

**MICROBIOLOGICAL EVALUATION OF COLONIAL CHEESES PRODUCED IN
ROQUE GONZALES, NORTHWEST OF RIO GRANDE DO SUL STATE, BRAZIL**

*(AVALIAÇÃO MICROBIOLÓGICA DE QUEIJOS COLONIAIS PRODUZIDOS EM ROQUE
GONZALES, NOROESTE DO ESTADO DO RIO GRANDE DO SUL, BRASIL)*

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RESUMO

O queijo Colonial, nas perspectivas econômica e cultural, é um importante produto produzido a partir de leite cru por famílias rurais do Sul do Brasil. Considerando que a contaminação microbiana pode afetar negativamente a qualidade e a segurança dos alimentos, o presente trabalho objetivou avaliar a qualidade microbiológica de oito queijos Coloniais produzidos em Roque Gonzales, noroeste do Rio Grande do Sul, Brasil. Os coliformes totais, considerados contaminantes ambientais, variaram de $1,5 \times 10^2$ a $2,3 \times 10^5$ Número Mais Provável (NMP).g⁻¹. A enumeração de coliformes termotolerantes, usados como indicadores sanitários, variou de $<1,5$ NMP.g⁻¹ a $1,5 \times 10^4$ NMP.g⁻¹. De acordo com a legislação brasileira para queijos de alta umidade, três queijos apresentaram não conformidade em relação aos coliformes totais ($>1 \times 10^4$ NMP.g⁻¹), sendo um deles inadequado para consumo devido à contagem de coliformes termotolerantes ($>5 \times 10^3$ NMP.g⁻¹). As bactérias aeróbias mesófilas variaram de 10^7 a 10^9 Unidades Formadoras de Colônia (UFC).g⁻¹, e bolores e leveduras entre 10^3 e 10^4 UFC.g⁻¹. Bactérias mesófilas e fungos e leveduras são indicadores de contaminação ambiental, geralmente levando à deterioração do produto. Os resultados indicam condições higiênico-sanitárias insatisfatórias, sugerindo o uso de leite contaminado, deficiências na produção e/ou manuseio inadequado. Considerando o crescente interesse dos consumidores pelos queijos Coloniais, e também a importância desses queijos para as famílias rurais, ações que promovam práticas higiênicas na cadeia produtiva podem beneficiar a qualidade e segurança desses produtos.

Palavras-chave: contaminação, indicadores microbiológicos, coliformes termotolerantes, coliformes totais

ABSTRACT

Colonial cheese, from both economic and cultural perspectives, is an important product manufactured from raw milk by rural families in southern Brazil. Since microbial contamination might negatively affect the food quality and safety, the aim of the present study was to evaluate the microbiological quality of eight Colonial cheeses manufactured in Roque

Gonzales municipality, northwest Rio Grande do Sul state, Brazil. Total coliforms, considered as environmental contaminants, ranged from 1.5×10^2 to 2.3×10^5 Most Probable Number (MPN).g⁻¹. Enumeration of thermotolerant coliforms, used as sanitary indicators, ranged from <1.5 MPN.g⁻¹ to 1.5×10^4 MPN.g⁻¹. According to the Brazilian legislation for high moisture cheeses, three Colonial cheeses presented nonconformity regarding total coliforms ($>1 \times 10^4$ MPN.g⁻¹), and one of these was inadequate for consumption due to thermotolerant coliform counts ($>5 \times 10^3$ MPN.g⁻¹). Aerobic mesophilic bacteria ranged from 10^7 to 10^9 Colony Forming Units (CFU).g⁻¹, and molds and yeasts were between 10^3 and 10^4 CFU.g⁻¹. Mesophilic bacteria, and molds and yeasts, are indicators of environmental contamination, commonly leading to product deterioration. Results indicate unsatisfactory hygienic-sanitary conditions, suggesting the use of contaminated milk, deficiencies during production and/or improper handling. Considering the growing interest of consumers in Colonial cheeses, and also the importance of these cheeses for rural families, actions that promote hygienic practices in the production chain can benefit the quality and safety of these products.

Keywords: contamination, microbiological indicators, thermotolerant coliforms, total coliforms

INTRODUCTION

Cheese is a common component of the human diet, presenting a considerable importance on the consumption profile of the Brazilian population, integrating the habits and national culture (APOLINÁRIO et al., 2014). Colonial-type cheese is commonly manufactured from raw bovine milk by rural families in Southern Brazil (CARVALHO et al., 2019).

The northwest region of Rio Grande do Sul state (RS) stands out with important dairy basins, with expressive participation of family farms. Informal production of Colonial cheese by rural families is a routine practice, and these products are intended for at-home consumption and/or trade (SCHMITT et al., 2011). The production of artisanal cheeses also represents a source of income for small rural producers (PINEDA et al., 2021).

Even though the commercialization of cheeses made from raw milk is not allowed by the Brazilian legislation, except if they have been ripened for at least 60 days or laboratory tests indicate its safety, Colonial and other traditional Brazilian cheeses have been consumed for a long time without undergoing the 60-day ripening process (CARVALHO et al., 2019).

Colonial-type cheese is generally produced at the margin of legislation and without quality control. Therefore, these cheeses may not meet sanitary and hygienic standards (SCHMITT et al., 2011; SILVA et al., 2015). Indeed, the poor hygienic-sanitary conditions of both milk and cheese-making facilities appear as important factors resulting in the low microbiological quality of Brazilian cheeses (TESSER et al., 2016).

Microbial contamination of cheeses, in addition to potentially diminishing product quality and increasing economic losses due to spoilage, is particularly relevant from a food safety perspective (TESSER et al., 2016). Hence, indicator microorganisms are often used to detect the hygienic-sanitary status of cheeses (SALOTTI et al., 2006; OLIVEIRA et al., 2010).

In this sense, we aimed to evaluate the microbiological quality of Colonial cheeses manufactured in the Roque Gonzales municipality, RS, Brazil. The performed evaluations included the determination of total and thermotolerant coliforms, counts of total aerobic mesophilic bacteria, and molds and yeasts.

MATERIAL AND METHODS

Cheese samples and processing

A non-probability convenience sampling approach was utilized (APOLINÁRIO et al., 2014). A total of eight Colonial-type cheeses were purchased from informal producers in the Roque Gonzales municipality, RS. Cheeses were transported under refrigeration to the laboratory and kept under refrigeration until processing.

In the laboratory, 25 g of each sample (cheese) were weighed under aseptic conditions, placed on sterile plastic bags, and then 225 mL of sterile saline peptone water (8.5 g.L⁻¹ NaCl, 1 g.L⁻¹ peptone) were added. This mixture was homogenized in a stomacher-type homogenizer for 1 min. From this initial dilution (10⁻¹), decimal serial dilutions were carried out up to 10⁻⁶. The obtained dilutions were used for microbiological analysis.

Microbiological analysis

Enumeration of total and thermotolerant coliforms

Coliform analysis was performed through the multiple tube fermentation technique, using presumptive and confirmed tests (GARCIA et al., 2016). For presumptive tests, 1 mL of all dilutions obtained for each cheese were inoculated, in triplicate, in series of three tubes containing 10 mL of Lauril Tryptose Broth and an inverted Durham tube. Tubes were incubated in a bacteriological stove at 35 °C for 24-48 h and, following this period, they were observed for gas production.

Positive tubes (gas production) from the presumptive test were used to perform the confirmed tests. For total coliforms, a loopful of positive presumptive tubes was transferred to tubes containing 10 mL of Brilliant Green Bile Broth and an inverted Durham tube. For

thermotolerant coliforms, a loopful of positive presumptive tubes was used to inoculate tubes containing 10 mL of EC Broth and an inverted Durham tube.

Following incubation at 35 °C in a bacteriological stove (total coliforms) or at 45 °C in a water bath (thermotolerant coliforms) for 24-48 h, positivity was confirmed by gas formation. The Most Probable Number (MPN) for each of the confirmed tests was then determined, and results were expressed in MPN.g⁻¹ cheese.

Additionally, a loopful of confirmed thermotolerant coliform tubes (EC broth) was streaked onto Eosin Methylene Blue Agar (EMB) plates. After incubation at 37 °C for 24 h, plates were observed for the presence of bluish-black colonies with a metallic green glow, usually characteristic of *Escherichia coli* (GASPAROTTO et al., 2020).

Total aerobic mesophilic bacteria

Each cheese dilution (0.1 mL) was inoculated, in triplicates, onto the surface of Plate Count Agar (PCA) plates using the spread plate method. After incubation in a bacteriological stove at 35 °C for 24-48 h, colonies were counted and the results presented as Colony Forming Units (CFU) per gram of cheese (CFU.g⁻¹), taking into account the dilution factor (RODRIGUES and FERREIRA, 2016).

Molds and yeasts

Cheese dilutions (0.1 mL) were inoculated, in triplicates, onto acidified Potato Dextrose Agar (PDA) plates (SCHMITT et al., 2011). PDA was acidified to pH 3.5 using a sterile tartaric acid solution (10%). Colony counts were performed after incubation in a bacteriological stove at 25 °C for 5 days, and the results expressed as CFU.g⁻¹, considering the dilution factor.

Data analysis

In the MPN technique, total and thermotolerant coliforms (MPN.g⁻¹) were determined based on the number of positive/negative test tubes, considering sample dilutions, according to statistical tables with 95% confidence limits (BLODGETT, 2002). The results of the counts of total aerobic mesophilic bacteria (CFU.g⁻¹) and molds as yeasts are presented as means ± standard deviation. Obtained values were submitted to analysis of variance and, if adequate, means were compared by the Tukey's test at $p < 0.05$.

RESULTS AND DISCUSSION

Microbiological quality of traditional Brazilian cheeses, including Colonial cheese, is thoroughly investigated (IDE and BENEDET, 2001; SANTOS-KOELLN et al., 2009; CARVALHO et al., 2019; GASPAROTTO et al., 2020). However, to the best of our knowledge, this is the first report on Colonial cheese produced in the Roque Gonzales municipality, RS.

As for total coliforms, the results indicated variations between 1.50×10^2 MPN.g⁻¹ (cheese #2) to 1.50×10^5 MPN.g⁻¹ (cheese #7) (Table 1), with an average of 6.24×10^4 MPN.g⁻¹. In an evaluation of Minas Frescal cheese in the Minas Gerais state, 24 out of 31 samples had total coliforms above 1.1×10^3 MPN.g⁻¹ (APOLINÁRIO et al., 2014). The same results were obtained from the analysis of five samples of unlabeled Minas Frescal cheese, sold in the region of Campinas, São Paulo state (GARCIA et al., 2017).

Silva et al. (2015), evaluating eight Colonial cheeses commercialized in southwestern Paraná state, PR, reported total coliform counts ranging <3 MPN.g⁻¹ to $>1.1 \times 10^4$ MPN.g⁻¹. Based on 12 Colonial cheeses sold at an agricultural fair, Fava et al. (2012) found total coliform counts ranging from 6.0×10^3 to 2.0×10^7 CFU.g⁻¹. In 25 samples of Colonial cheeses produced in the Central region of RS, 19 had total coliform counts greater than 1.5×10^4 MPN.g⁻¹ (FARDIN et al., 2008). Casaril et al. (2017), evaluating 10 Colonial cheeses produced in southwestern PR, indicated total coliform counts above 1.1×10^3 MPN.g⁻¹ in seven samples.

Table 1. Enumeration of total and thermotolerant coliforms, and presumption of *Escherichia coli* in the evaluated Colonial cheeses

Sample (cheese)	Total coliforms (MPN.g ⁻¹ × 10 ²)	Thermotolerant coliforms (MPN.g ⁻¹ × 10 ⁰)	Presumption of <i>Escherichia coli</i> ^a
#1	11	<1.5	Negative
#2	1.5	75	Positive
#3	43	360	Positive
#4	2,300	15,000	Positive
#5	1,100	1,400	Positive
#6	24	20	Positive
#7	1,500	3,600	Positive
#8	15	430	Positive

^a Typical colonies on Eosin Methylene Blue (EMB) agar plates.

The evaluation of thermotolerant coliforms in Colonial cheeses indicated values from $<1.50 \text{ MPN.g}^{-1}$ (cheese #1) to $1.50 \times 10^4 \text{ MPN.g}^{-1}$ (cheese #4), as presented in Table 1. Through subcultures of positive thermotolerant coliform tests on EMB agar, the typical growth of *Escherichia coli* was observed for seven cheeses, except for cheese #1 (Table 1).

In an investigation with 28 Colonial cheeses commercialized in the Francisco Beltrão municipality, PR, 19 resulted in thermotolerant coliform counts above $5 \times 10^3 \text{ MPN.g}^{-1}$ (ANTONELLO et al., 2012). For eight colonial cheeses sold in Medianeira, PR, Lucas et al. (2012) demonstrated that, in four cheese samples, the counts were lower than 10^3 MPN.g^{-1} , while in the remaining samples the counts were equal to or higher than $2 \times 10^6 \text{ MPN.g}^{-1}$. From 35 samples of Colonial cheeses commercialized in open markets in São Miguel do Oeste, Santa Catarina state (SC), three were negative for thermotolerant coliforms; of the 32 positive samples, 11 had counts between 2 and 10^2 MPN.g^{-1} , two with of $1.1 \times 10^3 \text{ MPN.g}^{-1}$, and 19 with counts greater than $2.4 \times 10^3 \text{ MPN.g}^{-1}$ (ROSSI et al., 2008).

From 30 samples of colonial cheese commercialized in northwestern RS state, the minimum and maximum counts of thermotolerant coliforms were 7.3×10^3 and $2.4 \times 10^7 \text{ MPN.g}^{-1}$, with an average of $2.5 \times 10^6 \text{ MPN.g}^{-1}$ (SCHMITT et al., 2011), thus higher as compared to the average value ($2.6 \times 10^3 \text{ MPN.g}^{-1}$) in present study (Table 1). In 25 colonial cheese samples analyzed by Fardin et al. (2008), thermotolerant coliform counts ranged 3.6 MPN.g^{-1} to $>1.1 \times 10^7 \text{ MPN.g}^{-1}$, with values above $5.0 \times 10^3 \text{ MPN.g}^{-1}$ observed for 18 samples. For 25 samples of colonial cheeses produced in Três Passos, RS, average counts of thermotolerant coliforms were commonly lower than $1.1 \times 10^2 \text{ MPN.g}^{-1}$ (ROOS et al., 2005), that is, lower values in comparison to the average value in the current investigation.

Several studies indicate that Colonial cheeses usually have a high moisture content (LUCAS et al., 2012; OLIVEIRA et al., 2012; SILVEIRA JÚNIOR et al., 2012; SILVA et al. 2015). For high moisture cheeses, the Brazilian legislation establishes maximal values of 10^4 MPN.g^{-1} for total coliforms (BRASIL, 1996), and $5 \times 10^3 \text{ MPN.g}^{-1}$ for thermotolerant coliforms (BRASIL, 1996, 2001). Hence, cheeses #4, #5 and #7 extrapolated the total coliforms limit, and cheese #4 is not suitable for consumption based on thermotolerant coliforms analysis (Table 1).

Both total and thermotolerant coliforms, major contaminating agents in cheeses, are related to deterioration processes. The presence of total coliforms in foods does not necessarily indicate fecal contamination or the presence of enteropathogens. Total coliforms usually indicate contamination of environmental origin, related to unsatisfactory hygienic

conditions during cheese production (FARDIN et al., 2008; LUCAS et al., 2012; OLIVEIRA et al., 2017).

Thermotolerant coliforms constitute a group of bacteria able of fermenting lactose with CO₂ production at 44 to 45 °C. At these conditions, *Escherichia coli* represents nearly 90% of the bacteria present in a food sample (CARVALHO et al., 2019). Thus, thermotolerant coliforms are commonly found in the intestine of humans and animals, and its presence in cheeses might indicate a (in)direct contamination with fecal material and, therefore, the potential presence of enteropathogens (OLIVEIRA et al., 2010; APOLINÁRIO et al., 2014; GARCIA et al., 2016). Beyond its importance to evaluate the hygienic-sanitary conditions of the cheese manufacturing process, it should be noted that thermotolerant coliforms might be present in the raw milk, which is traditionally used to prepare Colonial cheeses (CARVALHO et al., 2019).

Counts of total aerobic mesophilic bacteria are shown in Table 2. In all cheeses, counts were equal to or higher than 10⁷ CFU.g⁻¹, with an average of 3.53 × 10⁸ CFU.g⁻¹. In 20 samples of Minas cheese, the average count was 6.31 × 10⁷ CFU.g⁻¹ (RODRIGUES and FERREIRA, 2016). Of 18 fresh artisanal cheeses evaluated by Garcia et al. (2016), counts were between 5.1 × 10⁴ to 5.0 × 10⁶ CFU.g⁻¹ for four cheeses, 5.1 × 10⁶ to 5.0 × 10⁸ CFU.g⁻¹ for seven cheeses, and higher than 5.0 × 10¹⁰ for the remaining cheeses. In an evaluation with eight colonial cheeses, aerobic mesophilic counts ranged from 1.1 × 10⁴ to 1.48 × 10⁷ CFU.g⁻¹ (SILVA et al., 2015).

Table 2. Counts of total aerobic mesophilic bacteria, and molds and yeasts, in the evaluated Colonial cheeses

Sample (cheese)	Total aerobic mesophilic bacteria (CFU.g ⁻¹ × 10 ⁷)	Molds and yeasts (CFU.g ⁻¹ × 10 ³)
#1	2.6 ± 0.6 d	15.3 ± 1.3 c
#2	20.9 ± 1.4 c	12.1 ± 0.4 d
#3	38.5 ± 2.1 b	26.0 ± 0.4 b
#4	35.5 ± 7.7 b	10.0 ± 1.5 de
#5	2.8 ± 0.6 d	93.0 ± 15.0 a
#6	25.2 ± 2.4 bc	1.75 ± 0.5 f
#7	1.0 ± 0.3 e	6.30 ± 0.4 e
#8	156.1 ± 11.0 a	15.9 ± 2.2 c

Means followed by the same letter in the columns are not different by the Tukey test ($p > 0.05$).

Schmitt et al. (2011) indicated that minimum and maximum aerobic mesophilic counts were 5.1×10^4 and 7.8×10^9 CFU.g⁻¹, respectively, with an average of 5.0×10^8 CFU.g⁻¹, for 30 colonial cheeses sold in the region northwest of RS, which is close to the average value demonstrated in the current work (Table 2). Wide variations, from 1.0×10^3 to 3.0×10^7 CFU.g⁻¹, were also previously demonstrated for 12 samples of Colonial cheeses (FAVA et al., 2012). Casaril et al. (2017) indicated counts between 5.0×10^2 and 2.25×10^5 CFU.g⁻¹ in 10 samples of Colonial cheese in southwestern PR. For 20 samples of Serrano cheese, Delamare et al. (2012) observed counts of mesophilic microorganisms between 8.12×10^7 to 2.95×10^9 CFU.g⁻¹. Also regarding this parameter, variations in the range of 1.5×10^7 to 2.5×10^{10} were reported for 25 samples of Colonial cheeses produced in Três Passos, RS (ROOS et al., 2005).

The observed high counts (Table 2) suggest hygienic inadequacies. Mesophilic microorganisms constitute the majority of milk contaminants, and may indicate, for instance, that cheeses were produced with contaminated milk and/or the cheeses were stored under inappropriate conditions (ROOS et al., 2005; FAVA et al., 2012; CASARIL et al., 2017). Although aerobic mesophilic counts are not covered by Brazilian legislation, the higher the counts, the shorter the cheese shelf-life, as these microorganisms favor product deterioration (GARCIA et al., 2016; SILVA et al., 2015).

The mold and yeast counts for the eight cheeses indicated a variation between 1.75×10^3 CFU.g⁻¹ (cheese #6) to 9.30×10^4 CFU.g⁻¹ (cheese #5) (Table 2), with an average of 2.25×10^4 CFU.g⁻¹. For comparison purposes, nine out of 10 samples of Minas Frescal cheese had mold and yeast counts equal to or higher than 3.6×10^5 CFU.g⁻¹ (GARCIA et al., 2017). In eight samples of the same type of cheese, counts were in the range of 4 CFU.g⁻¹ to 3.33×10^5 CFU.g⁻¹; in five samples, counts were higher than 9×10^3 CFU.g⁻¹ (BAIRROS et al., 2016).

From eight Colonial cheeses, mold and yeast counts ranged from 2×10^1 to 2.4×10^6 CFU.g⁻¹; in six samples, results equal to or greater than 1.44×10^3 CFU.g⁻¹ (SILVA et al., 2015). In an investigation with 30 Colonial cheeses, the minimum mold and yeast count was 2.2×10^4 CFU.g⁻¹ and the maximum was 1.3×10^9 CFU.g⁻¹ (SCHMITT et al., 2011). In a study with 10 Colonial cheeses, mold and yeast counts ranged from 1.89×10^4 to 3.00×10^5 CFU.g⁻¹ (CASARIL et al., 2017), similarly to the present work (Table 2).

As for aerobic mesophilic bacteria, there is no regulated limit for molds and yeasts in cheese. However, the presence of these microorganisms generally represents an undesirable situation. The properties of cheeses can be modified by the growth of fungi due to their deteriorating action, resulting in changes in flavor, texture, color and odor (BÁNKUTI et al., 2017; BAIRROS et al., 2016). In this sense, the higher the counts, the greater the inadequacies in terms of hygiene (FEITOSA et al., 2003).

CONCLUSIONS

Microbiological analysis of eight Colonial cheeses indicated that the levels of total coliforms in three cheeses, and of thermotolerant coliforms in only one cheese, were higher than the limits established by Brazilian legislation.

Even in this case, significant contamination by aerobic mesophilic bacteria, and molds and yeasts, was observed in all cheeses. Although these microbial groups are not foreseen in the Brazilian legislation, they indicate unsatisfactory hygienic practices, which may be related to milk quality, handling, equipment, and storage.

From the cultural and economic importance of Colonial cheese, adequate support and actions that encourage the training of cheesemakers regarding the adoption of adequate hygienic-sanitary strategies might result in enhanced microbiological quality, and thus an improved safety of these traditional products.

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