A microbiological and physical-chemical based evaluation of artisanal Minas cheese production in Rio Paranaíba city, Minas Gerais, Brazil

Avaliação microbiológica e físico-química na elaboração de queijo minas artesanal no município de Rio Paranaíba, Minas Gerais

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Abstract

The aim of this study was to evaluate the microbiological and physical-chemical features of the water and milk used in the preparation of artisanal cheese produced in seven dairies, located in Rio Paranaíba, Minas Gerais, Brazil, in order to ascertain the conformity of these parameters with the current legislation. Physical-chemical analyzes of water (alkalinity, acidity, residual chlorine, chlorine and pH), milk (fat, protein, total solids, nonfat dry extract, acidity, cryoscopy and density) and cheese (moisture content, presence of starch and alkaline phosphatase) were performed. Also, it was determined the most probable number of coliform at 30 °C, at 45 °C and mesophilic count in the samples of water, milk and cheese. Cheese weight, diameter and height were also determined to verify the patterning of the physical format of cheeses. The water used by dairies showed satisfactory microbiological characteristics. However, the water in properties 2 and 7 showed mean values of alkalinity approximately 2 times higher and lower than the recommended range (10 to 50 mg $CaCO_3.L^{-1}$), respectively. Considering the confidence intervals, no water sample showed satisfactory results for alkalinity and residual chlorine determinations. With regards to raw milk samples, three samples showed mesophilic bacteria count greater than 1 x 10^5 CFU/mL (property 1, 5 and 7), whereas four and two samples showed number of coliforms at 30 °C and at 45 °C higher than 1100 NMP/mL, respectively. The milk produced in the property 2 showed confidence interval values for density (1.033 to 1.035), above the recommended limit. In addition, the confidence interval for the freezing point of milk in all properties were out of recommended values (-0.550 to -0.530 °H). Seventy-five percent of the cheeses had coliform counts at 30 °C greater than 1 x 10^3 MPN/g and 100% of the samples had humidity value above 45.9% (maximum allowed after maturation). The weight of cheeses from the properties 2, 4, 5, 6 and 7 also exceeded the recommended limit (1-1.2 kg). The artisanal cheese-making process requires some adjustments to meet the standards of current legislation, aiming to bring added value and greater product safety.

Keywords: Artisanal cheese. Good manufacturing practices. Food safety.

Resumo

O objetivo desse trabalho foi avaliar características microbiológicas e físico-químicas da água e do leite utilizados na elaboração do queijo artesanal, produzido em sete queijarias localizadas em Rio Paranaíba, Minas Gerais, a fim de averiguar a conformidade desses parâmetros com a legislação vigente. Análises físico-químicas da água (alcalinidade, acidez, cloro residual, cloreto e pH), do leite (teor de gordura, proteína, extrato seco total, extrato seco desengordurado, acidez, crioscopia e densidade) e do queijo (teor de umidade, presença de amido e fosfatase alcalina) foram realizados. Também foi determinado o número mais provável de coliformes a 30 °C, 45 °C e a contagem de mesófilos nas amostras de água, leite e queijo. Para averiguar a padronização do formato dos queijos foi determinado o peso, diâmetro e altura. A água utilizada pelas queijarias apresentou características microbiológicas satisfatórias. No entanto, as propriedades 2 e 7 tiveram valores médios de alcalinidade aproximadamente 2 vezes maior e menor do que o limite recomendado (10 a 50 mg $CaCO_3.L^{-1}$), respectivamente. Considerando os intervalos de confiança, nenhuma amostra de água apresentou teores de alcalinidade e cloro residual satisfatórios. Das amostras de leite cru, três apresentaram contagem de mesófilos superior a 1 x 10^5 UFC/mL (propriedades 1, 5 e 7) e para coliformes a 30 °C e 45 °C foram detectadas contagens superiores (>1100 NMP/mL), em quatro e duas amostras, respectivamente. O leite produzido na propriedade 2 apresentou intervalo de confiança para valor de densidade (1,033-1,035), acima do limite recomendado. Além disso, todas as propriedades apresentaram o intervalo de confiança para a crioscopia fora dos valores recomendados (-0,550 a -0,530 °H). Setenta e cinco porcento dos queijos apresentaram contagens de coliformes a 30 °C superiores a 1 x 10^3 NMP/g e 100% das amostras estavam com umidade acima de 45,9% (máximo permitido após a maturação). O peso dos queijos das propriedades 2, 4, 5, 6 e 7 também extrapolou o recomendado (1-1,2 Kg). Conclui-se que o processo de elaboração dos queijos artesanais analisados necessita de ajustes para se adequar a legislação em vigor, visando agregação de valor e maior segurança do produto.

Palavras-chave: *Queijo artesanal. Boas práticas de fabricação. Alimento seguro.*

Introduction

The production of cow's milk in Brazil grew at a rate relatively constant from 1974 to the present day. According to the Municipal Livestock Research (IBGE), the country left the level of 7.1 billion liters of milk produced in that year, reaching the 35.174 billion liters of milk in 2014 (growth of over 350% in the period). The production of Minas Gerais accounts for 30% of this volume, and the Triangulo and Alto Paranaíba together now account for 23% of the state's production, followed by the South and Southwest regions occupying 18% and the Zona da Mata with 12% (IBGE, 2016). A great potential for growth in revenue and volume associated with milk agribusiness is achieved through the production of cheese.

Brazilian cheese production has great economic and social importance and is practiced by a large number of small producers. Minas cheese is the most traditionally cheese produced in the Minas Gerais state, being produced by small farmers and by large dairy industries (Perin et al., 2015). Minas Gerais state is the largest producer of cheese in the country, and had an important feature handmade production. Cheese manufacturing in Minas focuses on five main microregions (Cerrado, Serro, Campos das Vertentes, Serra da Canastra and Araxá), which are considered to be traditional producers that are protected by Law nº. 14.185 of January 31, 2002 (IMA, 2002). This legislation considers the cheese from these regions to be part of the intangible patrimony of Minas Gerais state (Magalhães et al., 2009). There is a remarkable quantity of farming family in Alto Paranaíba, with diversity product characteristic and significant involvement in dairy business. This area produces approximately 1.270 tons/month of artisanal Minas cheese to supply local, regional and even neighboring state markets. One municipality that stands out for its production is Rio Paranaíba, which contributes approximately 1.328 tons of artisanal Minas cheese/year. Cheese production is an activity that has a history, and it has been part of the cultural knowledge of local families since people saw an alternative income and a way to survive by manufacturing cheese (Almeida and Souza, 2003).

Currently, the Institute of Agriculture of Minas Gerais (IMA) is responsible for supervising and

instructing producers regarding sanitary conditions and good practices in obtaining raw materials, handling and manufacturing. The IMA also determine the quality of water, which must report to drinking and have chlorination system; standards of health protection for milk suppliers herds, designed to ensure its correct vaccination and adequate control of the use of antibiotics; the basic requirements of the milking parlor facilities, dairies, storage, packaging and transport (IMA, 2002).

However, artisanal cheese is made from raw milk and usually produced in small farms, which mostly do not adopt good manufacturing practices. Consequently, this commercially available product may pose a risk to public health if it is not manufactured under rigorously hygienic and sanitary care (Pinto et al., 2009). Food safety is a concern for consumers and agencies responsible for public health. Failures in quality control of raw material as well as cheese processing and storage can result in a poor quality product with a risk increase of consumers infecting and intoxicating (Zaffari et al., 2007).

By considering dairy production as an alternative income and a diversification form within family farms, it is understood that the production of milk and the manufacture of dairy products meet legislative requirements, which should not exclude small production systems but should strive for the qualification and safety of these products. Thus, the aim of this study was to evaluate the microbiological and physical-chemical features of raw material (the raw milk and water used in the process) for the artisanal cheese manufacture and the finished product (artisanal Minas cheese) as manufactured in dairies located in the municipality of Rio Paranaíba, Minas Gerais, Brazil, in order to ascertain the conformity of these parameters with the current legislation.

Material and methods

Sample collection and transport

Three samples of water, raw milk and artisanal cheese were collected from seven dairies located in the municipality of Rio Paranaíba, Minas Gerais, which had registration and certification issued by the IMA. All analyzes were performed in triplicate.

For the microbiological analyses, 250 mL samples of water were collected and for the physical-chemical analyses 2 L of water were collected from each property (Brazil, 2011). For raw milk, two samples were taken (100 mL and 1 L) for the microbiological and physical-chemical analyses, respectively (Brazil, 2002). All samples were placed in sterilized bottles and transported in cold refrigerated boxes, at approximately 4 °C, to the Laboratory of Microbiology of the Federal University of Viçosa at Rio Paranaíba Campus and to the Laboratory of Milk Analyzes RioLac, which is located in the municipality of Rio Paranaíba.

After seven days of ripening, three samples of Minas cheese were collected from each property. Cheese samples were manufactured with raw milk, which was also analyzed, as described previously. The handlers washed their hands and used disposable gloves, cleaned with 70% alcohol to the collect of cheeses. The cheeses were collected and packed in sterile plastic bags, and samples were transported under refrigeration for both physical-chemical and microbiological analyses (Brazil, 2003).

Microbiological analysis

Tests for the most probable number (MPN) of coliforms at 30 °C and 45 °C were performed on water samples. In addition to these tests, it was performed a standard counting of colony forming units (CFU/mL or CFU/g) of mesophilic aerobes and facultative strict viable in the milk and cheese samples. All analyses were performed according to the technical guidelines from Instruction nº. 62 of 2003 from the Ministry of Agriculture, Livestock and Supply (Brazil, 2003).

Physical-chemical analysis

The water physical-chemical parameters of interest were alkalinity, acidity, residual chlorine, chloride and pH, which were analyzed by following the methodologies described in the Practical Handbook of Water Analysis (Brazil, 2009).

Milk sample fat contents, proteins, percentage of total solids content (TSC), percentage of nonfat

dry substances (NDS) and milk densities were analyzed using an Ekomilk M-Cap milk analyzer by Specialized Technology Laboratory. Milk freezing point measurements, also called cryoscopy, were taken in Cryoscope ITR-MK540. The antibiotics presence was determined using a Charm MRL Beta-Lactam Kit from Charm Sciences, block dry Hexis Scientific (Brazil, 2006).

Moisture content, starch and alkaline phosphatase were the physical-chemical parameters of the cheese samples analyzed (Brazil, 2006). The cheeses were also weighed with a digital scale from Bel Equipment, model MARK 1003, the diameter and height were measured with a graduated ruler, to verify the standardization of the physical format of the samples. All analyses were performed with three replicates for each sample.

Statistical analysis

Descriptive statistics (mean and standard deviation) were made for microbiological parameters data. Physical-chemical data and physical format of cheese were submitted a Shapiro-Wilk normality test. In sequence, an ANOVA was performed in a completely randomized design with treatments like properties (3 repeats). Confidence intervals (95%) were made using Fisher-LSD test with Bonferroni correction for multiple comparisons. All statistical analysis was performed with R Program (2015).

Results and discussion

An examination of water that was mainly intended for human consumption had the highest priority. Through this analysis, one can be sure that the water in question is reliable and free of microorganisms or chemical compounds that can be harmful to human health. The microbiological results of this study indicated an absence of coliforms at 30 °C and 45 °C in water samples from all rural properties under analysis. These results are in accordance with the current legislation (Brazil, 2011), which mandates an absence of both microbial groups in 100 mL samples.

This is important to determine whether total and fecal coliforms were absent in the water of the dairies, since the microbiological quality of water can affect the quality of milk, which makes it difficult to obtain foods that meet the microbial standards of existing legislation (Guerra et al., 2011). Moreover, there are numerous studies reporting the lack of sanitary quality of water used for human consumption and food preparation. A similar study from the Serro, another Minas Gerais region, found 50% of samples exceeding the legislative limits (Ferreira et al., 2004). Samples of water that were inappropriate for consumption (86%) were also reported by Martins et al. (2004) in a study of cheese dairies in Araxá. Araújo et al. (2009) found that 100% of water samples from cheese dairies in the city of Luz, Minas Gerais, fell outside the potability standards for microbiological quality as established by the Ministry of Health.

The physical-chemical parameters of the water used to prepare cheeses were analyzed in each one of the properties, as can be seen in Table 1. Water samples collected on the property 2 and 7 had average values of alkalinity approximately 2-fold higher and lower than the recommended limits (10 - 50 mg $CaCO_3$/L), respectively. Considering the confidence intervals, no sample shows satisfactory alkalinity value. Generally, when the alkalinity is too high, it is necessary to acidify the water until its alkalinity is sufficient to react with the aluminum sulfate or other products used in their treatment. When the alkalinity is very low, there is a need to apply sodium carbonate, to make possible the dosage of chemicals used in treatment (Brazil, 2009).

Samples from rural properties 1, 2 and 3 had excessive average of residual chlorine (Table 1). Administrative rule 2.914/2011 from the Ministry of Health requires that the minimum concentration of free residual chlorine should be kept at 0.2 mg. L^{-1} with a maximum of 2.0 mg. L^{-1} at any point within the distribution network (Brazil, 2011). However, when the confidence intervals are considered, all properties had residual chlorine values outside the recommended range, suggesting that manufacturers must take corrections in water chlorination system. Excessive dosage is responsible for bad smells and flavors in water drinking, which cannot be considered of good quality. Chlorine is the most biocide used in disinfection of water. In rural areas, forms of water treatment are usually filtration and

chlorination. After treatment with chlorine, remains in water, a certain quantity of residual chlorine, as well as by products of disinfection. The World Health Organization (WHO) and Ministry of Health consider that a concentration of 0.5 mg/L of residual free chlorine in water, after a minimum contact time 30 minutes, ensures a satisfactory disinfection. On the other hand, the WHO not observed any harmful health effects, if the chlorine concentration reaches 5 mg/L. This concentration was taken as a reference value and not as a value to be reached (OPS, 2016; Brazil, 2011).

The average values of pH of the water from all properties are within the limits established by the Administrative rule 2914/2011 of the Brazil Ministry of Health, which recommends that the pH of the water intended for human consumption or food preparation must be maintained between the range of 6.0 to 9.5 (Brazil, 2011). However, pH of samples the property 1, 3, 4 and 5 showed a confidence interval below the recommended minimum (Table 1). The average pH value found in this study was 6.5, which appears to be similar to the pH (6.62) of the water used for making artisanal Minas cheese in Araxá, Minas Gerais region (Martins et al., 2004). The parameters acidity and chlorides obtained were within the recommended limits by law.

In addition to water, the characteristics of the raw material used to make cheeses are among the main factors that influence the quality of the resulting dairy products, and in this context, the physical-chemical parameters and the bacterial count in milk were highlighted. Microbiological indicators from the raw milk samples are presented in Table 2. The average for the most probable number of coliforms at 30 °C, coliforms at 45 °C and standard mesophilic counts ranged from 175.3 to > 1100 MPN.mL^{-1}, 0 to > 1100 MPN.mL^{-1} and 2.0 x 10^3 to 1.0 x 10^6 CFU.mL^{-1}, respectively, among the different rural properties. Normative Instruction nº. 51 from the Ministry of Agriculture, Livestock and Supply established that the total bacterial count in raw milk samples from individual tanks be at most 1.0 x 10^5 CFU.mL^{-1} (Brazil, 2002). The IMA also proposed that the total microbial flora present in raw milk should be no more than 1.0 x 10^5 CFU.mL^{-1} in paragraph IV of Decree 42.645/2002 (IMA, 2002). By this measure, it was found that milk samples from rural properties 1, 5 and 7 did not meet the legal limit.

Table 1 - Physical-chemical parameters of water samples from artisanal Minas cheese producers, Rio Paranaíba, Minas Gerais, Brazil

Dairy farm	Alkalinity (mg $CaCO_3.L^{-1}$)	Acidity (mg $CO_2.L^{-1}$)	Residual chlorine (mg $Cl_2.L^{-1}$)	Chlorides (mg $Cl^-.L^{-1}$)	pH
1	14.91 b (1.01 - 28.8)	13.00 bc (10.96 - 15.04)	15.76 b (13.75 - 17.77)	22.71 b (18.58 - 26.8)	6.11 a (5.20 - 7.01)
2	86.86 a (72.95 - 100.77)	19.96 a (17.92 - 22.01)	26.53 a (24.52 - 28.54)	39.85 a (35.72 - 43.97)	7.76 a (6.85 - 8.66)
3	15.73 b (1.82 - 29.64)	12.91 bc (10.86 - 14.94)	3.43 c (1.42 - 5.44)	18.86 b (14.74 - 22.99)	7.04 a (5.44 - 7.25)
4	15.73 b (5.82 - 33.64)	15.30 ab (13.26 - 17.34)	0.2 c (-1.81 - 2.21)	23.86 b (14.74 - 27.99)	6.35 a (5.09 - 6.90)
5	18.30 b (4.39 - 33.64)	8.03 cd (5.99 - 10.07)	0.2 c (-1.81 - 2.21)	21.33 b (19.74 - 24.37)	6.13 a (5.23 - 7.04)
6	45.83 b (31.92 - 59.74)	8.06 cd (6.02 - 10.11)	0.82 c (2.81 - 6.83)	18.86 b (16.12 - 19.40)	6.11 a (6.14 - 7.94)
7	5.50 b (3.59 - 31.41)	4.7 d (2.72 - 6.81)	0.2 c (-1.81 - 2.21)	15.27 b (11.15 - 19.40)	6.00 a (6.25 - 8.05)
CV (%)	35.01	14.04	22.02	14.38	11.23

Legend: CV % - Coefficient of variation.

Note: Different letters in the same column differ statistically by Fisher's LSD test ($p<0.05$). Values in parenthesis represent the confidence interval for the mean.

Table 2 - Microbiological analyses for raw milk used to make artisanal Minas cheese, Rio Paranaíba, Minas Gerais, Brazil

Dairy farm	Coliforms at 30 °C (MPN. mL^{-1})	Coliforms at 45 °C (MPN. mL^{-1})	Mesophilic bacteria (CFU. mL^{-1})
1	386.0	321.0	$1.0 \times 10^6 \pm 1.1$
2	>1100	>1100	$5.5 \times 10^3 \pm 2.7$
3	>1100	118.6	$8.6 \times 10^3 \pm 8.6$
4	477.6	ND	$2.0 \times 10^3 \pm 2.0$
5	>1100	273.3	$1.8 \times 10^5 \pm 10.1$
6	886.6	40.5	$2.9 \times 10^3 \pm 29.2$
7	>1100	>1100	$1.5 \times 10^5 \pm 9.6$

Legend: ND - not detectable.
Note: Mean ± Standard deviation.

Lima et al. (2006) reported a total bacterial count of 4.0×10^9 CFU.mL^{-1} in samples of raw milk from the state of Pernambuco and Nero et al. (2005) observed a standard mesophilic count above the limit in Normative Instruction nº. 51 in raw milk samples that were collected from four different Brazilian regions. Moreover, high coliform MPN values at 30 °C and at 45 °C were observed in these samples, indicating hygiene-related failures in the milking, collection and transportation tools. One important consideration is that samples were collected from unrefrigerated storage tanks after milking, which indicates contamination during this procedure. Similar data were also found in 48% of raw milk samples from Londrina, Paraná, which showed coliform counts at 30 °C above 10^3 CFU.mL^{-1} (Nero et al., 2005).

The fat, protein, total solids content, nonfat dry contents and acidity (Table 3) in raw milk samples from seven cheese dairies in the city of Rio Paranaíba were in accordance with the standards established by Normative Instruction nº. 51/2002 (Brazil, 2002) and State Decree nº. 44.864/2008 (IMA, 2008). It was also not detected presence of antibiotics in milk samples of the seven properties in the study.

According to the results shown in Table 3, the milk produced in the property 2 showed confidence interval of density parameter above the recommended (1033-1035). Current legislation requires that the density of milk must be between 1.028 and 1.034 (Brazil, 2002). Values below this range can indicate the addition of water, and values above can indicate fraud, which is committed by adding other substances or skim milk (Silva et al., 2008). The same results were observed in samples of pasteurized milk from the state of Alagoas, where 1.4% of the samples had densities outside the legal limit (Silva et al., 2008).

Cryoscopy analyses indicated that only milk samples of properties 2 and 3 exhibited values below the minimum recommended limit (Table 3). However, when the confidence intervals are considered, all properties had cryoscopy values outside the recommended range. Brazilian law recommends a freezing point of milk maximum -0.530 °H and minimum -0.550 °H (Brazil, 2006). The freezing point depression, which is also called the cryoscopy index, corresponds to the milk freezing point. This parameter provides a way to test for milk adulterations. Milk fraud is common, and most cases are related to the addition of water, which changes the cryoscopy index. The addition of any other substance may also change the physical and chemical parameters such as density, acidity and nonfat solids (Agnese et al., 2002).

Cheese sample microbiological analysis results are shown in Table 4. From all rural properties analyzed, 75% had coliform at 30 °C count greater than 1.0×10^3 coliforms.g^{-1}, which represents the

maximum limit stablished by IMA (IMA, 2008) for artisanal cheese. For the coliform at 45 °C count was observed that samples of cheese in all properties presented below the required count (5.0 x 10^2 coliforms.g^{-1}). Fernandes et al. (2011), evaluated the microbiological aspects of artisanal Minas cheese marketed in Rio Paranaíba, observed coliform counts at 30 °C that were above the legal limits, and 66% of those samples had counts above 5.0 x 10^2 MPN.g^{-1} for thermotolerant coliform.

Table 3 - Physical-chemical parameters of raw milk used for artisanal Minas cheese production, Rio Paranaíba, Minas Gerais, Brazil

Dairy farm	Fat (%)	Protein (%)	TSC (%)	NDS (%)	Acidity (°Dornic)	Cryoscopy (°H)	Density
1	3.98 a (3.70-4.20)	3.15 b (3.12-3.17)	13.07 a (12.84-13.29)	9.09 b (9.02-9.15)	16.38 a (16.01-16.77)	-0.540 a (-0.597 to-0.484)	1.031 c (1.030-1.032)
2	3.46 a (3.18-3.73)	3.30 a (3.27-3.32)	12.98 a (12.76-13.21)	9.52 a (9.45-9.59)	16.26 a (15.89-16.63)	-0.554 a (-0.611 to-0.498)	1.034 a (1.033-1.035)
3	3.74 a (3.46-4.01)	3.18 b (3.16-3.21)	12.95 a (12.73-13.18)	9.21 b (9.14-9.28)	15.44 a (15.07-15.80)	-0.562 a (-0.618 to-0.505)	1.033 abc (1.032-1.033)
4	3.34 a (3.07-3.61)	3.05 c (3.02-3.07)	12.20 b (11.98-12.43)	8.83 c (8.76-8.90)	16.27 a (16.62-17.36)	-0.532 a (-0.588 to-0.475)	1.034 ab (1.033-1.034)
5	3.56 a (3.28-3.83)	3.19 b (3.16-3.21)	12.77 a (12.54-12.99)	9.20 b (9.13-9.45)	16.99 a (16.62-1.36)	-0.535 a (-0.548 to-0.521)	1.033 abc (1.032-1.033)
6	3.33 a (3.06-3.60)	3.25 a (3.22-3.27)	12.71 ab (12.49-12.94)	9.38 a (9.31-9.45)	16.55 a (16.18-16.91)	-0.544 a (-0.600 to-0.487)	1.032 bc (1.032-1.033)
7	3.48 a (3.15-3.69)	3.25 a (3.22-3.27)	12.81 a (12.58-13.04)	9.38 a (9.31-9.45)	16.10 a (16.73-17.47)	-0.545 a (-0.601 to-0.488)	1.033 ab (1.033-1.034)
CV (%)	6.21	0.61	1.43	0.59	1.81	1,99	0.06

Legend: CV % - Coefficient of variation; TSC - Total solids content; NDS - Nonfat dry substances.

Note: Different letters in the same column differ statistically by Fisher's LSD test ($p < 0.05$). Values in parenthesis represent the confidence interval for the mean.

Table 4 - Results for microbiological analysis of artisanal Minas cheeses, Rio Paranaíba, Minas Gerais, Brazil

Dairy farm	Coliforms at 30 °C (MPN. g^{-1})	Coliforms at 45 °C (MPN. g^{-1})	Mesophilic bacteria (MPN. g^{-1})
1	> 1100	19.3	1.5 x 10^8
2	> 1100	5.0	4.1 x 10^6
3	> 1100	242.6	9.7 x 10^5
4	> 1100	40.4	9.4 x 10^6
5	155.0	87.0	1.1 x 10^6
6	> 1100	63.3	9.2 x 10^6
7	886.6	14.1	1.0 x 10^7

Note: Mean ± Standard deviation.

With regards to microbial indicators of hygienic conditions for the cheese and its manufacturing process, the population of mesophilic aerobic or facultative microorganisms ranged from 9.7 x 10^5 to 1.5 x 10^8 CFU.g^{-1} (Table 4). There are no standard limits for these microorganism populations in Brazil; however, their presence in food indicates the existence of favorable conditions for pathogen multiplication. Moreover, many of these microorganisms have great spoilage potential, which can promote changes in the organoleptic properties of these products.

Samples of cheese were collected and evaluated after seven days of production. Possibly, due to the short maturation time, the confidence interval obtained for all samples, with respect to the moisture content was above the standard established for marketing (maximum moisture content of 45.9% after ripening for artisanal cheese), as shown in Table 5 (IMA, 2008). Only cheese from property 3 showed average moisture content within the limit (45.83%). Brazilian federal law requires that products derived from raw milk be sold after 60 days of ripening. However, in the region of Alto Paranaíba, artisanal cheeses are usually delivered to dealers after only two days of ripening, which raises concerns about the product quality. It is believed that the maturation time associated with lactic acid bacteria, which are known as "pingo", is able to reduce the proliferation of pathogenic bacteria that may be present in cheese, due to reduction of moisture and water activity of product (Perry, 2004).

Study cheese samples had satisfactory results relating to the absence of starch and presence of alkaline phosphatase activity (Table 5). The IMA considers artisanal Minas cheese to be made according to historical and cultural traditions from the region in the state where it is produced by using whole cow's milk that is fresh and raw, with milking and processing taking place at the rural property of origin (IMA, 2002). The absence of starch and the presence of alkaline phosphatase in all samples indicated that the milk used for making cheese has undergone no thermal treatment, a requirement for artisanal cheese (IMA, 2002). Alkaline phosphatase is a hydrolytic enzyme in raw milk that is sensitive to pasteurization temperatures. Measuring the residual phosphatase gives information on the

Table 5 - Physical-chemical parameters for artisanal Minas cheese samples, Rio Paranaíba, Minas Gerais, Brazil

Dairy farm	Moisture Content (%)	Weights (Kg)	Diameters (cm)	Heights (cm)	Starch	Alkaline Phosphatase
1	53.30 a (46.15-60.46)	1.08 bc (1.02-1.14)	15.00 d (14.97-15.02)	4.00 c (3.82-4.17)	-	+
2	48.91 a (41.75-56.06)	1.32 a (1.26-1.39)	16.00 b (15.97-16.02)	4.76 b (4.58-4.94)	-	+
3	45.83 a (38.67-52.98)	1.07 ab (1.01-1.14)	16.50 a (16.47-16.52)	4.53 b (4.35-4.71)	-	+
4	61.42 a (54.26-68.57)	1.27 bc (1.21-1.33)	16.00 b (15.97-16.02)	4.93 ab (4.75-5.11)	-	+
5	62.60 a (54.26-68.57)	1.04 bc (0.97-1.10)	15.50 c (15.47-15.52)	4.60 b (4.42-4.77)	-	+
6	47.72 a (55.45-69.76)	1.03 bc (0.96-1.09)	15.53 c (15.50-15.56)	4.60 b (4.42-4.77)	-	+
7	61.53 a (54.38-68.69)	1.19 c (1.13-1.25)	16.00 b (15.97-16.02)	5.26 a (5.08-5.44)	-	+
CV (%)	8.52	4.47	0.14	3.06		

Legend: CV % - Coefficient of variation; -: Absence; +: Presence.

Note: Different letters in the same column differ statistically by Fisher's LSD test ($p < 0.05$). Values in parenthesis represent the confidence interval for the mean.

presence or absence of thermal treatment (Brazil, 2006).

The weight, diameter and height values of cheeses are shown in Table 5. The average values for diameter and height of cheeses were within the limits established by IMA, which recommends that the diameter and height for cheeses produced in the region of Cerrado should be 15 to 17 cm and 4 to 6 cm, respectively (IMA, 2008). However, for the weight parameter, the confidence intervals cheeses produced in property 2, 4, 5, 6 and 7 extrapolate the range of 1-1.2 kg, the recommended IMA (IMA, 2008).

Conclusion

The water used by dairies showed satisfactory microbiological characteristics. However, the water treatment system should be reevaluated due alkalinity and high concentration of residual chlorine found. Due to high count of mesophilic and coliform in samples of raw milk and insufficient ripening time, 75% of cheeses presented NMP/g of coliforms at 30 °C higher than established by law. These results indicate that the process of making artisanal Minas cheeses requires some adjustments to meet current regulations, with an emphasis on a safer product and higher added value.

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