

**DETERMINATION OF WATER ACTIVITY, pH, COAGULASE POSITIVE *Staphylococcus* COUNTS, TOTAL AND FECAL COLIFORM COUNTS IN MINAS MEIA CURA CHEESE SOLD IN STREET MARKETS IN THE SOUTHERN REGION OF THE CITY OF SÃO PAULO**

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**ABSTRACT**

The objective of the present study was to determine the water activity and pH, as well as to enumerate coagulase positive *Staphylococcus*, total and fecal coliforms in 32 samples of *Minas meia cura* cheese purchased in street markets in the southern region of the city of São Paulo. In order to classify the product in terms of percentage moisture content, 5 samples were submitted to the analysis. Mean values obtained in physical-chemical analyses were 0.96 for water activity, 4.91 for pH. These values do not represent obstacles for the multiplication / survival of agents of hygienic-sanitary importance in the product. Considering that the mean moisture level was 40.31%, this cheese is classified as presenting medium moisture, according to the regulation RDC no.12. In the analyses performed, 40.62 % (13/32) of the samples presented fecal coliform counts above the standard determined by the regulation, and 28.12% (9/32) of them were above the standard for coagulase positive *Staphylococcus*. It should be pointed out that 3 (9.37%) of the samples were above the standards for both analyses. Thus, 19 (59.38%) samples were considered to be inadequate for consumption because they presented at least one of the microbiological requirements above the legal standards determined for the product, and therefore, were classified as “products in inadequate sanitary conditions”.

**Key words:** *Minas meia cura* cheese; coagulase positive *Staphylococcus*; coliforms, water activity, pH.

**DETERMINAÇÃO DA ATIVIDADE DE ÁGUA E pH, E ENUMERAÇÃO DE *Staphylococcus* COAGULASE POSITIVA, BEM COMO DE COLIFORMES EM QUEIJO, TIPO MINAS MEIA CURA, VENDIDO EM FEIRAS-LIVRES DA ZONA SUL DO MUNICÍPIO DE SÃO PAULO**

**RESUMO**

O presente estudo visou determinar a atividade de água e pH, a enumeração de *Staphylococcus* coagulase positiva, bem como coliformes totais e fecais em 32 amostras de

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queijos Minas meia cura vendidos em feiras-livres da zona sul do município de São Paulo. Para fins de classificação do produto quanto ao teor de umidade percentual, cinco amostras foram submetidas a essa análise. Os valores médios obtidos das análises físico-químicas foram 0,96 para a atividade de água e 4,91 para o pH; esses resultados não representam um obstáculo para a multiplicação/sobrevivência de agentes de importância higiênico-sanitária no produto. Considerando que o teor de umidade médio obtido foi 40,31%, o queijo é classificado como de média umidade, segundo RDC n.12. Assim, 40,62 % (13/32) das amostras apresentaram valores de coliformes fecais acima do máximo permitido e 28,12% (9/32) acima do valor para *Staphylococcus* coagulase positiva; ressalta-se que 3 (9,37%) encontravam-se com os limites excedidos para ambas as análises. Desta forma, 19 (59,38%) amostras são consideradas impróprias para o consumo por apresentar pelo menos um dos requisitos microbiológicos acima do padrão legal e, portanto, classificadas como “produto em condições sanitárias insatisfatórias”.

**Palavras-chave:** queijo Minas meia cura; *Staphylococcus* coagulase positivo; coliformes, atividade de água, pH.

## DETECCIÓN DE LA ACTIVIDAD DE AGUA, DEL pH Y NUMERACIÓN DE *Staphylococcus* COAGULASE POSITIVA Y COLIFORMES EN QUESO TIPO MINAS MEDIA CURA EN FERIAS LIBRE DE LA ZONA SUR DEL MUNICIPIO DE SÃO PAULO

### RESUMEN

Este estudio determina la actividad de agua, el pH, y enumeración de *Staphylococcus* coagulase positiva de coliformes totales y fecales en 32 muestras compradas en ferias libre de la zona sur del municipio de São Paulo; para los fines de clasificación del producto como la tasa de humedad porcentual, 5 muestras fueron sometidas a esa análisis. Los valores medios obtenidos del análisis físico químico fueron de 0,96 para a actividad de agua y 4,91 para el pH; esos resultados no representan un obstáculo para la multiplicación/sobrevivencia de agencia de importancia higiénico sanitaria en el producto. Considerando que el teor de humedad medio obtenido fue 40,31%, el queso es clasificado como de media cura, segundo RDC n.12. a si el 40,62% (13/32) de las muestras presentaron valores de coliformes fecales superior a lo máximo permitido y el 28,12 (9/32) superior a este valor para *Staphylococcus* coagulase positiva; se resalta que el 3 (9,37%) se encontraban con los límites excedidos para los dos análisis. De esta manera, 19 (59,38) muestras son consideradas inadecuadas para el consumo por presentar por lo menos uno de los requisitos microbiológicos superiores de lo padrones legales y por lo tanto se clasifica como producto en condiciones sanitarias insatisfechas.

**Palabras-clave:** queso Minas media cura; *Staphylococcus* coagulase positiva; coliformes, actividad de agua, pH.

### INTRODUCTION

*Minas meia cura* cheese is generally artisanally manufactured, without official inspection, using raw milk and unknown hygienic-sanitary conditions. Besides, it is traded at room temperature, without an adequate packaging, no indication of origin or expiring date. There is no official specific regulation that standardizes its production, and few scientific

reports on the sanitary quality of the product, its water activity ( $A_w$ ) and the influence of this parameter on microbial activity.

The quantity and variety of microorganisms in cheese is determined by the quality of raw material, thermal processing, hygienic conditions of manufacture, packaging, storage temperature, as well as the characteristics of the product that regulate bacterial survival and multiplication.

The present report is a study on water activity, pH, total and fecal coliform counts (MPN/g) and coagulase positive *Staphylococcus* (CFU/g) counts in *Minas meia cura* cheese sold in street markets in the southern region of the city of São Paulo.

## MATERIAL AND METHOD

Thirty two samples of *Minas meia cura* cheese purchased in street markets were analyzed in the microbiology and physical-chemical laboratory at the Department of Preventive Veterinary and Animal Health at Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (FMVZ-USP). Because most of the times the product has no identification of the manufacturer and/or batch and in order to minimize the possibility of repeating batches of the same producer, samples were purchased in different traders and on different days, in several street markets in the southern region of the city of São Paulo.

Baird Parker agar with potassium tellurite and egg yolk (35°C/48h) was used for the coagulase positive *Staphylococcus* analysis. Typical colonies were cultured in BHI broth (35°C/24h) and submitted to the coagulase test (35°C/ 4-24h). In the enumeration of coliforms, the MPN technique was used, with BGB broth with 2% lactose (presumptive test, 35°C/48h) and confirmation in EMB Levine agar (35°C/48h – total coliforms) and BVB with 2% lactose and triptone broths (44.5°C/48h – fecal coliforms), according to Brazilian official methods (BRASIL, 1991/1992).

For the water activity analysis, the sample was cut in small pieces and placed in the cuvette of the Decagon analyzer, 3TE. Direct reading was performed in the equipment after some minutes. Determination of pH was performed (BRASIL, 1981) with the aid of a digital pHmeter Digimed – DMPH 2.

Moisture was determined (BRASIL, 1981) in 5 samples of cheese in order to enable the comparison with legal parameters determined by RDC no. 12 (BRASIL, 2001).

Data gathered was submitted to descriptive statistical analysis. The analysis was carried out using SPSS (SPSS Inc.).

## RESULTS AND DISCUSSION

Based on data gathered in moisture analyses performed in 5 samples, *Minas meia cura* cheese was classified as presenting medium moisture, according to legal regulation RDC no. 12 (BRASIL, 2001). It presented, respectively, minimum, mean and maximum moisture equal to 37.50; 40.31 and 44.89%. Maximum tolerable counts for the product determined by the regulation are  $10^3$  MPN/g of fecal coliforms and  $10^3$  CFU/g for coagulase positive *Staphylococcus*. The regulation does not present any limit for total coliforms.

The values obtained for pH and  $A_w$  are showed in Table 1. It should be emphasized that the lowest values were, respectively, 4,61 and 0,938 and that all samples showed pH and  $A_w$  that give support for multiplication of several microorganisms important from hygienic-sanitary standpoint, including *Staphylococcus aureus* and *Escherichia coli*, based on Franco; Landgraf (1996); Forsythe (2002) and Germano & Germano (2003).

**Table 1** – Values of pH and Aw in *Minas meia cura* cheese samples purchased in street markets in the southern region of the city of São Paulo, May – August, 2004.

	Aw	pH
Minimum	0,938	4,61
Mean (standard deviation)	0,965 (0,012)	4,92 (0,15)
Maximum	0,983	5,34

From the samples that presented Aw lower than 0.96 (10/32), only one presented fecal coliform counts above  $10^3$  MPN/g, what is in agreement with literature information that this is the limit for the multiplication of *Escherichia coli* (FRANCO & LANDGRAF, 1996). It is interesting to notice that pH of this sample was 4,9, above the minimum described by the author for the multiplication of this agent.

Fifty-four samples of *Minas frescal* cheese was analyzed by Nascimento et al. (2001) and the mean pH observed was 5.34 ( $\pm 0.63$ ), value that coincides with the maximum observed in the present trial. It suggests that the curing developed in the *Minas meia cura* cheese results in a reduction on the pH what could be explained by the activity of the fermentative microorganisms during the curing.

Table 2 shows the number of samples per count interval for coagulase positive *Staphylococcus*, where it is observed that all samples showing growth (9/32; 28.12%) the counts were over  $10^6$  CFU/g, therefore, none of these samples complied with the standard for medium moisture cheese. It should be emphasized that over  $10^5$  CFU/g, enough toxin is produced to cause food born intoxication (FRANCO & LANDGRAF, 1996). All these samples revealed, respectively, pH and Aw up to 4,5 and 0,87, which are the minimum values for toxin production, according to Forsythe (2002). Considering that this kind of cheese is sold at room temperature and the toxin production occurs between 10 and 48°C, based on the author, these samples are, probably, at very high risk to cause food poisoning, depending on the presence of toxigenic strains.

**Table 2** – Distribution of the samples per interval of UFC/g of *Staphylococcus* positive coagulase in *Minas meia cura* cheese samples purchased in street markets in the southern region of the city of São Paulo, May – August, 2004.

	< $10^2$ *	$10^2$  -- $10^6$	$10^6$  -- $10^8$
	UFC/g	UFC/g	UFC/g
<i>Staphylococcus</i> positive coagulase	23 (71,88%)	-**	9 (28,12%)

\* corresponds to the detection limit of the technique

\*\* - means that no sample in this interval.

There was a great variation in total and fecal coliforms count. Mean values for these agents were, respectively,  $5.5 \times 10^6$  and  $9.7 \times 10^4$  MPN/g. As for the fecal group, 40.62% of the samples (13/32) were above the legal recommendation for medium moisture cheese. Table 3 shows the number of samples per count interval for coliforms.

**Table 3** – Distribution of the samples per interval of MPN/g of total and fecal coliforms in *Minas meia cura* cheese samples purchased in street markets in the southern region of the city of São Paulo, May – August, 2004.

	< 3	3  -- 10 <sup>1</sup> MPN /g	10 <sup>1</sup>  --10 <sup>2</sup> MPN /g	10 <sup>2</sup>  --10 <sup>3</sup> MPN /g	>10 <sup>3</sup> MPN /g
Total coliforms	-*	-*	3 (9.4%)	11 (34.4%)	18 (56.2%)
Fecal coliforms	2 (6.2%)	1 (3.1%)	6 (18.8%)	10 (31.2%)	13 (40.6%)

\* - No sample presented count in this interval.

Sousa et al. (2004) analyzed 31 samples of uninspected *Minas meia cura* cheese sold in the city of Jacareí-SP, and results obtained showed a greater number of samples inadequate for consumption: 96.8% (30/31) of them presented coagulase positive *Staphylococcus* and/or fecal coliforms above the standard set by RDC no.12. All samples presented Aw between 0.83 and 0.96, lower than observed in the present trial, but which still enable the multiplication of pathogenic agents of importance in cheese, such as *Staphylococcus aureus* and *Listeria monocytogenes*.

Moura Pedro (2003), analyzed *Minas meia cura* cheese sold in São Paulo and the results showed that 73,33% (44/60) of the samples were above the maximum limit determined by legal regulation RDC 12 for fecal coliforms and 26,66% (16/60) for *Staphylococcus aureus*.

There are few reports on *Minas meia cura* cheese, however, data on the contamination of Minas frescal cheese is abundant (PERESI et al., 2001; HOFFMANN et al., 2002; ALMEIDA FILHO et al., 2002; SALOTTI, 2004; BARROS et al., 2004; ALMEIDA FILHO & NADER FILHO, 2005).

In a research with *Minas curado* cheese (BORELLI et al., 2000) was observed 80% (8/10) of samples that did not comply with the regulation.

Results found in the present trial show that, although *Minas meia cura* cheese may present intrinsic factors that are less favorable to the survival/multiplication of microorganisms (lower pH and Aw) when compared to those of *Minas frescal* cheese, these intrinsic factors are not enough to ensure an acceptable microbiological characteristic. However, as expected, the literature reveals that the artisan-type *Minas frescal* cheese shows greater number of non-compliance with the RDC no.12 (PERESI et al., 2001; ALMEIDA FILHO et al., 2002) compared with *Minas meia cura* cheese analyzed in the present trial.

So, it is necessary that efforts are made in several sectors of society in order to improve the hygienic-sanitary quality of the product and reduce its risk to public health. These actions should include the government, milk producers, dairy manufacturers, cheese traders and consumers. The government should standardize and monitor all the dairy production chain, adopt public policies that incentive the production and adoption of good manufacturing practices, besides promoting public health education campaigns on the risks of the consumption of uninspected products. As for producers and traders, they should implement good food handling practices, and consumers should choose officially inspected products.

## CONCLUSION

It is concluded that intrinsic factors of the cheese as pH and Aw are not restrictive for microorganisms growth and the consumption of *Minas meia cura* cheese represents a great risk for the consumer.

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