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Avaliação da inocuidade e caracterização microbiológica do queijo minas artesanal produzido em Santa Vitória City, Brazil

Evaluation of the innocuousness and microbiological characterization of minas artisanal cheese produced in Santa Vitória, Brasil

Evaluación de la inocuidad y caracterización microbiológica del queso minas artesanal producido en la ciudad de Santa Vitória, Brasil

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Resumo

A fabricação de queijos artesanais constitui uma das mais importantes atividades da agroindús-tria familiar em Minas Gerais. Diante da importância econômica e cultural do Queijo Minas Artesanal, o objetivo deste estudo foi avaliar as características microbiológicas e a segurança do queijo artesanal de Minas (QMA) produzido em uma propriedade rural em Santa Vitória-MG. Doze amostras de queijo foram avaliadas em janeiro de 2019 (verão) e 12 amostras em julho de 2019 (inverno), em quatro tempos de maturação (1, 7, 14 e 21 dias). A evolução da umidade e do pH do queijo durante a maturação foi avaliada. Os coliformes foram contados a 30°C e 45°C, e a presença/ausência de *Staphylococcus aureus*, bactérias do ácido lático (BAL), bolores, levedura e *Salmonella* foram avaliadas. Também foram avaliadas as características microbiológicas do suprimento de água do queijo, leite, levedura endógena e superfície do queijo. Houve variação entre as duas estações do ano em termos de umidade e BAL. Diferenças significativas foram observadas nas contagens de coliformes a 30°C e 45°C, e a presença de *S. aureus*, BAL, bolores e leveduras no queijo foram avaliados durante a maturação nas duas estações. A maturação influenciou o teor de umidade, mas não influenciou o pH. Houve redução ao longo do tempo na contagem de patógenos. No final do amadurecimento, o queijo não apresentava parâmetros que garantissem sua segurança microbiológica. A maturação reduziu significativamente a contagem de microrganismos, mas o QMA não cumpriu a legislação vigente. Assim, o queijo não foi inofensivo e apresentou riscos quando consumido devido à possibilidade de causar doenças de origem alimentar.

Palavras-chave: Leite cru; Maturação; Boas práticas de fabricação.

Abstract

The manufacture of artisanal cheeses is one of the most important activities of the family agroindustry in Minas Gerais. Given the economic and cultural importance of Minas Artesanal Cheese, the objective of this study was to evaluate the microbiological characteristics and safety of Minas Artisanal Cheese (MAC) produced in a rural property in Santa Vitória-MG. Twelve cheese samples were evaluated in January 2019 (summer), and 12 samples were evaluated in July 2019 (winter) at four maturation times (1, 7, 14 and 21 days). Evolution of the moisture and pH of the cheese during maturation were evaluated. Coliforms were counted at 30 °C and 45 °C, and the presence/absence of *Staphylococcus aureus*, lactic acid bacteria (LAB), mold, yeast and *Salmonella* were evaluated. Microbiological characteristics of the cheese water supply, milk, endogenous yeast and the cheese surface were also evaluated. There was variation between the two seasons in terms of humidity and LAB. Significant differences were observed in the coliform counts at 30°C and 45°C, and the presence of *S. aureus*, LAB, mold and yeasts in the cheese were evaluated during maturation in both seasons. Maturation influenced the moisture content but did not influence the pH. There was a reduction over time in the pathogen count. At the end of ripening, the cheese did not exhibit parameters that would guarantee its microbiological safety. Maturation significantly reduced the microorganism counts, but the MAC did not comply with current legislation. Thus, the cheese was not harmless and presented risks when consumed due to the possibility of causing foodborne diseases.

Keywords: Raw milk; Maturation; Good manufacturing practices.

Resumen

La fabricación de quesos artesanales es una de las actividades más importantes de la agroindustria familiar en Minas Gerais. Dada la importancia económica y cultural del queso Minas Artesanal, el objetivo de este estudio fue evaluar las características microbiológicas y la seguridad del queso artesanal de Minas (QAM) producido en una propiedad rural en Santa Vitória-MG. Se evaluaron doce muestras de queso en enero de 2019 (verano) y 12 muestras en julio de 2019 (invierno), en cuatro tiempos de maduración (1, 7, 14 y 21 días). Se evaluó la evolución de la humedad y el pH del queso durante la maduración. Los coliformes se contaron a 30°C y 45°C, y se evaluó la presencia/ausencia de *Staphylococcus aureus*, bacterias del ácido láctico (BAL), mohos, levaduras y *Salmonella*. También se evaluaron las características microbiológicas del suministro de agua del queso, la leche, la levadura endógena y la superficie del queso. Hubo variación entre las dos estaciones en términos de

humedad y BAL. Se observaron diferencias significativas en los recuentos de coliformes a 30°C y 45°C, y se evaluó la presencia de *S. aureus*, BAL, mohos y levaduras en el queso durante la maduración en ambas estaciones. La maduración influyó en el contenido de humedad, pero no influyó en el pH. Hubo una reducción en el tiempo en el recuento de patógenos. Al final de la maduración, el queso no presentaba parámetros que garantizaran su seguridad microbiológica. La maduración redujo significativamente el recuento de microorganismos, pero el QAM no cumplió con la legislación vigente. Por lo tanto, el queso no era inofensivo y presentaba riesgos cuando se consumía debido a la posibilidad de causar enfermedades transmitidas por los alimentos.

Palabras clave: Leche cruda; Maduración; Buenas prácticas de fabricación.

1. Introduction

Minas Artisanal Cheese (MAC) is produced from fresh raw milk by the addition of rennet and endogenous natural yeast from cheese whey. The cheese is then shaped, hand-pressed, dry-salted and ripened. Because it is produced from raw milk and is highly manipulated, MAC can carry pathogenic bacteria. On the other hand, endogenous yeast is rich in lactic acid bacteria (LAB), which are technologically important for maturation, lactic acid production, competition for nutrients and the elaboration of antimicrobial substances such as bacteriocins (Castro et al., 2016; Monteiro, 2018; Pinto et al., 2009).

Several characteristic factors of MAC and its manufacturing process are fundamental for the control of spoilage and pathogenic microorganisms. Among these factors are the quality of the raw material, the application of good manufacturing practices (GMP), the temperature, the maturation time and the presence of LAB. These are extremely important due to their role in the development of the sensory and safety characteristics of cheese (Dores & Ferreira, 2012).

Ripening is a way to improve the microbiological quality of cheese, even with a high initial microorganism count. This is due to the physical, chemical and microbiological changes that occur in cheese during this stage. A wide variety of microorganisms participate in the cheese ripening process. The main group involved in maturation is LAB. However, ripening alone does not safely guarantee the microbiological quality of cheese (Fox et al., 2017; Martins et al., 2015; McSweeney, 2007; Soares et al., 2018).

As MAC is produced from raw milk, its microbiological characteristics are influenced by the raw material. The seasonality of the chemical and microbiological constituents of milk

results from the interaction of physiological, climatic and nutritional factors throughout the year and directly influences cheese quality. Thus, cheeses undergo changes that may impact their safety and safety (Castro et al., 2016; Costa Junior et al., 2009; Figueiredo et al., 2015). Thus, knowing the effects of maturation and seasonality on the endogenous and pathogenic microbiota of MAC is of fundamental importance to understanding its characteristics and microbiological safety. Thus, the objective was to evaluate the safety and microbiological characteristics of MAC produced in Santa Vitória-MG in summer and winter.

2. Materials and Methods

Researches are done to bring new knowledge to society as stated by Pereira et al. (2018). In that study, twelve samples of Minas Artisanal Cheese (MAC) were collected in January 2019 (summer), and 12 samples were collected in July 2019 (winter) in a cheese factory in Santa Vitória-MG on four different maturation days (1, 7, 14 and 21 days).

On the first day, cheese was collected after one day of production, and the endogenous yeast, milk and water from the cheese was obtained. Additionally, the surfaces of the milk transport bucket, the workbench and the maturation shelves were sampled. The cheese collections were carried out at 7, 14 and 21 days of maturation, and all were performed in the same production lot and stored in the cheese factory ripening room. Endogenous yeast, milk and water were collected only at the first visit.

For the MAC, pH was determined according to the Manual of the Official Methods for the Analysis of Animal Food (Brazil, 2018), the humidity was determined according to the International Organization for Standardization/International Dairy Federation (ISO, 2004) IDF 4 method. The coliform counts at 30 °C and 45 °C were obtained by the most likely number (NPM) method (Davidson et al., 2004; Kornacki et al., 2015); the NPM method was also used to obtain the *Staphylococcus aureus* count (Henning et al., 2004; Bennett et al., 2015), the presence/absence of *Salmonella* (Andrews et al., 2016), the lactic acid bacteria count (Frank et al., 2004; Njongmeta et al., 2015) and the mold and yeast count (Frank et al., 2004; Ryu & Wolf- Hall, 2015).

For the raw milk and endogenous yeast, the coliform counts were performed at 30 °C and 45 °C by NPM method (Davidson et al., 2004; Kornacki et al., 2015), and the NPM method was used to obtain the *S. aureus* count (Henning et al., 2004; Bennett et al., 2015) and to determine the presence/absence of *Salmonella* sp (Andrews et al., 2016). For milk, the aerobic mesophilic count was also performed (Laird et al., 2004; Ryser & Schuman, 2015).

For the water, coliform counts were obtained at 30 °C and 45 °C by the NPM method (Braun-Howland & Hunt, 2017b; Kornacki et al., 2015). In addition, for the surface samples, the aerobic mesophilic count (Ryser & Schuman, 2015; Moberg & Kornacki, 2015) and presence/absence of *S. aureus* (Bennett et al., 2015) were determined.

The experimental design was completely randomized. Analysis of variance was performed, followed by the means test and the Tukey test at a 5% significance level. When the F test result was significant, regression analysis was performed. Data were analysed using Sisvar version 5.7 software.

3. Results and Discussion

Table 1 shows the microbiological counts of the water used to make cheese in Santa Vitória-MG.

Table 1 - Microbiological water quality.

Season	Coliforms at 30 °C and 45 °C (Log NPM.mL^{-1})[a]
Summer	0.04
Winter	0.04
IMA Ordinance No. 1.837/2018	Absence/100mL

a = counting was done by NPM and all positive tubes for total coliforms confirmed for thermotolerant coliforms. For this reason a single result was displayed.
Source: Author (2019).

The results for the water (Table 1) were outside the required standards for coliforms at 30 °C and 45 °C (Minas Gerais, 2018). Maintaining the microbiological quality of the water used in the cheese industry is essential to ensure the quality of the cheese. High coliform counts indicate environmental contamination, inefficient chlorination or recontamination. In addition, it is an important source of contamination, as water was used to sanitize the handler's hands, sanitize the utensils and wash the cheese (Castro et al., 2016; Dias et al., 2012).

Almeida et al. (2013), who are cheese makers in the region of Montes Claros-MG, found all water samples to be within the standards of legislation. However, Martins et al. (2015), when evaluating the microbiological water quality of Serro-MG and the Santos et al (2017) cheesemakers in Uberaba-MG, found samples with results that were outside the required standards for coliforms. Thus, even low coliform counts indicate that water is unfit

for use in MAC production; even if the producer employs GMP, this does not produce a microbiologically safe cheese (Santos et al., 2017).

Table 2 shows the aerobic mesophilic counts of the surfaces used for different stages of milk production and the cheese making process.

Table 2 - Aerobic mesophil count (Log CFU.cm-²) in utensils and surfaces used in Minas Artisanal Cheese production.

Season	Surfaces					
	Brass A	Brass B	Workbenchs	Endogenous yeast maturation shelves	7 days maturation shelves	14 days maturation shelves
Summer	3.04	3.15	5.58	5.57	5.74	6.73
Winter	4.23	5.41	4.23	4.67	5.68	6.08

Source: Author (2019).

In the absence of a microbiological standard for artisanal cheese equipment and utensil surfaces, the standards set by the American Public Health Association (APHA) of 0.30 Log CFU.cm-² (Moberg & Kornacki, 2015) and those of the World Health Organization (WHO) of 1.70 Log CFU.cm-² for food contact surfaces (Cosby et al., 2008) were used.

The surfaces showed high aerobic mesophyll counts, indicating that the surfaces were poorly sanitized and a potential source of contamination for milk and cheese. Galinari et al. (2014) found high mesophyll counts on wooden shelves in Serro-MG cheese shops (6.18 and 6.20 Log CFU.cm-²) and on Canastra-MG cheese ripening shelves (4.18 and 4.93 Log CFU.cm-²). Miranda et al. (2016) found high mesophyll counts in cans and artisanal cheese workbench in Teixeiras-MG, with values of 2.11 Log CFU.cm-² and 2.79 Log CFU.cm-².

The wooden shelves had higher mesophyll counts, which are favoured by the transfer of the cheese microbiota to the surfaces of the shelves. As the shelves are made of wood, which is a porous and difficult to clean material, contact with cheese components favours the development of biofilms. In addition, as there are always ripening cheeses, hygiene is compromised (Ferreira and Ferreira, 2011; Galinari et al., 2014; Lortal et al., 2009).

The results presented in Table 3 indicate the presence or absence of *S. aureus* on surfaces used in different stages of the cheese manufacturing process.

Table 3 - Presence/Absence of *Staphylococcus aureus* on surfaces used at different stages of Minas Artisanal Cheese manufacturing process.

Season	Surfaces			
	Workbenchs	Endogenous yeast maturation shelves	7 days maturation shelves	14 days maturation shelves
Summer	Absence	Presence	Presence	Presence
Winter	Presence	Presence	Presence	Presence

Source: Author (2019).

In a study by Zegarra et al. (2009) of artisanal cheese shops in Rio de Janeiro, *S. aureus* was isolated from brass, utensils and the hands of handlers. Galinari et al. (2014) found maturation shelves contaminated with *S. aureus* in Canastra-MG cheese shops. Thus, the presence of *S. aureus* on the surfaces indicates that they are in unsatisfactory hygienic condition and may be the source of cross contamination. Inadequate hygiene practices within cheese factories, combined with the ability of *S. aureus* strains to form biofilms, contributes to the permanence of biofilms (Martin et al., 2016). Thus, the presence of this group of microorganisms on surfaces confirms that it is widespread in the production environment, contributing to the poor quality of the cheese.

Microorganism counting for raw milk is important in MAC quality control. The high nutritional value of milk, its high water content and its almost neutral pH allow the growth of various microorganisms (Chambers, 2005). Table 4 shows the microbiological counts for raw milk used in the manufacture of MAC in Santa Vitória-MG.

Table 4 - Microorganism counting for raw milk.

Season	Aerobic mesophylls (Log CFU.mL^{-1})	Coliforms at 45°C (Log CFU.mL^{-1})[a]	*S. aureus* (Log CFU.mL^{-1})	*Salmonella* (absence /presence at 25g)
Summer	4.60	6.66	0.00	Presence
Winter	4.08	6.32	0.00	Absence
Standard[b]	≤ 5.00	≤ 2.00	≤ 2.00	Absence

a = counting was made by the Most Likely Number and all positive tubes for total coliforms confirmed for thermotolerant coliforms. For this reason a single result was displayed.
b = According to State Decree No. 44864 of 2008 (Minas Gerais, 2008).
Source: Author (2019).

Aerobic mesophilic counts (Table 4) met the legislative requirements in both seasons, but the summer counts were higher than the winter counts. Figueiredo et al. (2015), when analysing raw milk from Serro-MG cheese houses, found non-standard aerobic mesophyll

counts in summer (5.78 - 5.83 Log CFU.mL^{-1}) and in winter (5.08 - 5.11 Log CFU.mL^{-1}). Castro et al. (2016) also found that non-standard milk samples were used for cheese production in Campos das Vertentes-MG in summer (6.52 Log CFU.mL^{-1}) and in winter (5.99 Log CFU.mL^{-1}). According to Chambers (2005), the aerobic mesophilic counts of raw milk are a useful indicator to monitor the sanitary conditions during milk production; thus, high counts suggest hygiene deficiencies in milk production.

The milk had counts above those allowed for coliforms at 45 °C in summer and winter. Figueiredo et al. (2015) found coliform counts at 45 °C above the standard in summer (5.38 - 5.41 Log CFU.mL^{-1}) and within the standard in winter (1.58 - 1.62 Log CFU.mL^{-1}). Castro et al. (2016) obtained average counts of 2.68 Log CFU.mL^{-1} for coliforms at 45 °C for raw milk in summer and 0.95 Log CFU.mL^{-1} for raw milk in winter. In rainy seasons, such as summer, there is a greater risk of cross contamination due to the higher solubilization of organic matter, which, when combined with high temperatures, favours the proliferation of microorganisms in the environment. On the other hand, in winter, when there is lower rainfall and low temperatures, environmental microbial proliferation tends to be decreased, resulting in decreased cross contamination. Counts of indicators, such as aerobic and coliform mesophylls at 45 °C in milk, indicate the presence of inadequate conditions (Castro et al., 2016; Chambers, 2005; Figueiredo et al., 2015; Soares et al., 2018; Verdier- Metz et al., 2009).

S. aureus contamination was not observed in raw milk. In raw milk evaluated in Serro-MG, *S. aureus* counts were within the standards required by law in all cheese factories when evaluated in summer (1.53 –1.57 Log CFU.mL^{-1}) but were not within the standards in winter. (2.04 Log CFU.mL^{-1}) (Figueiredo et al., 2015). Castro et al. (2016) found high S. aureus counts in raw milk from Campos das Vertentes-MG (3.89 Log CFU.mL^{-1} in winter and 3.85 Log CFU.mL^{-1}) in summer. High *S. aureus* counts are associated with the natural microbiota of animals, inadequate hygiene or cleaning of utensils used during milking, and a lack of personal hygiene on the part of the milker (Carvalho et al., 2018).

In summer, the presence of *Salmonella* spp was detected. In MAC, poor hygiene when obtaining milk will affect the final product, as the raw material will be of low quality due to the initially high level of contamination introduced during its production (Verraes et al., 2015; Yoon et al., 2016). Despite the absence of *S. aureus*, the high coliform count at 45 °C and the presence of *Salmonella* spp. show that milk is a source of contamination for MAC and that milking hygiene measures are necessary to obtain milk of high quality.

Endogenous yeast gives the cheese its identity, reflecting the natural environmental conditions in which the raw milk used in cheese making is produced due to the presence of LABs (Kamimura et al., 2019). Table 5 shows the microbiological counts of endogenous yeast used in the manufacture of MAC in Santa Vitória-MG.

Table 5 - Microbiological quality of endogenous yeast.

Season	Coliforms at 30°C and 45°C (Log NMP.mL^{-1})[a]	*Staphylococcus aureus* (Log CFU.mL^{-1})	*Salmonella* (absence /presence at 25g)
Summer	5.66	1.80	Presence
Winter	6.04	1.04	Absence

a = counting was made by the Most Likely Number and all positive tubes for total coliforms confirmed for thermotolerant coliforms. For this reason a single result was displayed.
Source: Author (2019).

MAC legislation does not define microbiological parameters for endogenous yeast. However, endogenous yeast can be a source of desirable bacteria, such as LAB, as well as undesirable (pathogenic) bacteria. This situation can cause problems, as the endogenous yeast collected from a cheese batch will be used in the preparation of cheese the next day, and if contamination exists, the quality of the products prepared the next day will be compromised. However, the presence of LAB in endogenous yeast is important because it provides much of the typical MAC sensory characteristics and reduces the pathogen count in the product (Galinári et al., 2014; Castro et al., 2016).

High coliform counts at 30 °C and 45 °C were found in both seasons. Martins et al. (2015) found higher endogenous yeast counts in summer than in winter for coliforms at 30 °C (3.36 Log CFU.mL^{-1} and 3.08 CFU.mL^{-1}) and 45 °C (2.23 Log CFU.mL^{-1} and 2.18 Log CFU.mL^{-1}). Castro et al. (2016) found higher endogenous yeast counts in summer for coliforms at 30 °C (3.83 Log CFU.mL^{-1} and 0.87 Log CFU.mL^{-1}) and higher counts for coliforms at 45 °C in winter compared to that in summer (0.51 Log CFU.mL^{-1} and 0.46 Log CFU.mL^{-1}). According to Castro et al. (2016), the increased coliform count in summer is explained by the high temperature and humidity, which are favourable for microbial development. In the present study, the highest coliform count occurred in winter, indicating failures in the cheese cleaning processes used during the handling and collection of endogenous yeast.

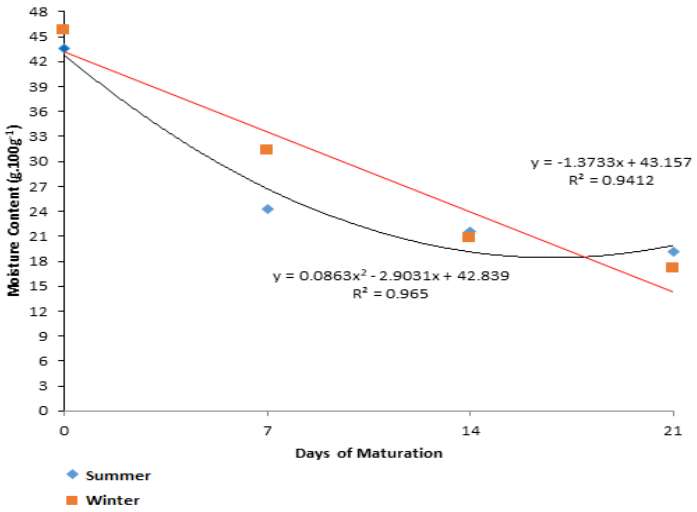
Endogenous yeast produced counts for *S. aureus*. This may be due to its higher resistance to low pH conditions and high salt concentration, which are characteristics of

endogenous yeast that favour the survival of this microorganism (Seo & Bonach, 2007). The counts in summer were higher than those in winter. Martins et al. (2015), in endogenous yeast, found higher *S. aureus* counts in winter compared to summer (2.46 Log CFU.mL^{-1} and 2.41 Log CFU.mL^{-1}). Castro et al. (2016) found higher *S. aureus* counts in summer compared to winter (5.38 Log CFU.mL^{-1} and <3.0 Log CFU.mL^{-1}). In summer, the highest concentration of these microorganisms may occur for the same reasons described for coliforms. In addition, humans are the main reservoir of *S. aureus* and may be a source of contamination during MAC manipulation (Borges et al., 2008; Seo & Bonach, 2007).

Salmonella spp. were present in summer and absent in winter, which were results similar to those obtained for raw milk. The presence of *Salmonella* spp. may be associated with contamination linked to improper hygienic practices. Thus, although endogenous yeast has the function of replacing industrial lactic cultures in artisanal productions, it can represent an important source of cheese contamination (Castro et al., 2016; Soares et al, 2018).

The results for the MAC moisture content differed ($p<0.05$) during maturation in the two production seasons (Figure 1).

Figure 1 - Evolution of moisture content of Minas Artisanal Cheese during 21 days of maturation.



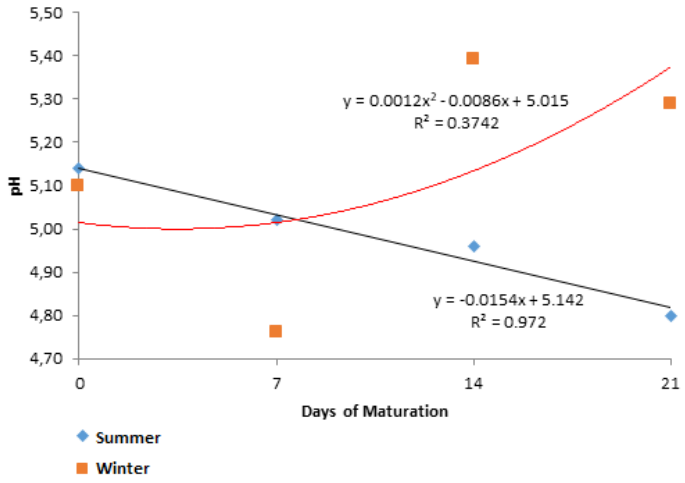
Source: Author (2019).

Determining the moisture content of MAC is very important because it is related to microbiological counts, as cheese moisture is linked to water activity (aw) and moisture reduction makes water unavailable for microbial metabolism (Beresford et al. al., 2001).

During maturation, in summer and winter, the moisture content decreases progressively to values below 20 g.100 g^{-1} due to the influence of ambient temperature and evaporative water loss (Fox et al., 2017). In addition, the moisture content was lower in MAC in summer compared to winter. Dores et al. (2013) stated that higher temperatures facilitate water loss during cheese ripening.

In summer, moisture loss was more intense compared to that in winter. In addition, the moisture content in the first 14 days of maturation was higher in winter. Similar results for MAC were found by several other authors (Costa Junior et al., 2009; 2014; Martins et al., 2015). According to Costa Jr et al. (2014), the higher moisture content of MAC in winter was a result of the production technology adopted to compensate for the low relative humidity, since the maturing of cheeses at room temperature tends to dehydrate them. This practice involves increasing the moisture in MAC by increasing the grain size, breaking the dough, and/or decreasing manual pressure during hanging. However, Silva et al. (2011) observed different behaviour in MAC, which resulted in a lower moisture content in winter compared to summer. The authors claim that the lower moisture content of cheese is due to the reduction in the relative air humidity, causing greater moisture loss during ripening (Silva et al., 2011). Figure 2 shows the evolution of MAC pH during maturation.

Figure 2 - Evolution of Minas Artisanal Cheese pH during 21 days of maturation.



Source: Author (2019).

There was no difference between seasons ($p>0.05$); however, pH was influenced by the number of maturation days ($p<0.05$). According to Beresford et al. (2001), the ideal pH for the growth of the most common bacteria is almost neutral, and microbial growth at pH <5.0 is very low.

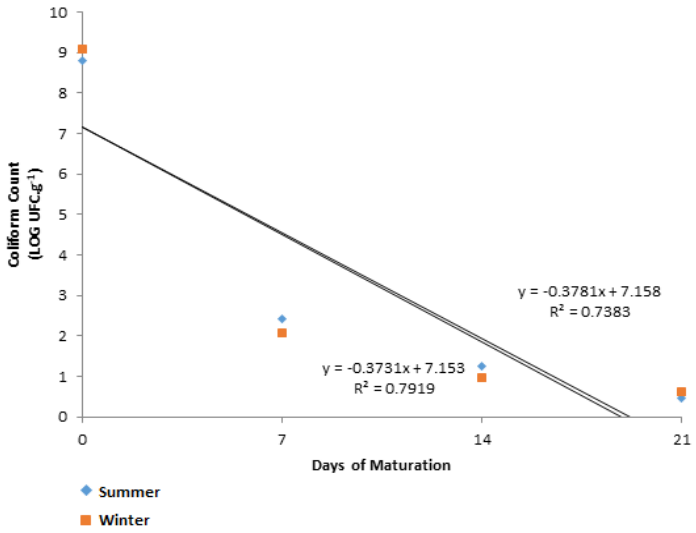
In summer, there was a linear decrease ($p<0.05$) in pH throughout maturation (Figure 2). Costa Junior et al. (2014) state that high temperatures favour the fermentation of cheese mass, resulting in decreased pH values. The decrease in pH is due to the presence of bacteria in the endogenous yeast that convert lactose to lactic acid, acidifying the medium. Increasing lactic acid production and decreasing the pH inhibit the growth of pathogenic microorganisms (Fox et al., 2017; Oliveira et al., 2017).

In winter, there was a decrease ($p<0.05$) in the pH in cheeses after 7 days of ripening and an increase in the pH after 14 days, and the pH was stable until after 21 days of ripening. According to McSweeney (2004), the pH increase in cheese during ripening is a consequence of the formation of nitrogen compounds resulting from proteolysis, which neutralize, to different degrees, the hydrogen protons released during the conversion of lactose to lactic acid. For this reason, during cheese ripening, it is common for the pH to be lowered, stabilized and subsequently raised (McSweeney, 2004).

The behaviour of pH during maturation has been described by other authors. The results found by Costa Júnior et al. (2014), Figueiredo et al. (2015) and Martins et al. (2015) show that there was a correlation between maturation and production time ($p<0.05$) in MAC. The authors reported a linear increase ($p<0.05$) of pH throughout ripening and found that the pH values of cheeses made in summer were slightly higher than the pH values of cheeses produced in winter.

The number of maturation days had a negative linear effect on coliform counts at 30 °C and 45 °C (Figure 3). However, the average coliform counts at 30 °C and 45 °C for cheese collected in summer and winter showed no differences ($p>0.05$).

Figure 3 - Evolution of coliform count 30 °C and 45 °C of Minas Artisanal Cheese during 21 days of maturation.



Source: Author (2019).

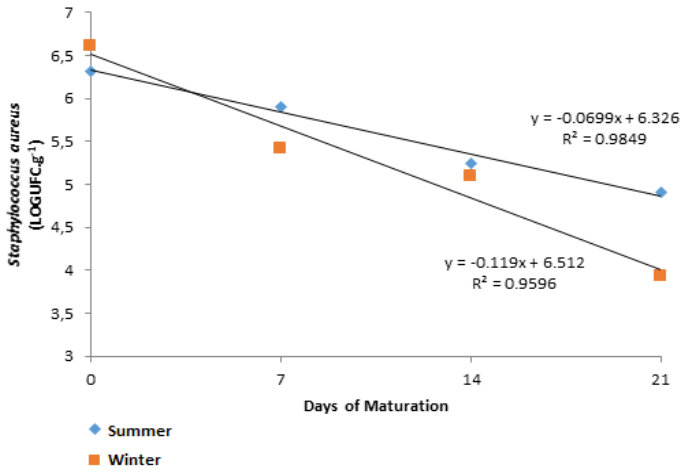
Coliform counts were increased in freshly produced cheese (8.79 Log CFU.g^{-1} in summer and 9.08 Log CFU.g^{-1} in winter). The coliform counts of milk and endogenous yeast both high counts, which may be responsible for the high counts in cheese. The presence of coliforms at 30 °C, in addition to indicating poor processing hygiene, may cause cheese to deteriorate, as hydrogen and carbon dioxide are formed in a process called early stuffing (McSweeney, 2007; Sobral et al., 2017).

The coliform count recommended by law at 30 °C is 3.7 Log CFU.g^{-1} and at 45 °C is 2.7 Log CFU.g^{-1} (Minas Gerais, 2018). The legal limits for MAC coliforms were reached at 7 days (2.43 Log CFU.g^{-1} in summer and 2.08 Log CFU.g^{-1} in winter). Maturation, in summer and winter, was efficient ($p<0.05$) in reducing the coliform count, considering that on the first day of production the value was above the state legal limit and decreased during maturation.

In MAC evaluated by Figueiredo et al. (2015), coliform counts at 30 °C did not vary ($p>0.05$) during maturation in winter, but in summer, there was a reduction ($p<0.05$) in the coliform population at 30 °C. Figueiredo et al. (2015) observed that coliform counts at 45 °C increased ($p<0.05$) during the first 15 days of maturation, followed by a decline in both summer and winter. Martins et al. (2015), in MAC, observed a linear decrease ($p<0.05$) in the coliform counts at 30 °C and 45 °C throughout maturation. According to Teshome (2015), the activity of LAB during ripening, the decrease in the humidity and the increase in the NaCl concentration were determining factors contributing to the decrease in the cheese coliform count.

MAC *S. aureus* counts did not vary (p>0.05) according to season. The negative linear evolution of the *S. aureus* counts during maturation is shown in Figure 4. There was a difference (p<0.05) between the maturation days in both seasons.

Figure 4 - Evolution of *Staphylococcus aureus* count of Minas Artisanal Cheese during 21 days of maturation.



Source: Author (2019).

At 21 days of maturation, the *S. aureus* counts were 3.77 and 2.93 Log CFU.g^{-1} in summer and winter, respectively. The legislation recommends a maximum count of 2 Log CFU.g^{-1} (Minas Gerais, 2018). Thus, the cheeses did not meet the legal requirements at the end of ripening. The presence of *S. aureus* in MAC can be explained by endogenous yeast contamination. However, it is likely that the main cause of *S. aureus* in cheese is improper handling during cheese production because contamination by *S. aureus* in food is generally associated with handlers (Seo & Bonach, 2007). Moreover, during research of these microorganisms in workbench and shelves used for maturation, the results suggested the possible contamination of the cheese during its production.

Dores et al. (2013) observed a difference (p>0.05) in *S. aureus* counts in MAC in summer and winter, which decreased linearly during maturation. Figueiredo et al. (2015) observed a reduction (p<0,05) in the population of *S. aureus* during maturation in both seasons, but the counts did not reach the value recommended by legislation at the end of maturation. Martins et al. (2015) also observed a reduction in the *S. aureus* counts (p<0.05) during MAC maturation in winter and summer. According to Seo & Bonach (2007), an important feature of *S. aureus* that deserves attention in research is its ability to produce

enterotoxins. Thus, it is necessary in future studies to research staphylococcal enterotoxins in these cheeses to confirm their safety.

Salmonella spp. were detected in freshly manufactured MAC and in cheese after 14 days of maturation in summer and in freshly manufactured cheese in winter (Table 6).

Table 6 – Presence/Absence of *Salmonella* in Minas Artisanal Cheese throughout Maturation

Season	Maturation days			
	0	7	14	21
Summer	Presence	Presence	Presence	Absence
Winter	Presence	Absence	Absence	Absence

Source: Author (2019).

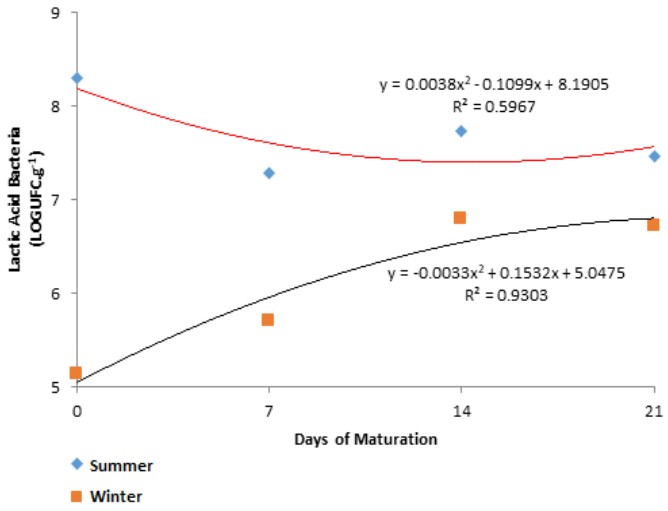
The presence of *Salmonella* spp. in MAC (Table 6) in summer is in agreement with the results obtained for milk and endogenous yeast. However, in winter, milk and endogenous yeast did not show the presence of *Salmonella* spp., which rules out the contamination of cheese by the raw material. This result suggests that contamination occurred during production.

Legislation advocates for the absence of *Salmonella* spp. (Minas Gerais, 2018), so cheeses produced in summer are innocuous at 21 days of maturation, and cheeses produced in winter become innocuous at 7 days of maturation. The absence of *Salmonella* spp. in cheeses it is due to a decrease in aw, an increased salt concentration and the presence of LAB that contribute to increased lactic acid concentrations and bacteriocin production (Fox et al., 2017; McSweeney, 2007; Verraes et al., 2015; Yoon et al., 2016).

In MAC evaluated by Dores et al. (2013), Cardoso et al. (2013) and Castro et al. (2016), the presence of *Salmonella* spp was detected. Martins et al. (2015) detected the presence of *Salmonella* spp. only in MAC samples obtained after 8 and 15 days of maturation in summer. MAC GMPs must address all stages of production, and any failure can lead to cheese contamination.

LAB are essential for MAC maturation and safety. These microorganisms play an important role in the technological and sensory quality of cheese (Castro et al., 2016; Yoon et al., 2016). LAB were the predominant microorganisms in the Santa Vitória-MG MAC, and their counts were high ($p<0.05$) in summer (7.70 Log $CFU.g^{-1}$) and in winter (6.09 Log $CFU.g^{-1}$). The changes in the LAB count in cheese during ripening can be observed in Figure 5.

Figure 5 - Evolution of lactic acid bacteria cout of Minas Artisanal Cheese during 21 days of maturation.



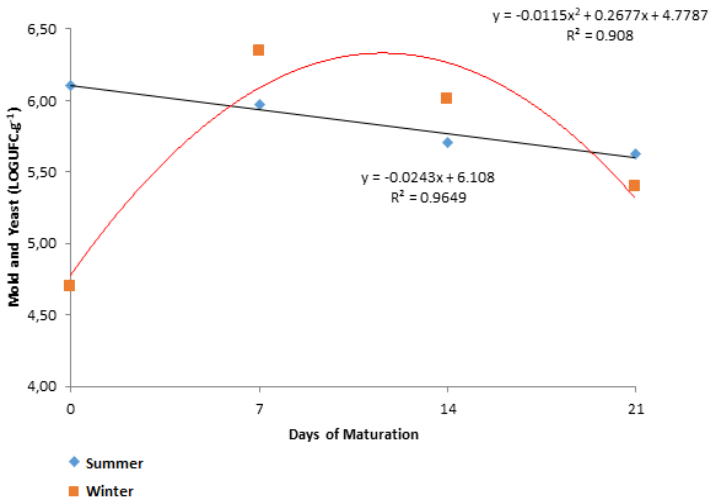
Source: Author (2019).

Results were described by Figueiredo et al. (2015) and Luiz et al. (2016) in which the LAB counts in MAC were similar in summer and winter. However, Castro et al. (2016) found lower LAB counts in summer (8.51 Log CFU.g^{-1}) than in winter (8.70 Log CFU.g^{-1}). According to the author, in winter, producers add a higher volume of endogenous yeast to milk during MAC production to maintain the normal milk coagulation process, as low temperatures slow down coagulation, resulting in inconsistent cheese (Castro et al., 2016).

Distinct changes were observed in the LAB counts in summer and winter. The LAB count decreased ($p<0.05$) between 0 and 7 days of maturation in summer. After this period, there was no difference ($p>0.05$) until after 21 days of maturation. In winter, the LAB count increased during maturation ($p<0.05$). This can be explained by the low winter temperatures. Because LAB are mesophilic microorganisms, their growth at temperatures below optimal temperatures, such as those encountered in winter, may be affected. LAB are desirable microorganisms in cheese for the production of lactic acid and bacteriocins capable of reducing or controlling the proliferation of pathogenic microorganisms. In addition, the ability of LAB to produce acid rapidly is a very important property, as a rapid pH drop is essential for coagulation, curd firmness and pathogen control (Favaro et al., 2015; Figueiredo et al., 2015; Campagnollo et al., 2018).

In summer, there was a linear ($p<0.05$) reduction in the mold and yeast counts during maturation (Figure 6). However, in winter ($p<0.05$), it was observed that the mold and yeast counts were initially lower, increased after 7 days of maturation and then decreased.

Figure 6 - Evolution of mold and yeast count of Minas Artisanal Cheese during 21 days of maturation.



Source: Author (2019).

In Serrano cheese produced from raw milk, as evaluated by Souza et al. (2003), there was a decrease in the mold and yeast counts during maturation. According to the authors, during ripening, there is an increase in cheese matrix compaction, reducing the diffusion of oxygen necessary for the multiplication of these microorganisms and reducing their counts (Souza et al., 2003). Distinct behaviour was observed by Cardoso et al. (2015) in MAC ($p<0.05$), who observed an increase in counts in summer and winter.

Banjara et al. (2015) stated that high counts are expected in cheeses with longer ripening times, as there is usually a reduction in pH and humidity, which is a condition that favours the growth of mold and yeast. The presence of mold and yeast in MAC is expected, since products matured in uncontrolled environments can be subject to this type of contamination (Castro et al., 2016). Figueiredo et al. (2015), in MAC, found high mold and yeast counts in summer (7.5 Log CFU.g^{-1}) and in winter (8.1 Log CFU.g^{-1}). Similar results were found by Castro et al. (2016), who observed lower counts in summer (6.15 Log CFU.g^{-1}) than in winter (7.05 Log CFU.g^{-1}).

Higher mold and yeast counts in summer may be associated with increased LAB counts in this period. Because of this, there may be an intensification in the lactose fermentation process due to the LAB present in the cheese, which may result in the formation of a large amount of lactic acid. The high acid concentrations in cheese inhibit most mold and yeast pathogens and competitors. However, those species that are tolerant of acidity have

found a favourable environment in which to survive and multiply (Castro et al., 2016; Irlinger & Mounier, 2009; Ryu & Wolf-Hall, 2015).

According to Figueiredo et al. (2015), the presence of mold and yeast in MAC is undesirable; because of spoilage, some species are mycotoxin-producing producers associated with food poisoning. However, although in most cases the presence of fungi is seen as undesirable in foods, they may play a relevant role in the ripening of artisanal cheeses. For example, yeast-associated filamentous fungi used in cheese production are responsible for the development of characteristic flavours and aromas (Banjara et al., 2015; Irlinger & Mounier, 2009; Takashi et al., 2017).

4. Conclusion

The quality of the water supplying the cheese factory was not satisfactory. The counts of microorganisms on the surfaces of the brass, workbenchs and maturation shelves suggest that they were not properly sanitized and may have contaminated the cheese. Milk and endogenous yeast presented low microbiological quality. It was found that the microbiological quality of cheese was influenced by the milk and yeast conditions.

Summer and winter seasons influenced the microbiota in raw milk and endogenous yeast. However, they did not influence the microbiota present in cheese, except for LAB. Maturation significantly reduced the microorganism counts, but the MAC did not comply with current legislation. Thus, the cheese was not harmless and presented risks when consumed due to the possibility of causing foodborne diseases.

Production of cheese with high quality raw materials and hygienic sanitary care during production are fundamental for its innocuousness. It can be concluded by means of the present work that it is necessary a greater strictness in the quality control during the elaboration of the milk, that go from the water to be used in the washing of the utensils until obtaining and correct storage of the milk until the elaboration of cheese, without quality raw materials and processing following good manufacturing practices, cannot obtain quality products, even after ripening.

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