

Room temperature aging to guarantee microbiological safety of brazilian artisan Canastra cheese

Maturação em temperatura ambiente como garantia da segurança microbiológica de queijos artesanais brasileiros da Serra da Canastra

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Abstract

Canastra cheese is one of the oldest and most traditional cheeses made from raw milk in Brazil. However, this type of practice may have severe consequences for human health. According to the current legislation, any cheese made from raw milk must be aged for at least 60 days. Traditionally, Canastra cheese is consumed after different ripening periods, but consumers usually prefer those that are aged less than eight days. This study aimed to evaluate the effects of physicochemical and microbiological parameters, with emphasis on the pathogenic microbiota regulated by law, on cheese aged at room temperature and under refrigeration. Cheese samples were collected from eight different cheese producers located in the Serra da Canastra region twice a year (rainy and dry seasons) and analyzed with 8, 15, 22, 29, 36, and 64 days of ripening. Room temperature aging effectively reduced pathogens, reaching the total count established by law in 22 days, regardless of the season. However, ripening under refrigeration, it was ineffective in reducing the *Staphylococcus aureus* counts to the legislation limits, even after 64 days. Therefore, Canastra cheese should be ripened for at least 22 days at room temperature in order to fulfill the safety regulatory limits.

Keyword: food safety; cheese; raw milk; *Staphylococcus aureus*.

Resumo

O queijo Canastra é um dos mais antigos e tradicionais queijos produzidos com leite cru no Brasil, no entanto, sua produção pode gerar consequências graves para a saúde. De acordo com lei vigente no País, o queijo feito com leite cru deve ser submetido a um período mínimo de maturação de 60 dias. Tradicionalmente o queijo canastra é consumido após diferentes períodos de maturação, sendo priorizados pelo consumidor os queijos com menos de 8 dias de fabricação. O objetivo deste estudo foi avaliar a modulação de variáveis físico-químicas e microbiológicas, com ênfase na microbiota patogênica regulamentada por lei, no queijo maturado em temperatura ambiente e sob refrigeração. Amostras de queijos foram coletadas em oito propriedades na região, em dois períodos do ano (chuvoso e seco), e analisadas com 8, 15, 22, 29, 36 e 64 dias de fabricação. A maturação em temperatura ambiente foi decisiva para redução da microbiota patogênica para níveis permitidos em 22 dias, nos dois períodos analisados, ao passo que, sob refrigeração, os níveis de *Staphylococcus aureus* (espécie limitante) permitidos somente seriam atingidos após 64 dias. Dessa forma, mantendo-se a tradição, o queijo canastra maturado por 22 dias em temperatura ambiente apresenta os requisitos legais de segurança.

Palavras-chave: segurança alimentar; queijos; leite cru; *Staphylococcus aureus*.

1 Introduction

Canastra is a traditional variety of Minas farmhouse cheese produced on small scale by a large number of producers in the Serra da Canastra region located in the state of Minas Gerais, Brazil (Altitude from 637 m to 1,485 m above sea level, average temperatures of 22.2 °C, and rainfall of 1,390 mm). It is made from raw milk from local herds of dairy cows. It is characterized by the addition of natural endogenous starter to fresh raw milk in order to assist the beginning of the fermentation process. Artisan cheeses have been produced using the same technique since the seventeenth century. Nowadays, in order to keep up the production of this important product, the Brazilian legislation requires some technique adjustments to meet the standards of hygiene and sanitation established. Since it is frequently touched by human hands and produced with raw milk, artisanal Minas cheese can have serious consequences on public health due to the risk of conveying pathogens. Therefore, the Brazilian

legislation (BRASIL, 1996) allows the sale of cheese made from raw milk only when it is ripened for 60 days at a temperature above 5 °C. However, this long ripening period affects the sale of the product, both economically, for small producers, and in terms of quality due to changes in the cheese taste. Since the product is usually sold fresh (immediately after it's been manufactured), vacuum-packed, and refrigerated, it does not meet the microbiological standards established by legislation (PINTO et al., 2004).

Few scientific studies have been made to date confirming the role of temperature and aging on the physicochemical and microbiological characteristics of artisanal cheeses produced in the Minas Gerais state (MARTINS, 2006). The knowledge of the effects of aging and temperature on the modulation of pathogenic microorganisms, in addition to the physical and

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chemical characteristics of these cheeses, is necessary in order to guide the adjustments to be made in the production units as well as in producers' training programs and help to bring these products out of informality and clandestinity. This information would also be used to legalize the sale of these products. Without proper definition of ripening time, the artisanal cheese from "Serra da Canastra", a widely consumed and enjoyed product, may pose serious risks to the public health.

This study aimed to evaluate the effect of ripening time on the physicochemical and microbiological parameters, with special emphasis on the pathogenic microbiota regulated by the legislation, on cheese made in the rainy and the dry seasons and aged at room temperature and at refrigeration for a period of 64 days.

2 Materials and methods

2.1 Sample collection and storage

The Canastra cheese samples were randomly collected from eight production units in two seasons: the rainy season – May 2006 and in the dry season – September 2006. A total of 16 samples were collected in duplicate. The cheese samples were collected two days after manufacturing in order to complete the salting process. The samples were then transported to the ripening chambers located in the Laboratory of Lactic Cultures of Federal University of Viçosa. They were stored either under ambient conditions, at a temperature of 25 °C and relative humidity (RH) around 68% in the rainy season and 62% in the dry season, or under refrigeration, at a temperature of 8 °C and RH around 77% in the rainy season and 75% in the dry season. The samples were analyzed in the pre-determined times of 8, 15, 22, 29, 36 and 64 days of ripening.

2.2 Physicochemical analysis

The cheese pH was determined according to previously described methodology (BRASIL, 1996). Water activity (aw) was determined in a AquaLab CX2 aw meter (Decagon Devices, Inc., Pullman, Wash). The content of sodium chloride was determined according to the method of Pereira et al. (2001).

2.3 Microbiological analysis

Twenty five g of cheese were mixed with 225 mL of 2% sterile peptone water and then homogenized in a stomacher for 1 minute. Decimal dilutions were made, followed by plating in duplicate for each dilution.

The counts of coliforms and *Escherichia coli* (*E. coli*) at 30 °C were made by using petrifilms (3M Petrifilm™ EC method – 3M Company, St. Paul, MN, EUA). The total count of mesophilic bacteria in the artisanal cheese was determined by the pour plate method in Petri dishes using the culture medium Plate Count Standard Agar – PCA (Merck). After 48 hours, the plates were counted and the results expressed in cfu.g⁻¹ (LANCETTE; BENNET, 2001).

For the analysis of *Staphylococcus aureus* (*S. aureus*), 3M Petrifilm™ RSA (3M Company, St. Paul, MN, EUA) was used in

accordance with the procedures established by the manufacturer (PONSANO et al., 2000; SCHÖELLER; INGHAM, 2001).

The Reveal - *Salmonella* Test System (®Neogem corporation, USA/Canada) was used in the analysis of *Salmonella* sp. For the detection of *Listeria* sp., the Reveal Test for *Listeria* (®Neogem corporation, USA/Canada) was used in accordance with the procedures established by the distributor.

2.4 Statistical analysis

The physicochemical and microbiological data were subjected to analysis of variance. When necessary, a regression analysis was performed. The regression coefficients were tested using the t-test at 5% significance level. All statistical analyses were performed using the *Statistical Analysis System* (STATISTICAL..., 2003).

3 Results and discussion

The microbiological counts and physicochemical values are shown in Figures 1 and 2. According to the results, the physicochemical and microbiological data were significantly influenced ($p < 0.05$) by the time of ripening, regardless of the season that the cheese was made or the storage temperature (room temperature or refrigerated).

In artisanal cheeses stored under refrigeration, ripening time had no significant effects ($p < 0.05$) on any of the variables analyzed (Figure 2). This lack of change could be attributed to decreased enzymatic and metabolic reactions of the microorganisms caused by the low temperature.

During aging at room temperature, a decrease in water activity (A_w) was observed. During the rainy season, the water activity was 0.98 at the beginning of the ripening period, and it decreased to 0.93 at the end of the ripening period. For the dry season, water activity started at 0.98 and decreased to 0.92 in the end of the ripening period. The values of A_w of cheese stored under refrigeration remained at 0.98 for the entire ripening period in both seasons.

The values of sodium chloride (NaCl) increased during aging at room temperature. The salt content increased from 1.43 to 2.47% NaCl in the rainy season and from 1.50 to 2.74% in the dry season. Under refrigeration, this variation was lower, ranging from 1.49 to 1.69% NaCl in the rainy season and from 1.55 to 1.76% NaCl in dry season. It is important to point out that the chloride content of the different cheeses is fairly irregular due to the salting technique used (MARTINS, 2006).

An increasing pH trend was observed during ripening; no change was as high as that observed at room temperature in the dry season (Figure 1). The increase in pH may be related to protein degradation due to activity of a natural milk protease (plasmin) and the proteases present in the endogenous starter resulting in the formation of alkaline nitrogenous compounds. Deamination and proteolysis that occur in the decarboxylation of amino acids and dissociation of acids, mainly lactic acid, are factors that contribute to the increase in pH (KOSIKOWSKI; MISTRY, 1997; KONGO et al., 2007).

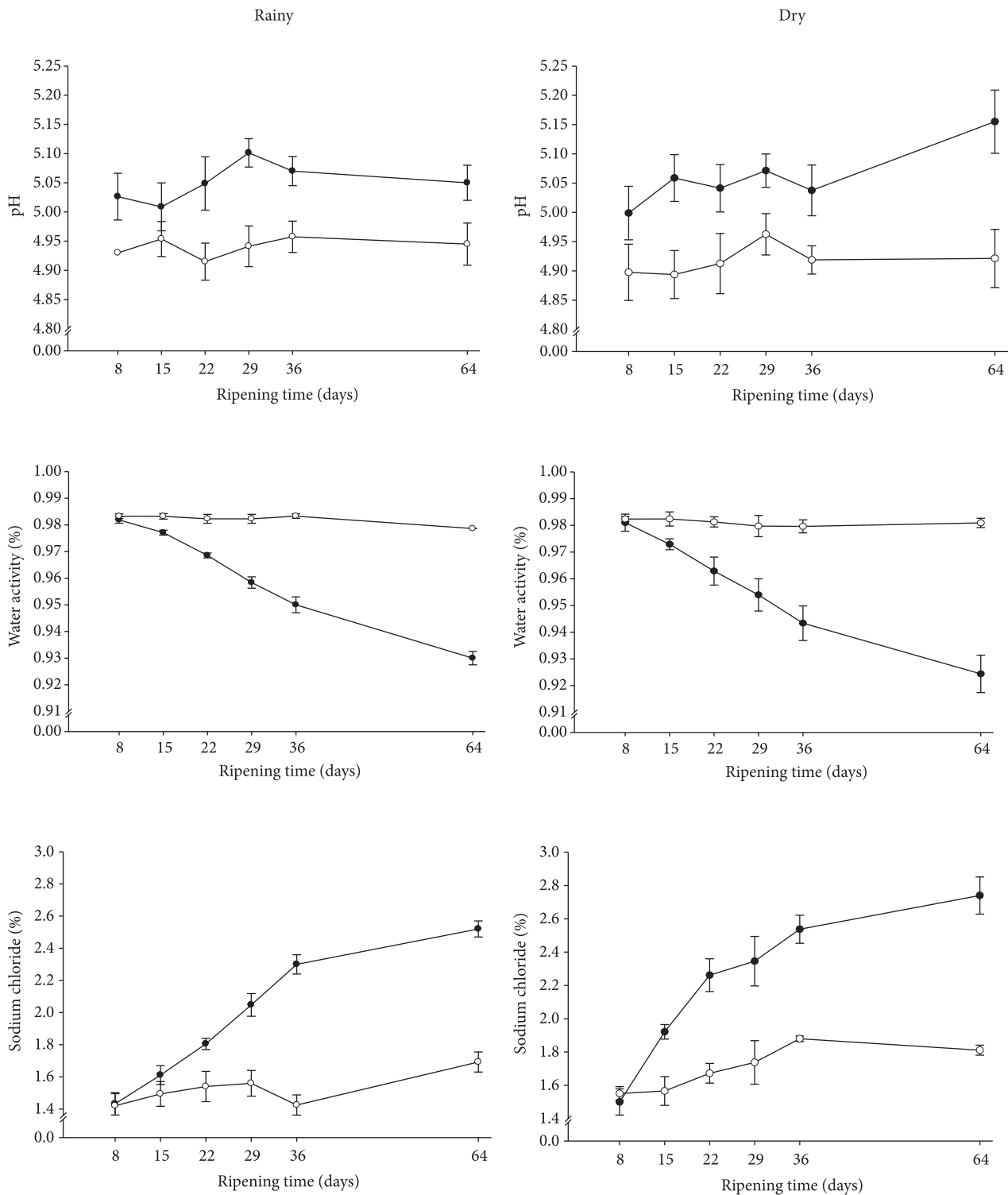


Figure 1. pH, water activity, and sodium chloride of Canastra cheese as a function of ripening time at room temperature (●) and under refrigeration (○) in the dry season and rainy season.

The development of the microbial groups was monitored during ripening (Figure 2). Room temperature and ripening time caused a linear effect on total aerobic counts, coliforms at 30 °C, *E. coli* and *S. aureus*. This effect was also observed under refrigeration conditions, although at lower rate.

The presence of *Listeria* sp. and *Salmonella* sp. was not detected in any of the samples, even though they are commonly detected in cheese made with raw milk (MORGAN et al., 2001; PAK et al. 2002; VITAS; AGUADO; GARCIA-JALON, 2004; PINTO, 2008; MARTINS, 2006).

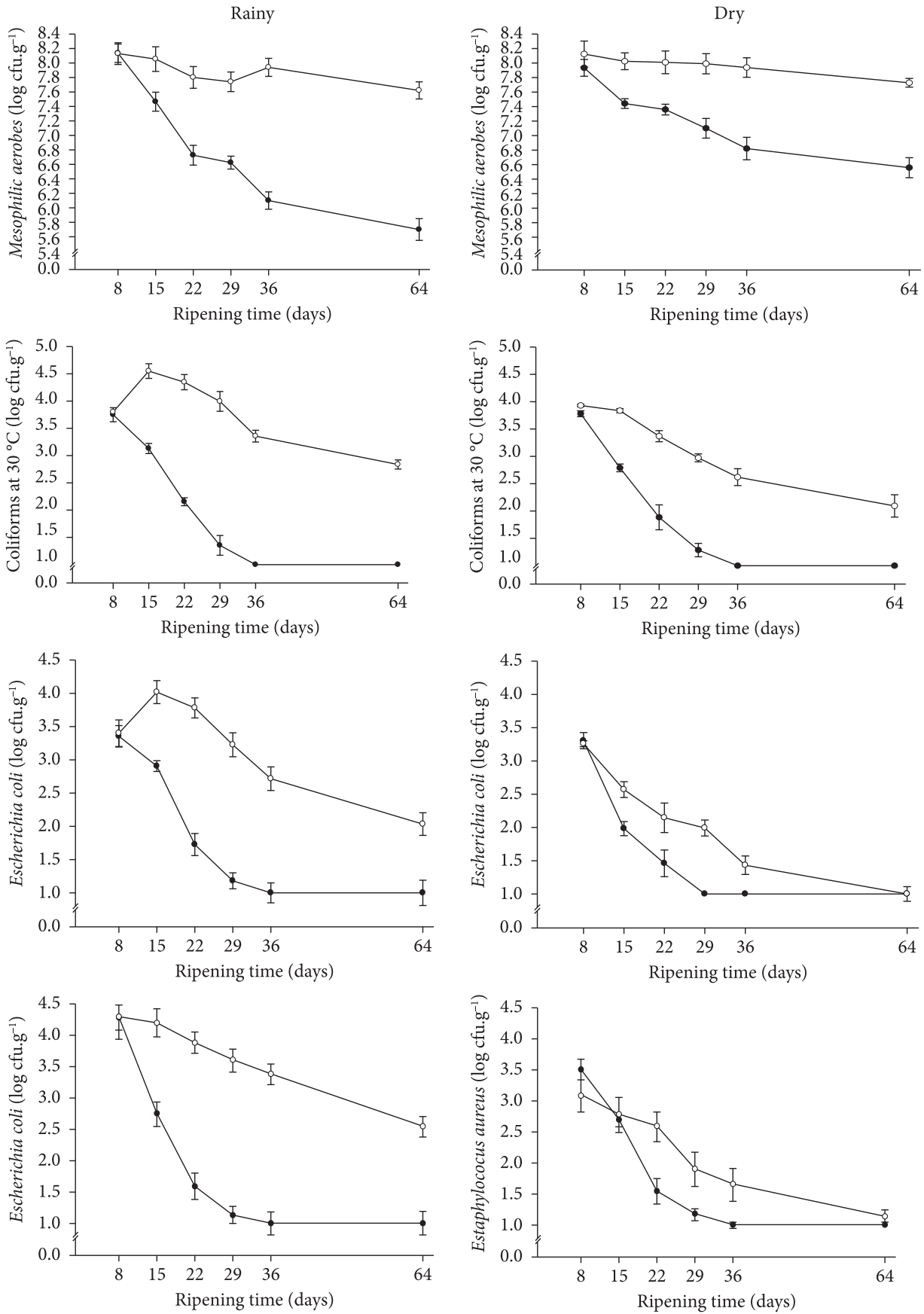


Figure 2. Estimate of counts of mesophilic aerobes, coliforms at 30 °C, *Escherichia coli*, and *Staphylococcus aureus* in Canastra cheese as a function of ripening time at room temperature (●) and under refrigeration (○) in the dry season and rainy season.

At eight days of ripening, all samples of Canastra cheese showed coliform counts at 30 °C, *E. coli* and *S. aureus* counts higher than those established by the Brazilian legislation. These results may be associated with the absence of heat treatment and improper handling of milk used in the production of such cheeses. Other sources of contamination that can also be mentioned are the low quality of water used in manufacturing and inadequate manufacturing practices by food handlers. However, the reduction of these microorganisms observed during ripening suggests that they could be inhibited by highly competitive lactic acid bacteria which are able to withstand ripening conditions. It is well known that ripening acts as a natural selector, during which lactic acid bacteria normally inhibit pathogens (CARIDI et al., 2003). The rate of reduction in the pathogen counts may also be related to the reduction of water activity, increased NaCl concentration, and higher ripening temperature. In a study conducted by Nardes (2006), it was found that the higher the maturation temperature, the greater the loss of moisture in cheese leading to a decreased Aw and increased solid concentration, which increases the chloride content. The salt content is one of the factors that directly interfere in the Aw of cheese, following an inverted proportional tendency (FURTADO; LOURENÇO NETO, 1994). Thus, the higher the concentration of salt in the cheese, the lower the water activity of this product (ECK, 1987).

Table 1 shows the period when the collected cheese samples would be fit for consumption, according to the Brazilian laws (BRASIL, 2000). *S. aureus* was the species that survived longer under the conditions used. Cheeses aged at room temperature were suitable for consumption after 22 days of ripening in both seasons. However, the cheese stored under refrigeration during ripening required at least 35 days of aging in the dry season, whereas in the rainy season, 64 days of aging were not sufficient to reduce the counts of *S. aureus* to acceptable levels.

It is worth to remember that when the cheese samples were kept refrigerated, no change on the Aw values was observed during ripening. This is important because even after 64 days of ripening these samples were considered high moisture

Table 1. Estimated minimum period of Canastra cheese ripening for microbiological adequacy requirements established by Brazilian legislation.

Counts	Season	Ripening temperature	Legislation
Coliforms at 30 °C	Wet	Ambient	16 days
		Cooling	35 dias
	Dry	Ambient	21 days
		Cooling	13 days
<i>E. coli</i>	Wet	Ambient	22 days
		Cooling	32 days
	Dry	Ambient	18 days
		Cooling	13 days
<i>S. aureus</i>	Wet	Ambient	22 days
		Cooling	>64 days
	Dry	Ambient	21 days
		Cooling	35 days

cheese, and therefore they did not meet the law specifications. Thus, the cooling temperature was not sufficient to maintain the microbiological quality of cheese, and it did not reduce the microbial load during ripening. It is known that temperature is a primary factor in food preservation, especially those foods with high nutrient content and high Aw values such as cheese.

Therefore, refrigeration maintains the initial microbiota in foods produced with raw milk and consequently with the potential to for microbial contamination. This fact is usually ignored or not even recognized by business that transport and commercialize cheese made from raw milk. Similar results were also observed by Martins (2006) when analyzing artisanal cheese produced in the Serro region (MG, Brazil).

4 Conclusion

The influence of the ripening period on the physicochemical characteristics of the cheeses aged at room temperature significantly influenced the microbiota, with a reduction in the counts of mesophilic aerobes, coliforms at 30 °C, *E. coli* and *S. aureus*. The genus *Listeria* sp. and *Salmonella* sp. were not detected in any of the samples. The species *S. aureus* survived longer under conditions above the legal limit and therefore defined the minimum time required for the ripening of these cheeses. Therefore, the ripening temperature was crucial to reducing pathogenic microorganisms regardless of the season that the cheese was produced. On the other hand, the cheeses made in the dry season that were aged under refrigeration only reached the levels established by law after 37 days of ripening, while in the rainy season, 64 days of ripening were not sufficient to reduce the counts of pathogens to levels acceptable by the Brazilian legislation.

The results of this study indicate that aging under refrigeration was not enough to reduce pathogenic microorganisms at levels below the legal limit, producing cheeses after 64 days of ripening with low microbiological quality and low sensory attributes. The use of refrigeration for cheese ripening should be reviewed by government agencies since this type of treatment does not guarantee the microbiological safety of the product.

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