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Microbiological and physicochemical characterization of Minas artisanal cheese from the Campo das Vertentes region (Brazil) during ripening in rainy and dry seasons

Caracterização microbiológica e físico-química do queijo artesanal Minas da região de Campo das Vertentes (Brasil) durante o período de maturação nas estações chuvosa e seca

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ABSTRACT

Introduction: Minas artisanal cheese is produced from raw milk. Legislation establishes a minimum 22-day ripening period for the cheese from Campo das Vertentes region. However, the lack of studies does not allow to attest whether this period is adequate to comply with the parameters. **Objective:** Based on this, the aim of this survey was to evaluate the microbiological and physicochemical quality of these cheeses throughout the ripening period at rainy and dry seasons. **Method:** Cheese samples throughout ripening were collected at four cheesemaking facilities, and were submitted to microbiological and physicochemical analyses. **Results:** The quality of cheeses was influenced by the ripening period and counts of coliforms at 45°C and molds and yeasts were affected by the season. **Conclusions:** The results met the official legislation requirements for cheeses at 60 and 22 days of ripening at rainy and dry seasons, and demonstrate the need for improvements in the control of coliforms and *Staphylococcus*.

KEYWORDS: Inspection; Food Quality; Food Safety; Foodborne Pathogens; Raw Milk Cheese

RESUMO

Introdução: O queijo Minas artesanal é produzido a partir de leite cru. A legislação estabelece um período mínimo de maturação de 22 dias para o queijo da região de Campo das Vertentes. No entanto, a falta de estudos não permite atestar se esse período é adequado para cumprir com os parâmetros. **Objetivo:** Avaliar a qualidade microbiológica e físico-química desses queijos ao longo do período de maturação nas estações chuvosa e seca. **Método:** Amostras de queijo durante a maturação foram coletadas em quatro queijarias e submetidas às análises microbiológicas e físico-químicas. **Resultados:** A qualidade dos queijos foi influenciada pelo período de maturação e as contagens de coliformes a 45°C e de fungos e leveduras foram afetadas pela estação. **Conclusões:** Os resultados atenderam aos requisitos da legislação oficial para queijos aos 60 e 22 dias de maturação nas estações chuvosa e seca, e demonstram a necessidade de melhorias no controle de coliformes e *Staphylococcus*.

PALAVRAS-CHAVE: Inspeção; Patógenos Transmitidos por Alimentos; Qualidade Alimentar; Queijo de Leite Cru; Segurança Alimentar

INTRODUCTION

Minas artisanal cheese (MAC) is a traditional Brazilian product from Minas Gerais state, which is obtained from raw milk, coagulated by the action of rennet, added of endogenous starter cultures, popularly known as "pingo"[1]. Due to the artisanal character of its production, MAC is widely manipulated throughout its elaboration and may present a potential risk to consumer health. Besides that, raw milk and endogenous starter cultures have an abundant and diverse microbiota[2]. This may contribute to the presence of undesirable microorganisms to the final product, such as coliforms, *Salmonella* spp. and *Staphylococcus aureus*[3].

On the other hand, lactic acid bacteria (LAB) found in cheese contribute to the food safety. Sant'Anna et al.[4] showed that LAB isolated from MAC, produced in Campo das Vertentes region were able to inhibit, *in vitro* and *in vivo*, the activity of pathogenic microorganisms. They also play an important role in determining the sensory characteristics of cheeses, due to the production of compounds from fermentation, proteolysis, and lipolysis processes[5].

The physicochemical changes that occur during cheese ripening also contribute to its safety and sensory characteristics. The loss of moisture by the evaporation and the draining process of the whey cause the concentration of solids compounds. In addition, organic acids accumulated due to the fermentation process, results in a decrease in the pH[5].

In this context, climatic conditions change the microbiological and physicochemical characteristics of cheeses[2]. The Campo das Vertentes region has two well-defined seasons: one rainy and with high temperatures (October to March), and another dry with milder temperatures (April to September)[6]. Thus, it becomes essential to investigate the ripening of cheeses in different seasons, in order to analyze how seasonality influences the transformations that occur during this process.

The Brazilian legislation establishes that raw milk cheeses must be ripened for 60 days or less, based on the results of scientific studies[7]. According to this prerogative, MAC produced in the Campo das Vertentes region must be subjected to 22 days of ripening to eliminate health hazards to consumers[8]. The study of the microbiological and physicochemical parameters allows determining whether the proposed ripening period is adequate. Based on this, the aim of this survey was to evaluate the microbiological and physicochemical characteristics of MAC, from the Campo das Vertentes region, during ripening at dry and rainy seasons. The results will assist the official inspectors determine the appropriate ripening time for the MAC from the studied region.

METHOD

Sampling

After production stage, the cheeses were stored in a specific area dedicated to ripening, placed on wooden shelves, under natural conditions of temperature and humidity. The ripening room was equipped with windows for air circulation, sealed with insect screens.

Cheeses were aseptically sampled at 1, 7, 14, 22 and 60 days of ripening: two samples for period, from the same batch. They were transported in polystyrene thermal boxes containing recyclable ice bars, maintaining a temperature of approximately 4°C until processing at the laboratory. The sampling process was carried out in November 2019 (rainy season) and July 2021 (dry season).

Microbiological analyses

The analyses of the most probable number (MPN) of coliforms at 30°C and at 45°C; counts of *Staphylococcus* spp., coagulase-positive *Staphylococcus* and mold and yeast; and presence of *Salmonella* spp. were carried out following the recommendations of the American Public Health Association[9]. Suspected *Salmonella* colonies were submitted to the MALDI-TOF mass spectrometry (Bruker Daltonik MALDI Biotyper, Bruker, United States) for confirmation[10]. Enumeration of LAB was performed following the International Dairy Federation procedure[11].

Physicochemical analyses

The physicochemical analyses were carried out in triplicate. The parameters analyzed for the MAC were: contents of moisture by the gravimetric method[12], ashes and chloride[13], fat by the butyrometric method[14], protein by the micro-Kjeldahl method[15], as well as titratable acidity and pH[13]. The fat in dry matter content (FDM) was calculated by the following formula: (fat content x 100)/solids content.

Statistical analyses

Data normality and homoscedasticity were analyzed using the Shapiro-Wilk test and the Bartlett test, respectively. The parametric data were evaluated by the Tukey test (between ripening periods) and Sidak test (between seasons). The non-parametric data were evaluated by the Friedman test (between ripening periods and seasons of the year). All analyses were performed considering a significance level of 5%, using the Graphpad Prism 7.0 software.

RESULTS AND DISCUSSION

Microbiological parameters

As can be seen in Table 1, LAB were the most prevalent micro-organisms in MAC, regardless of the period of ripening and climatic season.

The medians found were similar to the counts of these bacteria related by Castro et al.[2]. The initial counts of these microorganisms were high in the dry period, although the statistical analysis did not show significant difference ($P > 0.05$). Costa Júnior et al.[1] observed that during the dry period, cheesemakers add a greater volume of endogenous starter culture and, consequently, inoculate more LAB to the cheese. The high counts of LAB observed during the first 14 days of cheese ripening, in both seasons, are associated with the fermentation process

Table 1. Microbiological quality of Minas artisanal cheese produced in the Campo das Vertentes region, Minas Gerais state, Brazil, during ripening.

Parameter	Season	Days of ripening				
		1	7	14	22	60
Coliforms at 30°C (MPN/g)	Rainy	> $1.1 \times 10^{5\,Aa}$	$6 \times 10^{4\,Aa}$	$1.4 \times 10^{4\,Aa}$	> $1.1 \times 10^{5\,Aa}$	$3.3 \times 10^{2\,Aa}$
	Dry	$5.6 \times 10^{4\,Aa}$	$1.4 \times 10^{4\,Aa}$	$2.4 \times 10^{4\,Aa}$	$3.1 \times 10^{3\,Aa}$	< $3.0 \times 10^{2\,Aa}$
Coliforms at 45°C (MPN/g)	Rainy	$5.7 \times 10^{4\,Aa}$	$1.2 \times 10^{3\,Aa}$	$1.1 \times 10^{3\,Aa}$	$9.0 \times 10^{2\,Aa}$	< $3.0 \times 10^{2\,Aa}$
	Dry	< $3.0 \times 10^{2\,Ba}$	< $3.0 \times 10^{2\,Aa}$	< $3 \times 10^{2\,Ba}$	< $3.0 \times 10^{2\,Aa}$	< $3.0 \times 10^{2\,Aa}$
Lactic acid bacteria (CFU/g)	Rainy	$2.5 \times 10^{6\,Aa}$	$2.2 \times 10^{8\,Aa}$	$1.4 \times 10^{8\,Aa}$	$8.2 \times 10^{7\,Aa}$	$5.6 \times 10^{7\,Aa}$
	Dry	$1.5 \times 10^{8\,Aa}$	$1.1 \times 10^{8\,Aa}$	$1.7 \times 10^{8\,Aa}$	$1.6 \times 10^{8\,Aa}$	$2.5 \times 10^{6\,Aa}$
Molds and yeasts (CFU/g)	Rainy	$8.0 \times 10^{4\,Ba}$	$3.6 \times 10^{7\,Aa}$	$8.7 \times 10^{6\,Ba}$	$5.8 \times 10^{6\,Ba}$	$6.0 \times 10^{6\,Aa}$
	Dry	$1.4 \times 10^{8\,Aa}$	$1.5 \times 10^{8\,Aa}$	$1.4 \times 10^{8\,Aa}$	$7.1 \times 10^{7\,Aa}$	$2.8 \times 10^{6\,Aa}$
Staphylococcus spp. (CFU/g)	Rainy	$2.5 \times 10^{5\,Aa}$	$6.0 \times 10^{6\,Aa}$	$3.6 \times 10^{7\,Aa}$	$2.6 \times 10^{7\,Aa}$	$4.0 \times 10^{7\,Aa}$
	Dry	$3.9 \times 10^{5\,Aa}$	$9.7 \times 10^{6\,Aa}$	$1.6 \times 10^{7\,Aa}$	$5.1 \times 10^{7\,Aa}$	$5.8 \times 10^{6\,Aa}$
CPS (CFU/g)	Rainy	$1.5 \times 10^{4\,Aa}$	< $1.0 \times 10^{4\,Aa}$	< $1.0 \times 10^{4\,Aa}$	< $1.0 \times 10^{4\,Aa}$	< $1.0 \times 10^{4\,Aa}$
	Dry	$1.6 \times 10^{5\,Aa}$	$3.5 \times 10^{4\,Aa}$	$2.9 \times 10^{4\,Aa}$	< $1.0 \times 10^{3\,Aa}$	< $1.0 \times 10^{3\,Aa}$
Salmonella spp. (presence/25g)	Rainy	Absence	Absence	Absence	Absence	Absence
	Dry	Absence	Absence	Absence	Absence	Absence

Source: Prepared by the authors, 2024.
MPN: most probable number; CFU: colony-forming unit; CPS: coagulase-positive *Staphylococcus*
[a-b]Medians within a row with distinct superscripts differ significantly by Friedman test ($P < 0.05$); [A-B]Medians within a columns with distinct superscripts differ significantly by Friedman test ($P < 0.05$).

and the consequent increase in cheese acidity. Additionally, during their metabolism, LAB produce antagonistic compounds, such as bacteriocins, which may contribute to the control of undesirable microorganisms in these products[16]. The tendency of the LAB population to decrease in the more advanced stages of ripening can be explained by the increase in acidity and decrease in the moisture content[17].

Coliforms had high counts in fresh MAC, mainly in the rainy season. Considering only coliforms at 45°C, their count was statistically higher in this period ($P < 0.05$). Factors related to this season, such as mud formation, high temperatures and environmental humidity, may have contributed to this result[2]. In general, coliform counts showed a tendency to decrease throughout ripening, which can be explained by the inhibitory mechanisms exerted by LAB[4]. However, in some specific moments, such as at 22 days of ripening during the rainy season, an abrupt increase in the value of this indicator was observed. The most likely cause was the recontamination of cheeses due to inadequate handling.

Salmonella spp. was not detected in any MAC sample, regardless of the ripening period and season. Care with personal hygiene associated with the competitive microbiota of the cheese, with a predominance of LAB, also ensured that the environment was inappropriate for the establishment of these bacteria.

Staphylococcus spp. was found in high counts in MAC, regardless of the ripening period and season of the year. The persistence of *Staphylococcus* spp. over ripening may indicate recontamination of the cheeses, which may be caused by lack of good manufacturing practices by the handlers. From 20 to 60% of the human beings are asymptomatic carriers of this bacterium on the hands, in oropharynx, nasal vestibules and/or nail bed[18]. *Staphylococcus* spp. can tolerate acidity and desiccation, which also contributes to its persistence throughout cheese ripening[19].

Coagulase-positive *Staphylococcus* is considered of health interest, since it may also produce enterotoxins. Counts of *Staphylococcus* spp. higher than 10^5 CFU/g in cheeses were recorded throughout the ripening period. Scientific literature states that from 1×10^4 CFU/g there can be a significant production of enterotoxins[20].

Coagulase-positive *Staphylococcus* was detected in MAC from the 1st to the 14th day of ripening in the rainy and dry seasons, respectively. Although counts of this bacterium remained high throughout ripening, it decreased to undetectable levels at 7th (rainy season) and 22th (dry season) days of ripening. However, this reduction does not eliminate the hazard. Due to tolerance to factors such as temperature, pH and proteolytic activity of enzymes, enterotoxins are able to persist in cheeses even after the elimination of producing microorganisms[20].

Count of molds and yeast was the parameter with the greatest difference of results due to the season of year ($P < 0.05$). The dry period can favor these microorganisms due to the optimal growth temperature and the greater dispersion of spores in the air[21]. Molds and yeasts are also more resistant to acidity and low humidity, which justifies their high counts throughout the ripening period[22]. Although these microorganisms can be related to the development of desirable sensory characteristics to cheeses, they can also be indicators of hygienic-sanitary conditions. Thus, it is important to carry out studies to characterize the fungi found in cheeses.

Physicochemical parameters

As seen in Table 2, there was no significant effect (P > 0.05) of the season on the physicochemical parameters of the MAC. Although unexpected, this result indicates that the cheeses were made under technological conditions in order to maintain a quality standard throughout the year.

The titratable acidity of the MAC showed a tendency to increase during ripening. It was expected that the development of cheese acidity would be more accentuated in the rainy season, but the higher initial LAB count (Table 1) may have contributed to the obtained result. The development of acidity observed during ripening can be considered one of the main factors that led to a decrease in the counts of undesirable microorganisms and influenced their viability (Table 1). The pH of the MAC was not significantly affected by the ripening period (P > 0.05). A decrease in pH throughout ripening was expected as a consequence of lactic fermentation. However, products from the proteolysis process and the degradation of acids by molds and yeasts increase the pH[23].

Similar reduction of moisture content of MAC was observed in both seasons (P > 0.05). Costa Júnior et al.[1] described some strategies that are used to prevent excessive drying of cheeses during the dry period, such as cutting the mass into larger grains and applying less force during mass pressing. From days 1st to 7th of ripening, moisture reduction was more intense. At the beginning of ripening, the cheese loses moisture in two ways: surface evaporation and syneresis. With advancing ripening, syneresis tends to decrease until it stops[24].

The tendency of the fat content to rise (P < 0.05) during ripening is also a consequence of the of moisture reduction in cheeses. The same cannot be attributed to the increase (P < 0.05) observed in the fat in total solids content in the rainy season. It can occur due to the reduction in the content of some solid components of the cheese, as probably the degradation of proteins as a result of the proteolysis process[25]. Although the protein content increased (P < 0.05) throughout ripening, resulted from the reduction of moisture, this rising was less intense compared to that related to the fat content. This finding also reinforces the hypothesis of a probable effect of proteolysis to degrade protein molecules.

The concentrations of mineral residues and chlorides also tended to increase along the ripening period due to the loss of moisture by the cheeses, although the statistics showed significant differences (P < 0.05) only for the mineral content in the dry period. Cheese minerals are composed of several chemical elements and compounds, the main ones being calcium from milk and sodium chloride from salting. Some dairy minerals are water-soluble and can be leached from the cheese during whey drainage and cleaning, preventing the concentration effect during ripening from being more intense. Chloride content showed great variation

Table 2. Physicochemical quality (mean and standard deviation) of Minas artisanal cheese produced in the Campo das Vertentes region, Minas Gerais state, Brazil, during ripening.

Parameter	Season	Days of ripening				
		1	7	14	22	60
Acidity (g/100 g)	Rainy	$0.14^{Aa} \pm 0.06$	$0.24^{Ab} \pm 0.05$	$0.31^{Ab} \pm 0.05$	$0.48^{Abc} \pm 0.11$	$0.54^{Ac} \pm 0.07$
	Dry	$0.16^{Aa} \pm 0.06$	$0.31^{Aab} \pm 0.10$	$0.38^{Abc} \pm 0.09$	$0.51^{Acd} \pm 0.15$	$0.56^{Ad} \pm 0.03$
pH	Rainy	$5.48^{Aa} \pm 0.38$	$5.31^{Aa} \pm 0.21$	$5.44^{Aa} \pm 0.14$	$5.31^{Aa} \pm 0.14$	$5.25^{Aa} \pm 0.19$
	Dry	$5.28^{Aa} \pm 0.32$	$5.12^{Aa} \pm 0.28$	$5.08^{Aa} \pm 0.15$	$5.10^{Aa} \pm 0.21$	$5.39^{Aa} \pm 0.15$
Moisture (g/100 g)	Rainy	$55.03^{Aa} \pm 2.67$	$42.98^{Ab} \pm 3.38$	$41.30^{Ab} \pm 2.34$	$37.70^{Ab} \pm 2.45$	$26.13^{Ac} \pm 0.93$
	Dry	$57.90^{Aa} \pm 6.89$	$49.47^{Aab} \pm 3.92$	$44.74^{Abc} \pm 6.56$	$39.09^{Acd} \pm 7.63$	$28.8^{Ad} \pm 7.66$
Total solids (g/100 g)	Rainy	$44.97^{Aa} \pm 2.67$	$57.02^{Ab} \pm 3.38$	$58.70^{Ab} \pm 2.34$	$62.30^{Ab} \pm 2.45$	$73.87^{Ac} \pm 0.93$
	Dry	$42.10^{Aa} \pm 6.89$	$50.53^{Aab} \pm 3.92$	$55.26^{Abc} \pm 6.56$	$60.91^{Acd} \pm 7.63$	$71.20^{Ad} \pm 7.66$
Fat (g/100 g)	Rainy	$22.13^{Aa} \pm 5.14$	$29.79^{Aab} \pm 2.90$	$32.79^{Ab} \pm 2.65$	$34.75^{Abc} \pm 1.47$	$43.33^{Ac} \pm 2.38$
	Dry	$21.63^{Aa} \pm 6.59$	$25.17^{Aab} \pm 3.69$	$29.67^{Aab} \pm 4.38$	$31.25^{Abc} \pm 5.12$	$39.75^{Ac} \pm 6.00$
FDM (g/100 g)	Rainy	$48.85^{Aab} \pm 8.91$	$47.46^{Aa} \pm 3.09$	$55.81^{Aab} \pm 2.90$	$55.79^{Aab} \pm 1.13$	$58.67^{Ab} \pm 3.41$
	Dry	$50.64^{Aa} \pm 6.54$	$49.70^{Aa} \pm 4.91$	$53.86^{Aa} \pm 7.03$	$51.17^{Aa} \pm 3.37$	$53.13^{Aa} \pm 5.51$
Protein (g/100 g)	Rainy	$16.16^{Aa} \pm 0.78$	$22.32^{Ab} \pm 1.95$	$23.47^{Abc} \pm 2.00$	$23.55^{Abc} \pm 1.61$	$26.79^{Ac} \pm 4.00$
	Dry	$15.21^{Aa} \pm 1.45$	$18.37^{Aab} \pm 1.03$	$20.24^{Ab} \pm 2.20$	$21.82^{Ab} \pm 2.02$	$26.48^{Ac} \pm 2.34$
Ashes (g/100 g)	Rainy	$3.15^{Aa} \pm 0.36$	$3.95^{Aa} \pm 0.76$	$3.34^{Aa} \pm 0.46$	$3.86^{Aa} \pm 0.39$	$4.05^{Aa} \pm 1.27$
	Dry	$3.17^{Aa} \pm 0.88$	$4.86^{Aab} \pm 1.44$	$4.51^{Aab} \pm 1.45$	$5.04^{Aab} \pm 1.34$	$6.08^{Ab} \pm 2.09$
Chlorides (g/100 g)	Rainy	$1.05^{Aa} \pm 0.29$	$1.07^{Aa} \pm 0.11$	$1.02^{Aa} \pm 0.32$	$1.26^{Aa} \pm 0.43$	$1.47^{Aa} \pm 0.87$
	Dry	$1.33^{Aa} \pm 0.94$	$2.19^{Aa} \pm 1.09$	$2.31^{Aa} \pm 1.34$	$2.35^{Aa} \pm 1.48$	$2.40^{Aa} \pm 1.47$

Source: Prepared by the authors, 2024.
FDM: Fat in dry matter.
[a-d]Means within a row with distinct superscripts differ significantly by Tukey test (P < 0.05); [A-B]Means within a columns with distinct superscripts differ significantly by Sidak test (P < 0.05).

among cheesemaking facilities. As cheese production is an artisanal process, there is no standardization of the salting step, which may have contributed to this result.

Compliance with legislation

Considering the general values shown in tables 1 and 2, the MAC produced during the rainy season only met all legal requirements after 60 days of ripening. The dry season MAC, in turn, met the legal parameters at 22 days of ripening[26].

The main obstacle to a reduction in the ripening period in the rainy season were the microbiological standards established by legislation (counts of coliforms at 30°C, coliforms at 45°C and coagulase-positive *Staphylococcus*). Sanitary-hygienic failures in the production process and raw material quality may be resulting in contamination and recontamination of the cheeses. Therefore, it is suggested that the precepts of good manufacturing practices are carefully followed, as a way to minimize the occurrence of this situation.

In the dry period, the moisture content remained above the maximum allowed value (45.9%) before the cheeses reached 22 days of ripening[26]. Some strategies used by the producer to avoid excessive drying of the cheeses in this season (such as cutting the curd into larger grains and less force applied when pressing the curd) may have contributed to the cheeses remaining with a high moisture content[1].

CONCLUSIONS

Counts of coliforms at 45°C and yeast and molds were the only parameters statistically different between seasons. Ripening was a phenomenon capable of significantly modifying the physicochemical quality of the MAC, in addition to decreasing its microbial load. The results of sanitary evaluation of MAC were in line with the legislation at 60 (rainy season) and 22 (dry season) days of ripening. It is believed that hygienic failures in the cheeses manipulation may have resulted in recontamination, which made it difficult to determine an earlier ripening period according to the up-to-date legislation.

The ripening of MAC significantly enhances both the sensory quality and safety of the products. This process reduces microbial load, hindering the growth of pathogenic microorganisms, while also improving desirable characteristics such as flavor and texture. These transformations underscore the importance of maturation in ensuring compliance with sanitary standards and preserving the identity of artisanal cheeses.

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Authors' Contribution

Valente GLC, Silva AM, Penna CFAM, Souza MR - Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work. Figueiredo RC, Brito RF, Fonseca LM - Analysis. Lana AMQ – Planning (study design), analysis, data interpretation. Souza BMS - Writing of the work. Madureira AP – Planning (study design), analysis. All the authors approved the final version of the work.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.