Q3	43	Absence	Absence

The results found for pH and humidity are indicative of changes in the samples. According to Silva (2005), the average pH of minas frescal cheese is 5.0 to 5.3. Values outside this average may indicate changes in the dairy product, as found by this study (**Table 2**).

Table 2. Physical-chemical results obtained by analyzing the samples of minas frescal cheese sold at a municipal fair in Ji-Paraná-RO.

Cheese samples	pH	Cheese moisture % (m/m)
Q1	6.27±0.17	74.89%±1.44
Q2	5.98±0.05	68.97%±0.76
Q3	5.53±0.02	62.78%±1.69

Based on the results summarized and presented in Tables 1 and 2, it is possible to detect changes in the quality of the production conditions of the minas frescal cheese. Above all, there was inadequate storage and inadequate handling. Thus, a stricter inspection of the production environment is required, such as better monitoring of the materials used, herd health and the storage methodology. These suggestions are not limited to just the municipal fair studied in Ji-Paraná, but to all public fairs in the country.

DISCUSSION

The microbiological criteria established by the Ministry of Agriculture, Livestock and Supply (MAPA) (BRASIL, 2018a) for chilled raw milk is 9×10^5 CFU/mL, for standard plate count, before using for the production of dairy products. ANVISA's RDC n° 12 of 2001 (BRASIL, 2001) establishes microbiological standards for very high humidity cheeses (humidity > 55 %), including minas frescal cheese: thermotolerant coliforms at 45.0°C, 5×10^2 per gram, *Salmonella sp* absent in 25,0 g.

Reis et al. (2019) evaluated the quality of minas frescal cheese produced in the city of Guarapuava-PR and it was found that 37.5 % of the analyzed samples were outside the commercialization standards for thermotolerant coliforms. A similar result was found by Dias et al. (2016), where 50 % of the industrialized samples used in the study were contaminated with thermotolerant coliforms above the current legislation, while 20 % of the samples of artisanal minas frescal cheese were also above the tolerable limit.

The results obtained by Nespolo et al. (2009) and Eckert and Webber (2016) are similar to those presented in Table 1 of this paper, where it is possible to visualize a high contamination by thermotolerant coliforms, as well as the presence of *Salmonella sp*, indicating a lack of hygiene in cheese production, thus, making the product unfit for consumption. Other results also indicated this problem, as in the study performed by Pinto et al. (2011), who observed that 100 % of the analyzed samples of minas frescal cheese (artisanal cheese with state inspection) were contaminated with thermotolerant coliforms. Also, Salotti et al. (2006) and Saleh et al. (2019) found that more than 80 % of the samples of minas frescal cheese sent for microbiological analysis of thermotolerant coliforms presented values above the limit allowed by the current legislation, in addition to the presence of *Salmonella sp*.

Therefore, we can emphasize that the dairy product derivatives marketed at public fairs and routinely used for the manufacture of artisanal cheeses do not go through the pasteurization process, so the contamination of the milk can occur due to the fact that the milking is not hygienic, due to the use of contaminated storage containers and even due to the time of cheese production. The lack of compliance with the storage temperature after production also contributes to microbial multiplication (it is recommended that the minas frescal cheese should be storage at a temperature less than 8.0 °C (BRASIL, 1997)).

Vieira et al. (2008), Nespolo et al. (2009) and Faval and Pinto (2010) detected, in minas frescal cheeses, the presence of *Escherichia coli* in over 90 % of the minas frescal cheese samples, being a strong indication of the poor quality of cheese production, taking into account the type of milk used, as well as the production method. Soares et al. (2018) evaluated samples of minas frescal cheese manufactured in three dairy industries in the state of Minas Gerais, and detected a population of thermotolerant coliforms of 106 (NMP)/g, a result well above the tolerable limit.

Regarding physical-chemical tests, Dias et al. (2016) in their studies, found a pH variation from 4.3 to 6.7, very similar to the variations shown in Table 2 of this work. Similar values were found by Gomes et al. (2011) where cheeses were stored at 8.0°C, and obtained a pH variation between 5.38 to 6.16, thus, similar to the data obtained in the present study. As for the moisture values, they are predicted by MAPA (BRASIL, 2004) for minas frescal cheese, which is classified as high humidity with values above 55 %, in agreement with Gomes et al. (2011) and Dias et al. (2016) and with the results found in this study.

In general, it is possible to observe that there is a high microbial load found in minas frescal cheeses marketed in different areas of Brasil, mainly at municipal public fairs (CAVADA et al., 2010; MORAES and BENDER FILHO, 2017). Also, public health problems are confirmed in all seasons, which is a very strong indication of contamination from the handlers, as well as the null or insufficient heat treatment of raw milk, not forgetting the poor quality of the raw material, which is one of the main factors (SOUZA et al., 2017). It is also possible to produce Minas Frescal cheese using raw milk, which is a major carrier of microorganisms. In addition, the place of storage and handling without hygiene, as well as inadequate preservation temperature also integrate the contributing factors with the poor quality of minas frescal cheese (MORAES and BENDER FILHO, 2017).

CONCLUSIONS

The results presented in this study indicate poor quality of minas frescal cheese production conditions, especially in storage and handling, since patterns outside the current legislation were found. Thus, a stricter inspection of the production region is required, such as better monitoring of the materials used, herd health and the storage methodology. In addition, it should be emphasized the importance of producers being trained and aware of the implementation of good manufacturing practices, such as the insertion of municipal seals for sale, so there can be a better possibility of ensuring consumer health.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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