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Quality assessment of artisanal cheeses in the northwest region of Paraná

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Research Article

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Abstract

Artisanal unripened cheeses produced in northwestern Paraná, Brazil, were studied for microbiological quality and sensory quality. The cheeses analyzed showed high counts of aerobic mesophilic microorganisms and *S. aureus*. However, even with the results showing poor microbiological quality, from a sensory point of view, consumers considered cheeses acceptable (high acceptance index). The results may indicate that there is still a lack of training and knowledge of production procedures to reduce microbiological contamination of artisanal cheese produced in northwestern Paraná.

Milk production is one of the main agribusiness activities in Brazil, with the state of Paraná being the second largest producer and accounting for 12.9% (4.53 billion liters) of the national production in 2018 (IBGE, 2019). Artisanal cheese production is an important economic alternative for small farmers, as it boosts the rural economy and the permanence of men in the countryside (Carvalho *et al*., 2019). Most of the cheese produced is popular, such as unripened cheese, mozzarella, ricotta, prato cheese and hard cheese. However, heterogeneity is still found in the management and control of production, which can lead to variations in product quality, mainly influencing the microbiological and compositional aspects (Bemfeito *et al*., 2016).

Unripened 'fresh' cheese, also known as 'minas cheese' is widely consumed in Brazil, being considered a cheese with short shelf life Queiroz *et al.* (2017). Due to its high moisture content, this cheese is often associated with cases of high microbiological contamination and risk to the consumer, so its production must be continuously monitored to recognize weaknesses and improve product quality (Dores *et al*., 2013). To the best of our knowledge, artisanal unripened cheeses from northwestern Paraná have never been tested for their microbiological and sensory quality, which is performed in the present work.

Materials and methods

Cheese samples

Twenty unripened fresh cheese samples produced by small producers in the Northwest region of Paraná were randomly and aseptically collected by EMATER (Technical Assistance and Rural Extension Company- Brazil). Two cheeses were collected from each producer, collected in 2 different days of production, corresponding to 30% of sampling. These cheeses were prepared according to the protocols adopted by each producer, using raw or pasteurized milk, enzymatic coagulation with rennet and without heating the curd, and in some of them they used culture. The cheeses were pressed and vacuum packed (15–17 cm in diameter and 6 in height) before being transported under refrigeration (on ice, at 5°C) to the Mesoregional Center for Excellence in Milk Technology–UEM. It is important to highlight that this transportation step never exceeded 5 h. The samples were identified numerically and analyzed immediately upon arrival with respect to microbiological quality.

Microbiological analysis

The microbiological quality of cheeses was analyzed for aerobic mesophiles, total coliforms, coagulase-positive staphylococci (CPS), and *Salmonella* sp. Aerobic mesophiles was determined by surface inoculation on PCA agar (KASVI®, Brazil), until dilution 10^5, and incubation at 32°C for 48 h. The total coliforms were determined by inoculation on VRB agar (Himedia Laboratories, India) and incubation at 35°C for 48 h. Coagulase-positive Staphylococci group were determined by inoculating on mannitol salt agar and analyzing the colony characteristics.

Salmonella sp. was determined in 25 g of sample, using lactose broth for non-selective enrichment followed by RV broth for selective enrichment stage, both incubated at 35°C for 24 h. Typical colonies of *Salmonella* were isolated on plates containing SS agar (Salmonella Shigella) and xylose lysine deoxycholate agar (XLD) by streaking and incubating at 35°C for 24 h. The confirmatory test was performed in TSI agar (35°C for 24 h) to determine the typical reactions.

Cheeses instrumental color and consumer acceptability

Cheeses with acceptable microbiological quality ($n = 3$) were evaluated for their instrumental color of rind and core using a colorimeter (Konica Minolta, Tokyo, Japan) according to Cielab coordinates. Data was expressed as the angle Hue (h°), which gives the predominant wavelength composing the color, and chroma, which corresponds to color saturation (Gaglio *et al.*, 2019).

Sensory evaluations were carried out in two days where 108 consumers (untrained evaluators) of both genders between 18 and 60 years of age were recruited. The three samples were placed in disposable white plastic cups that were identified with three-digit numbers and served at random. The consumers evaluated sample acceptability through the structured 9-point hedonic scale (1 = detested, 9 = loved) for appearance, color, texture, overall appearance and taste. Purchase intention of cheeses was evaluated in a 3-point scale (would not buy, indifferent, would buy). The project was approved by the Ethics and Research Committee – UEM, according to CAAE: 25081613.8.0000.0104. The acceptance index (IA) was also calculated by considering only the overall appearance attribute, equal to the average of each sample divided by the maximum grade, and multiplied by 100, according to Deolindo *et al.* (2019) and Pieretti *et al.* (2019).

Statistical analysis

The results were subjected to analysis of variance (ANOVA) and Tukey test using XLSTAT 7.5.3, 2007.

Results and discussion

Microbiological analysis

The results of microbiological analysis of the 20 artisanal cheeses are presented in Table 1. Among the analyzed products, 85% presented counts above 10^3 for *S. coagulase-positive* bacteria, 50% had aerobic mesophile counts above 10^6 and 30% had counts above 10^4 for total coliforms. These are values that endanger food safety (Yoon *et al.*, 2016). Although Brazilian legislation does not provide for limits for aerobic mesophile counts and total coliforms in cheese, such classes of microorganisms are indicators of sanitary hygienic quality in food. Brazilian law establishes maximum limits of 10^3 CFU/g for Minas cheese. Therefore, the percentage of samples that present counts above those permitted by law is alarming, indicating a potential risk to the consumer. High counts of coagulase-positive staphylococci are usually related to milk obtained from mastitic cows in raw milk cheeses or, in pasteurized milk cheese, due to inadequate thermal process or post-processing recontamination (Prates *et al.*, 2017). In many cases, and in contravention of Brazilian law, artisanal fresh cheeses are made with raw milk (Carvalho *et al.*, 2019), such that any initial milk contamination carries through to the products.

Table 1. Results of microbiological analyses of cheeses

Sample	Total mesophilic counts (CFU/g)	Total coliforms (CFU/g)	Coagulase-positive *Staphylococci* (CFU/g)
P01	6.30×10^4	3.00×10^2	8.50×10^3
P02	1.18×10^7	8.70×10^4	3.21×10^4
P03	2.80×10^4	1.95×10^3	1.00×10^4
P04	1.53×10^7	$>1.00 \times 10^3$	4.60×10^3
P05	1.08×10^7	$>1.00 \times 10^3$	2.30×10^3
P06	$<1.00 \times 10^4$	$<1.00 \times 10^2$	$<1.00 \times 10^2$
P07	7.10×10^5	$<1.00 \times 10^2$	7.10×10^3
P08	1.20×10^5	1.00×10^3	2.23×10^3
P09	$<1.00 \times 10^3$	2.30×10^3	3.00×10^3
P10	2.00×10^2	$<1.00 \times 10^2$	1.70×10^4
P11	1.70×10^7	$>1.00 \times 10^4$	$<1.00 \times 10^2$
P12	1.90×10^4	4.00×10^3	1.97×10^4
P13	1.97×10^5	2.80×10^4	9.80×10^3
P14	1.98×10^6	1.00×10^2	1.40×10^3
P15	1.60×10^6	4.30×10^4	2.28×10^4
P16	1.20×10^6	3.00×10^3	7.50×10^3
P17	8.70×10^6	7.00×10^3	2.04×10^4
P18	2.50×10^4	3.00×10^2	6.00×10^2
P19	1.00×10^6	1.30×10^4	$<1.00 \times 10^3$
P20	2.15×10^6	4.20×10^4	$<1.00 \times 10^3$

The contamination that we observed is likely to also include post-processing cross contamination due to unhygienic handling of the finished products (Prates *et al.*, 2017).

We did not detect *Salmonella* sp. in any of the cheeses analyzed. Nevertheless, the high counts of other microorganisms indicating hygienic failures suggest that the conditions could also be suitable for the growth of this pathogenic enterobacteria. The growth of fastidious pathogenic bacteria like *Salmonella* sp. is often inhibited by an antagonistic microbiota, which compete for nutrients for their development in the product and although it is not possible to completely remove potential pathogen contamination in this way to ensure food safety, the detection limits of the microbiological techniques used are not suitable for their isolation (Yoon *et al.*, 2016).

Artisanal cheeses are important for regional development and family support in the interior of Brazil, but most producers of these foods are not aware of microbiological hazards and do not follow good manufacturing practices. As a result of this scenario, it is still frequently the case that microbiological analyzes of artisanal cheeses show high contamination and are outside acceptable hygienic-sanitary standards (Queiroz *et al.*, 2017; Carvalho *et al.*, 2019). Similar results to ours were observed in other studies regarding the microbiological quality of high or very high humidity cheeses. Queiroz *et al.* (2017) reported that 40% of the samples analyzed were outside the microbiological standard for coliforms at 45°C and 12% were considered to have high counts for coagulase-positive staphylococci. Dores *et al.* (2013) also reported high *S. aureus* counts with

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Table 2. Results of sensory and instrumental color analyses of selected artisanal cheeses

		Sensory acceptability scores				
	Color	Overall appearance	Taste	Texture	Purchase intention	Acceptance index (%)
P06	7.31	7.33	6.90	7.38	2.28	81.44
P18	7.49	7.43	7.00	7.19	2.49	82.56
P09	7.45	7.62	7.40	7.58	2.43	84.67

	External surface (rind)			Internal (core)		
Sample	L	Chroma surface	Hue angle	L	Chroma center	Hue angle
P06	89.44^{a}± 0.01	29.54^{ab}± 0.53	76.75^{a}± 0.23	89.33^{a}± 0.11	29.37^{a}± 0.12	76.78^{a}± 0.03
P18	89.34^{a}±0.21	27.68^{a}±0.57	75.94^{a}±0,28	89.24^{a}±0.16	28.80^{a}±0.84	76.54^{a}±0.24
P09	88.19^{a}±0.07	31.47^{b}±1.12	78.35^{b}±0.46	88.60^{a}±0.01	33.24^{b}±0.29	78.88^{b}±0.33

Values with equal letters, in the same column, do not differ significantly at the 5% level of significance.

staphylococcal enterotoxins. On the other hand, Prates *et al.* (2017) stated that pasteurization coupled with good manufacturing practices was enough to maintain the microbiological quality of cheese. All these studies indicate that hygienic failures are recurrent and that there are still many points to be improved regarding the microbiological safety of cheese processing in Brazil.

Cheese instrumental color and consumer acceptability

In the sensorial analysis (Table 2), consumers also did not differ significantly on the acceptability of flavor, texture and color of the analyzed cheeses. Bemfeito *et al.* (2016) reported that artisanal cheeses from the Serra da Canastra (Minas Gerais, Brazil) also presented high acceptability (grades between 6 and 7) even with variations in attributes such as taste and texture.

Sample P9 presented the highest acceptance index (IA = 84.67%), followed by P18 and lastly, P6, but all were well accepted (IA >70%). Purchase intention (scale 1 to 3) was above 2 (Table 2), revealing that consumers were likely to buy. Cheese with high counts of contaminating microorganisms may suffer the action of proteolytic and lipolytic enzymes with production of short hydrophobic bitter peptides and volatile compounds, which negatively affects the sensory quality of cheese (Khattab *et al.*, 2019). However, for Soares *et al.* (2017), cheese consumption is largely related to cultural issues which focuses on subjective aspects of the product. Thus, it is understood that consumers recognized in the cheeses evaluated in this work products that are part of their daily lives, reiterating their desire to buy. It is important to highlight that the good acceptance of the products due to the sensory and microbiological characteristics found for cheese reflects that the consumer cannot identify the risks that may be subject to ingesting these products. Regarding the color parameters, the cheeses did not show lightness differences, although sample P9 was more intensely yellow.

In conclusion, microbiological findings on the safety and hygiene of artisanal cheeses in northwestern Paraná revealed dangers to consumers, mainly through high counts of positive coagulase staphylococci, which may indicate the risk of enterotoxin production. From the sensory point of view, no differences were found between the analyzed cheeses, which were well accepted by consumers, probably due to their identification as well known, everyday products. The study reveals that consumers may be at risk when consuming artisanal cheeses as many hygienic flaws have been raised. However, relatively simple techniques and producer training can potentially solve these problems.

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