



Microbiological analysis of half-cured Minas cheese sold informally in the city of Rio Branco, AC

Danielle Saldanha de Souza Araújo^{1*}, Cássio Toledo Messias², Patrícia Gelli Feres de Marchi², Bruna Laurindo Rosa², Carlos Augusto dos Santos Souza²

¹Graduada em Medicina Veterinária, Universidade Federal do Acre, Rio Branco, Acre, Brasil, ²Docente da Universidade Federal do Acre, Centro de Ciências Biológicas e da Natureza, Rio Branco, Acre, Brasil.
*dani-saldanha@live.com

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ABSTRACT

The semi-cured minas cheese has a smooth flavor and pleasant texture, with good acceptance among consumers in the city of Rio Branco. Produced from raw milk and with indefinite curing time, it does not meet the minimum curing time in the production of cheeses from raw milk established in the current legislation. This research aimed to evaluate the microbiological quality of 20 samples of semi-cured Minas cheese sold in the city of Rio Branco, AC. The samples were processed at the microbiology laboratory of the Food Technology Unit - UTAL, being fractionated, diluted, and inoculated in Petri dishes for standard agar counts, to estimate the amount of UFC/g of viable and mesophilic microorganisms. To determine the NMP/g of the total and thermotolerant coliforms, a multiple dilution test was performed, as a presumptive test and the confirmatory tests in Bile Brilliant Green Agar broth for total coliforms and EC broth for thermotolerant coliforms, where 70% of the samples were considered unfit for consumption, serving as an alert for the implementation of good manufacturing practices in the manufacture and regularization of their sale, since the lack of control of the production chain represents a risk to public health.

Keywords: Coliforms. Milk. Sale.

Análise microbiológica de queijos Minas meia cura comercializados informalmente no município de Rio Branco, AC

RESUMO

O queijo minas meia cura possui sabor suave e textura agradável, tendo grande aceitação entre os consumidores da cidade de Rio Branco. Produzido a partir de leite cru e com o tempo de cura indeterminado, não atende o tempo mínimo de cura na produção de queijos advindos de leite cru estabelecido na legislação vigente. Esta pesquisa teve como objetivo avaliar a qualidade microbiológica de 20 amostras de queijos minas meia cura comercializados na cidade de Rio Branco, AC. As amostras foram processadas no laboratório de microbiologia da Unidade de Tecnologia de Alimentos – UTAL, sendo fracionadas, diluídas e inoculadas em placas de Petri para contagem padrão em ágar, para estimar a quantidade de UFC/g de microrganismos facultativos e mesófilos viáveis. Para determinar o NMP/g dos coliformes totais e termotolerantes foram realizados o teste de diluições múltiplas, como teste presumitivo e os testes confirmativos em caldo Ágar Verde Brilhante Bile para coliformes totais e caldo EC para coliformes termotolerantes. Aonde 70% das amostras foram consideradas impróprias para o consumo, servindo de alerta para a implantação das boas práticas de fabricação na fabricação e regularização da sua venda, visto que a ausência de controle da cadeia produtiva representa um risco à saúde pública.

Palavras-chave: Coliformes. Leite. Venda.

INTRODUCTION

The semi-cured minas cheese has good acceptance among the Brazilian population consumed among people of all age groups. It is a matured cheese, of semi-cooked, pressed and salted dough, it is classified as a semi-fat to fat cheese and of medium humidity.

It should be cylindrical in shape and have a thin, slightly yellowed, crack-free shell (BRASIL, 2019).

Information on the physicochemical data of minas half-cured cheese is scarce, probably due to the difficulty of standardizing this product since it is an informal product, which presents variations in the manufacturing technique, as a consequence, the final result of the product (RAIMUNDO, 2013).

Little is known about the real health status of minas cheese sold as half cured because although there is theoretical knowledge about the potential risk that these cheeses made from raw milk represent to consumers, there is no specific inspection in place that meets their specification. There is a need to know the risk to public health that these products represent the population (SOUZA et al., 2006).

According to the public health perspective, the population should have the right to have access to good quality food, free of contaminants and within pre-established standards by the competent agencies, not only in nutritional value but also in terms of hygienic requirements that ensure safety for consumer health (CORREIA; RONCADA, 1997).

The sale of animal food at free fairs, in booths without cover or refrigeration, without barrier against dust and insects, can significantly modify its quality. Especially cheeses, which are sold in pieces, there is a risk of being contaminated, externally with contaminant materials such as insects and mites or earth. It may be due to product handling failures during the sale to the fraction, the unsatisfactory hygiene of the stalls and utensils used by the fair, or even by cross-contamination between the exposed products (CORREIA; RONCADA, 1997).

This research work aimed to evaluate the microbiological quality of minas half-cured cheeses sold in the city of Rio Branco, CA since the absence of current legislation keeps this product informal.

MATERIAL AND METHODS

Twenty samples of minas half cured cheese were collected, acquired at different commercial points and fairs in Rio Branco, Acre during the months of October and November 2019. Where data were evaluated in the form of indicative sample analysis, which according to RDC No. 12 of ANVISA 2001, is composed of several sampling units lower than that established in a sampling plan contained in the specific legislation.

The results of the 20 samples obtained for total coliforms, thermotolerant coliforms, and aerobic or facultative heterotrophic microorganisms and viable mesophiles were observed. These results are compared to RDC No. 12 and ANVISA in No. 60, December 23, 2019.

The samples weighed approximately 200 grams, packed in a plastic bag, stored in most places without refrigeration. Immediately after collection, the packaged samples were packed in an isothermal pouch containing reusable artificial ice and sent to the laboratory for analysis.

The analyses were performed in the Food Technology Unit (UTAL), the integrating organ of UFAC in the laboratory the samples were aseptically weighed, where 25g of the cheese was added to the Erlenmeyer bottle containing 225 mL of 0.1% sterilized and homogenized peptone saline solution. Then, decimal dilutions up to 10^{-3} , were prepared (BRASIL, 2017).

The standard counting procedure of aerobic or facultative heterotrophic microorganisms and viable mesophiles was poured placement. With the inoculation of 1mL of each dilution in the bottom of Petri dishes sterilized, in duplicate, and distributed in two series.

Then, 15 to 20 mL of standard agar for counting (PCA) was added and the temperature cooled around 45°C. After cooling and homogenizing and solidifying the agar at room temperature for 20 minutes, the plates were incubated inverted in an oven at 36°C for 24 hours (CETESB, 2018).

The count was performed on the inoculated plate with dilution 10^{-2} whenever possible, without duplicate, calculating the number of CFU/g multiplying the number of colonies by the inverse of the inoculated dilution (SILVA et al., 2010), providing the number of mesophilic microorganisms per gram of the analyzed sample.

However, the procedure for the Determination of MPN of total coliforms/g dilutions 10^{-1} to 10^{-3} , were inoculated with 1mL, respectively, three tubes of Tryless Lary Sulphate Broth with inverted Durhan tube. After inoculation these tubes were

incubated at 36°C for 24 hours, and those that were revealed to be gas production or effervescence were considered positive when gently agitated (BRASIL, 2017).

After confirming the presence of total coliforms, two drops of culture were incubated for corresponding tubes containing 2% Lactose Bile Bright Green Broth and inverted Durhan tube, with subsequent incubation at 36C° for 24 hours (BRASIL, 2017).

RESULTS AND DISCUSSION

The results of all microbiological analyses carried out on the 20 samples of minas half cured cheese, table 1, which was designated from 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

Table 1 - Results of analyses carried out with minas half cured cheese marketed in Rio Branco, AC.

Sample	Mes (UFC/g ou mL)	Col Tot (NMP/g ou mL)	Col Ter (NMP/g ou mL)
01	1x10 ¹	3,0	3,0
02	8x10 ¹	2,4x10 ²	2,4x10 ²
03	1,1x10 ³	2,4x10 ³	2,4x10 ³
04	4,5x10 ³	2,4x10 ³	1,1x10 ³
05	3x10 ⁴	2,9x10 ²	2,4x10 ³
06	3,5x10 ⁴	2,9x10 ²	2,9x10 ²
07	7x10 ¹	2,4x10 ³	2,4x10 ³
08	2x10 ⁴	2,4x10 ³	2,4x10 ³
09	1,1x10 ³	2,4x10 ²	2,4x10 ³
10	6x10 ³	2,4x10 ³	1,1x10 ¹
11	3x10 ²	2,4x10 ³	1,1x10 ³
12	4,8x10 ⁴	2,4x10 ³	2,9x10 ²
13	1,07x10 ⁵	2,9x10 ²	1,1x10 ³
14	3,9x10 ²	2,4x10 ³	2,4x10 ³
15	4,8x10 ⁴	2,4x10 ³	2,4x10 ³
16	1,2x10 ⁵	2,4x10 ³	2,4x10 ³
17	1x10 ²	2,4x10 ³	2,4x10 ³
18	6x10 ²	2,9x10 ²	2,9x10 ²
19	5x10 ²	2,4x10 ³	2,4x10 ³
20	1,3x10 ³	2,4x10 ³	2,4x10 ³

Mes: mesophiles; Col tot: Total coliforms; Col ter: Thermotolerant coliforms.

The samples collected presented mostly different visual appearance and coloration, such as textural and color differences. These results suggest a lack of control of the manufacturing process of this type of cheese, which was expected because it is an informal product production.

The result of the presumptive test for total and fecal coliforms was measured by the result presented by the presence of the air bubble in the inverted Durham tube in the Lauryl Sulfate Tryptose Broth. Each sample identified as positive was submitted to the NMP investigation of total and thermotolerant coliforms by the test with EC broth and Bile Bright Green Agar Broth.

The presence of coliforms at 35°C and coliforms at 45°C in number beyond what is allowed by the legislation is related to the lack of pasteurization of the raw material and an excessively high initial microbial load from low-quality milk. It may also be associated with contamination of packaging, handlers with poor hygienic and/or inefficient habits, contaminated equipment, among others (SANTOS, 2016).

The results for total and thermotolerant coliforms ranged from 3.0 to >2400 MPN/g. According to ANVISA RDC No. 12 of 2001, the maximum acceptable count of thermotolerant coliforms for medium humidity and high humidity cheeses is 10^3 NMP/g and 5×10^3 NMP/g, respectively. The Regulation does not specify the number of total coliforms allowed in that food. This normative instruction was replaced by ANVISA's IN no. 60 in December 2019, while it became more rigid with the number of microorganisms found and started to use *Escherichia coli* as a biological marker, as it was verified the existence of other types of fecal coliforms present in the environment, and not only in the intestinal tract with before it was thought, the maximum amount of *E. coli* was stipulated at 10^3 and there is no mention of the allowed amount for mesophiles, total coliforms and fecal coliforms in general.

However, normative instruction no. 60, of December 23, 2019, does not present a maximum value for the presence of total or thermotolerant coliforms, only values for the presence of *E. coli*, which for cheeses with humidity equal to or above 46% humidity – which is where the half-cure cheese fits – has a limit of 10^3 for experimental units (ANVISA, 2019).

The total coliform count and thermotolerant coliforms remained at a similar percentage. Total coliforms presented 65% of the samples with a count greater than 2.4×10^3 NMP/g and the thermotolerant coliforms showed close results, with 70% of the results equal to or greater than 2.4×10^3 NMP/g, according to Table 2.

Table 2 - Distribution of samples by NMP/g interval of total and fecal coliforms in half cured minas cheese samples purchased in the city of Rio Branco, in October and November 2019.

	<3	3 -- 10 ¹ NMP/g	10 ¹ -- 10 ² NMP/g	10 ² -- 10 ³ NMP/g	>10 ³ NMP/g
Total coliforms	- *	1 (5%)	-*	6 (30%)	13 (65%)
Thermotolerant coliforms	-*	1 (5%)	1 (5%)	7 (35%)	11 (55%)

* - No samples shown are in this range.

According to the results, 65% of the total coliform samples and 70% of the samples of thermotolerant coliforms were above the legislation, having as parameter the IN no. 60 of December 23, 2019, and adopting humidity equal to or above 46% for this type of cheese.

The standard count in plates of viable mesophilic or facultative heterotrophic micro-organisms ranged from 1×10^1 to 1.2×10^3 CFU/g, presenting in 24 hours. The results of 35% of the samples presented results lower than 6.0×10^2 CFU/g and 65% of the samples indicating higher indices, from 1.1×10^3 to 1.2×10^5 UFC/g. According to Santos (2016), the measurement of these microorganisms helps in the evaluation of the quality of the product and the efficiency of its conservation method.

The establishments that presented the product to the consumer without indication of origin, expiration date, packed in a plastic bag without mandatory information, at room temperature and absence of adequate hygiene practices, confirming the practices observed in the commercial points evaluated in the works of Correia and Roncada (1997); Pedro (2003); Favaro et al., (2006); Sousa et al., (2006); Raymond (2013); Santos André and Sturion (2015).

The sale of this product is commonly carried out in fractional form and the washing of the entire part or parts in tap water was carried out in two establishments. The cut was made with a kitchen knife, without any previous asepsis, and packed in an unmarked plastic bag, as observed in our work.

Starikoff (2011) in his work, found that 60 days of cure in cheese made of raw milk, as determined by the legislation, are not enough to eradicate pathogenic bacteria, which despite the lower incidence of water activity (Aw) acting, have an affinity with fat and the concentration of solids in cheese.

On the other hand, in the work of Martins et al. (2015), 256 samples of artisanal minas cheese were followed, it was necessary 29 days of cure at room temperature in the rainy season and 17 days in the dry period to promote the reduction of *E. coli* below

10³. While curing under refrigeration of 8°C was necessary 64 days in the rainy season and 33 days in the dry season to obtain the same result, a time longer than that required by the legislation regarding the use of raw milk in the production of cheeses. The cure at room temperature caused a decrease in moisture content, thus, causing an increase in sodium chloride concentration and reducing water activity (Aw), significantly decreasing contaminant microorganisms.

A similar study was conducted by Cardoso et al. (2013), where 50 cheese samples were cured in a cold chamber at 10°C and another 50 samples at room temperature (22 to 27°C). In this study, it took 30 days of curing for cheese manufactured during the dry season to present MPN/g of total and fecal coliforms less than 10³ and 60 days for the cheese produced in the rainy season to reach the same standard season.

Therefore, it was verified that the higher temperatures during the curing process can be combined in the ripening of cheese, promoting the metabolism of lactic acid bacteria (BAL) and the accumulation of metabolites responsible for inhibition and elimination of contaminating microorganisms, serving as a natural selector. This process can be optimized with the use of milk with good microbiological quality in cheese production (CARDOSO et al., 2013; MARTINS et al., 2015).

However, "drop" can be one of the causes of high counts of contaminating microorganisms, because the serum taken from contaminated cheese is reserved and used as yeast for the next day's cheese. Consequently, other sources of contamination may be, such as the materials used in production, which must be carefully controlled for cleaning to improve this traditional cheese (CARDOSO et al., 2013). This is added to the manipulation, often performed by people without any understanding of hygiene, since the manufacture of this product involves a lot of handling and results in microbiota with a high number of indicator microorganisms (especially *Staphylococcus aureus* and total and thermotolerant coliforms (MORICONI, 2013).

Studies have been done on the use of yeast enriched from BAL strains with antilisterial activity, with a great possibility of use in fermented dairy products as a starter or adjunct cultures. In the study by Campagnollo et al. (2018) six strains of *Lactobacillus Brevis* and *Lactobacillus Plantarum* and 4 *Enterococcus faecalis* with antilisterial activity were isolated. Its addition in artisanal cheeses half healing mines was carried out in raw milk and added of selected endogenous BAL, where it resulted in the *L. monocytogenes* inactivation. These findings suggest that the addition of BAL

strains with antilisterial activity may constitute an additional viable obstacle to inhibit the growth of *L. monocytogenes* during the storage of this type of cheese and inactivate this pathogen during the maturation period. Taking into account the inactivation of *L. monocytogenes*, the application of this endogenous BAL would allow us to complete the ripening process of cheese at 22 °C within 22 days, thus ensuring food safety.

More effective measures are needed to establish good manufacturing practices in production since the curing time established for raw milk will only be sufficient if it is concomitant with the good quality of milk and hygiene and sanitization of the production environment. The lack of control of the production chain represents a risk to public health.

CONCLUSIONS

70% of the minas half cured cheeses sold in Rio Branco were described as unfit for human consumption, constituting a risk to the health of consumers due to inadequate product quality, raw materials, and/or improper conditions of processing, storage, and marketing of the product.

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