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Physico-chemical and microbiological characterization of rennet cheese made from pasteurized goat's milk and frozen for different periods of time

Antoniel Florencio da CRUZ[1], Carla Aparecida Soares SARAIVA[1], Mateus de Assis SANTOS[1], Ronaldo Gomes da SILVA JÚNIOR[2], Felipe Nael SEIXAS[2], Cleice Kelly dos Santos NASCIMENTO[1], Ana Beatriz Azevedo de MEDEIROS[1], Artur Araújo e ASSUNÇÃO[1], Aleff Walisson Rocha GOMES[1], Dyalla Correia DUARTE[1], Alberto Jefferson da Silva MACÊDO[1], Neila Lidiany RIBEIRO[3]*

Abstract

The aim of this study was to evaluate the nutritional composition (fat, fat in dry matter, protein, and total dry matter) and microbiological quality (total and thermotolerant coliforms) of rennet cheese produced from pasteurized goat's milk and frozen for different periods. A completely randomized design was used, with treatments consisting of rennet cheese made immediately after pasteurization and after 51, 90, and 128 days of freezing, for a total of four treatments and three replications. There were no significant changes in relation to total dry extract, fat in dry extract, and protein, with respective averages of 47.26, 46.83, and 21.54%, but there was a significant variation ($p < 0.05$) in relation to fat content, whose values were 23.16, 22.34, 21.78, and 21.18% for days 0, 51, 90, and 128 days of freezing, respectively. For the microbiological analysis, total coliforms > 1.1×10^4 NMP/g were observed, and for thermotolerant coliforms, values of 7.83×10^3; 8.6×10^2; 7.85×10^2, and 7.34×10^3 NMP/g were found for days 0, 51, 90, and 128 days of freezing, respectively. The production of rennet cheese from frozen goat's milk is a viable alternative, but adjustments need to be made to the process to improve its microbiological quality.

Keywords: dairy goat; total coliforms; thermotolerant coliforms; nutritional composition.

Practical Application: Among the wide variety of cheeses that can be made from goat's milk, rennet cheese is an alternative.

1 INTRODUCTION

Goat's milk has a high nutritional value and is highly digestible due to the smaller size of most of its fat globules. Additionally, its reduced content of α-s-1 casein protein favors the formation of fine, soft clots, promoting digestive processes. Because of these characteristics, this milk is considered an excellent food for children and the elderly, both fresh and in derivative forms. However, there is still a certain amount of prejudice regarding the quality and flavor of goat's milk, which shows that the milk and its derivatives are not very popular in the market (Bomfim et al., 2013).

Dairy products such as cheeses (Beltrão et al., 2022; Lima et al., 2021; Santos et al., 2022; Sobral et al., 2023), yogurts, dairy drinks (Santos et al., 2022), and dulce de leche are products that stand out in dairy farming and add value to milk products, offering a way for producers to increase their profitability. Curi and Bonassi (2007) state that goat's cheese is the most interesting product made from goat's milk. Among the wide variety of existing cheeses that can be made from goat's milk, rennet cheese is an alternative. This cheese is typically Brazilian and is of great value in the north-east of Brazil. It is also a product of great commercial value because it is easy to make and has a high yield in the process (Santos et al., 2011).

Although dairy goat farming is a widespread activity worldwide, it still has some bottlenecks, such as low productivity per animal and seasonal production. As a result, there will also be seasonality in the supply of its products, especially when they are derivatives that have a short shelf life, which is not very viable from the point of view of the consumer market since there will not be a constant supply of its products of interest in the supermarket, for example.

Normative Instruction Number 37 of October 31, 2000, which deals with the approval of the Technical Regulation for the Identity and Quality of Goat's Milk, states that processed goat's milk can be stored frozen at -18°C, provided that equipment is used to achieve this temperature in the shortest possible time.

In addition to being widespread in the Northeast and of interest for marketing and adding value to goat's milk, rennet cheese is easy to make and yields good results. Although there

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[1]*Universidade Federal da Paraíba, Areia, Paraíba, Brazil.*
[2]*Universidade Federal de Viçosa, Vicçosa, Minas Gerais, Brazil.*
[4]*Unidade Federal de Campina Grande, Campina Grande, Paraíba, Brazil.*
*Corresponding author: neilalr@hotmail.com
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are some studies evaluating the production of cheeses from frozen goat's milk (Curi & Bonassi, 2007), there are still many gaps to be filled, such as how long frozen milk can be stored without damaging the yield and flavor of the product, since IN 37 does not mention the maximum storage time for frozen milk. Furthermore, it is not known whether the practice of making cheeses from frozen goat's milk will produce the same result for all types of cheese.

The aim of this study was, therefore, to evaluate the physicochemical and microbiological quality of goat's rennet cheese subjected to different freezing periods.

2 MATERIALS AND METHODS

2.1 Materials used

Milk from Saanen goats was used to make the cheeses. The goats were part of an experiment being conducted in bioclimatic chambers in the Zootechnics Department of the CCA/UFPB. Milking was carried out manually in accordance with good milking practices.

The trial used a completely randomized design with four treatments consisting of rennet cheese made from milk that had been frozen for 0, 51, 90, and 128 days and three replications (milk collected over three consecutive days).

2.2 Pasteurization and freezing

After milking, the milk was immediately transported to the CCA/UFPB School Dairy, where it was pasteurized at a temperature of 65°C for 30 min, then cooled to around 4°C and frozen at -18°C for further manufacturing. This practice was carried out for three consecutive days for each treatment. When the cheeses were made from the frozen milk, it was thawed under refrigeration (4°C) for approximately 24 h. The cheese made from fresh and pasteurized milk occurred when the first batch of frozen milk was thawed for cheese making.

The treatments consisted of rennet cheese made immediately after pasteurization (day 0) and rennet cheese made from milk that had been frozen for 51, 90, and 128 days, with a total of four treatments and three replications.

2.3 Making rennet cheese

For the elaboration of artisanal raw coalho cheese, samples of 10 L of goat's milk from each experimental treatment were used. After milking, the milk was strained and immediately refrigerated at 5–7°C for 1 day. Subsequently, the milk was heated to 32–37°C and the liquid coagulant was added at a rate of 1 mL per liter of milk. The clotting power of the rennet used was 1:3,000/75 IMCU. After adding the rennet, the milk was homogenized and allowed to clot for 40 min. The curd was then cut, stirred, heated to 45 °C, partially whey was removed, sodium chloride was added to the mass in a proportion of 1%, pressed into round plastic molds, and kept for 24 h at an average temperature of 10–12°C. After the elapsed time, the cheeses were removed, packaged, labeled, and kept at the same refrigeration temperature (Brasil, 2001).

2.4 Microbiological analysis of cheese

According to the recommendations of RDC Resolution No. 12 of January 2, 2001 (Brasil, 2001), the samples were analyzed according to the methodology described by the American Public Health Association (2001) for the determination of total coliforms and thermotolerant coliforms. The analyses of total and thermotolerant coliforms were performed using the most probable number technique. The analysis of viable aerobic mesophilic bacteria was performed using the depth plate technique with Plate Count Agar and incubation at 35 ± 2°C for a period of 48 h (Sobral et al., 2023).

2.5 Physicochemical analysis of cheese

After production, the cheese was analyzed for total dry extract (TDE), fat in dry extract (FDE), fat, and protein. All these analyses were carried out at the Food Analysis and Animal Nutrition Laboratory at the CCA/UFPB.

The curd cheese samples were analyzed in duplicate according to the methodology of MAPA Normative Instruction 68 (2006) for fat content (using Gerber's lactobutyrometer), protein (Micro-Kjedahl method), and total dry extract (oven drying at 105°C). FDE was obtained using the Equation 1:

$$FDE = \frac{fat(\%) \times 100}{TDE(\%)} \quad (1)$$

2.6 Sensory analysis

The sensory analysis was conducted in individual booths away from noise and odors at pre-established times, excluding 1 h before and 2 h after lunch. The samples were served at room temperature in cubes of approximately 3 cm^3, presented in coded disposable cups (50 mL), and accompanied by a cream cracker cookie, water, and an evaluation form.

For the sensory evaluation, the acceptance test was applied following the methodology described by Faria and Yotsuyanagi (2002). The following attributes were evaluated: appearance, smell, texture, taste, consistency, and overall acceptance, using a structured 9-point hedonic scale ranging from one (1. I disliked it extremely) to nine (I liked it extremely). Finally, the purchase intention test was carried out using a structured 5-point scale ranging from one (1. Would never buy) to five (5. Would buy).

2.7 Statistical analysis

The data were subjected to an analysis of variance, and the Tukey test compared the averages at 5% probability through the PROC GLM of the SAS statistical package (Statistical Analysis System Institute, 2012). The Ryan-Einot-Gabriel-Welsch test compared the means of the sensorial attributes at a 5% probability level.

3 RESULTS

With regard to microbiological analysis, total coliforms were found to be > 1.1 × 104 NMP/g, while thermotolerant coliforms were found to be between 7.85 × 102 and 7.83 × 103 NMP/g (Table 1).

There were no significant changes in total dry extract, fat in dry extract, or protein. However, there was a significant variation in the fat content ($p < 0.05$), i.e., as the milk freezing period increased, there was a decrease in the fat content of the cheese (Table 2).

From the results of the sensory evaluation (Table 3), it can be seen that there was no statistical difference ($p > 0.05$) between the treatments for rennet cheese made from pasteurized and pasteurized/frozen milk with regard to the attributes of appearance, color, aroma, flavor, texture, and overall acceptability. The scores for the sensory attributes indicate that consumer preference was between slightly liked and very much liked.

With regard to the percentage of intention to buy observed among the tasters, the majority of those interviewed would buy or perhaps buy all three types of cheese, while only 15% would not buy any of the three types of cheese. This behavior showed that the tasters preferred all the cheeses (Table 4). These results align with those obtained in the sensory tests, so it can be seen that, regardless of freezing, all the cheeses made in different periods do not present few restrictions to the consumer.

Table 1. Microbiological characteristics of rennet cheese produced from frozen goat's milk.

Microorganisms (MPN g^{-1})	Freezing days			
	0	51	90	128
Total coliforms	$> 1.1 \times 10^4$	$> 1.1 \times 10^4$	$> 1.1 \times 10^4$	$> 1.1 \times 10^4$
Tolerant coliforms	7.83×10^3	8.6×10^2	7.85×10^2	7.34×10^3

Table 2. Physico-chemical characteristics of rennet cheese made from frozen goat's milk.

Variables (%)	Freezing days				SEM	*p*-value
	0	51	90	128		
Fat	23.16a	22.34ab	21.78ab	21.18b	0.3305	0.0264
Fat in dry extract	48.54	46.15	46.80	45.83	1.2709	0.4925
Total dry extract	47.79	48.45	46.58	46.24	1.0435	0.4627
Protein	21.78	23.00	21.25	20.13	1.6386	0.6783

Averages followed by different letters on the same line differ by the Tukey test at 5% probability; SEM: standard error means.

Table 3. Acceptance averages of the values attributed to cheeses made with pasteurized goat's milk (time zero) and at 51 and 128 days of freezing.

Attributes	Freezing days			SEM	*p*-value
	0	51	128		
Appearance	7.80	7.82	7.73	1.60	0.987
Color	7.91	7.98	7.65	1.99	0.876
Aroma	6.85	6.92	6.79	1.82	0.534
Flavor	6.92	7.12	7.00	2.32	0.678
Texture	7.20	7.40	7.35	1.66	0.888
Global acceptance	7.05	7.40	7.32	1.54	0.543

SEM: standard error means. Means followed by different lowercase letters in the same row indicate significant differences according to the Ryan–Einot–Gabriel–Welsch test at a 5% significance level.

Table 4. Purchase intention (%) of rennet cheese made with goat's milk without freezing (time zero) and at 51 and 128 days of freezing.

Purchase intention (%)	Freezing days		
	0	51	128
Would buy	45	49	59
Maybe yes/Maybe no	40	36	26
Would not buy	15	15	15

4 DISCUSSION

The Technical Regulation on Microbiological Standards for Food (Brasil, 2001) establishes that for medium- to high-humidity cheeses, the maximum permitted values for thermotolerant coliforms should be 5×10^3 NMP/g. Therefore, with regard to thermotolerant coliforms, the cheeses produced with milk frozen for up to 51 and 90 days were found to be within the established standard and fit for consumption. However, the cheeses produced with fresh milk and frozen for 128 days showed values above the permitted level, showing errors during processing. As the microbiological characteristics of the milk were not assessed before and after pasteurization or even after it had been thawed, it was not possible to assess where the possible faults lay.

The values of the microorganisms observed were within the parameters established by RDC 12, with adequate microbiological quality from a hygienic-sanitary point of view, and could be safely consumed. Freire et al. (2022) and Lima et al. (2021) evaluated the microbiology of rennet cheese produced with goat's milk and observed that the Coliform 35 and 45°C values were < 3.0 MPN g^{-1}. Souza et al. (2013) obtained a quantification (MPN) of total and thermotolerant coliforms in milk samples ranging from < 3 to > 1,100 CFU/mL, regardless of the form of storage. However, when the authors determined the average count of total and thermotolerant coliforms, they found a significantly higher amount in samples kept under refrigeration.

According to Dutra et al. (2014), the storage of frozen milk maintains the initial microbiological qualities of the raw material, and they state that the guarantee of the quality of milk and dairy products is obtained through the use of milking management and hygiene procedures based on the principles of good practices and healthy animals.

According to the results obtained in this study, the value for fat in the total dry extract (TDS) is within the standard established by the Technical Regulation for the Identity and Quality of Rennet Cheeses (Brasil, 2001), which in turn establishes that rennet cheese should have TDS fat contents ranging from 35 to 60%.

According to Freire et al. (2022), total dry extract values are inversely proportional to moisture content. This is because when the moisture content increases, the total dry extract—which is represented by the sum of proteins, fat ash, and others minus moisture—decreases. Bearing this in mind, there is a variation in the moisture content of the cheeses, which did not affect the total dry extract.

According to Teixeira (2016), the control of the total dry extract should be advocated, since the content is one of the most important factors in the physicochemical characterization of cheeses. The values of fat in the extract were influenced by the goat's milk fed with double-distilled glycerin. The percentage of fat in the dry extract is one of the most expressive ways of quantifying the fat contained in different types of cheese (Brasil,

2001), indicating that the decrease in milk fat directly interfered with the values of total dry stratum and defatted dry stratum. Pinto Júnior et al. (2012) found no significant difference between freezing time and the parameters of fat, fat in dry extract, total dry extract,, protein, lactose, and cryoscopic index in the milk of Saanen goats.

However, research has shown that freezing can affect the fat and protein content of milk. Zhang et al. (2006) when assessing the chemical composition of goat's milk frozen for up to 6 months, found a progressive reduction in fat content from the second month of freezing, but other components such as protein and lactose remained unchanged. The authors state that during freezing, ice crystals can compact the retained fat globules, causing the release of lipoproteins from the fat globule membrane.

In this case, it can result in destabilization of the emulsion and coalescence of globules upon subsequent thawing, and the released fat, mainly triacylglycerols, undergoes lipolysis by lipoprotein lipase. They finally conclude that the destruction of fat globules, the enzymatic degradation of triacylglycerols, and microbial activities may be the reasons for the reduction in milk fat during frozen storage.

Another reason for this reduction in the fat content of the cheese may be the adherence of the fat globules to the utensils used throughout the processing, which begins during defrosting with a visible amount of fat attached to the wall of the containers and during the cheese-making process, a fact observed in this study.

Although there were no statistical differences between the treatments, the color attribute received the highest scores. According to Santos et al. (2011), goat cheese when fresh has a white color, which is totally related to the conversion of β-carotene into vitamin A. With regard to overall acceptance, it was found that the most accepted treatment among the tasters was the one frozen for 51 days, while the one with zero time was the most rejected.

Freezing milk can cause changes in its composition, especially in protein and fat. However, the instability does not seem to occur due to the freezing itself but is related to the freezing time and temperature, i.e., the longer the storage time, the greater the destabilization (Pinto Júnior et al., 2012). Therefore, the storage time of the frozen milk in this study was probably not enough to cause changes in its components and, consequently, in its sensory characteristics.

5 CONCLUSION

Freezing goat's milk did not influence the composition of the total dry extract, protein, or fat in the dry extract of the rennet cheese, but it did influence its fat content. As for the microbiological composition, there was a need for further research and analysis to check for possible flaws in the process of good milking practices or cheese making.

The freezing of goat's milk for different lengths of time at 51 and 128 days compared to time zero showed no significant differences and did not influence the sensory characteristics or the intention to buy. It is therefore a viable alternative for sustainability and profitability for the industry, as there are no significant changes and it can be marketed at different times of the year, avoiding greater seasonality.

REFERENCES

American Public Health Association (APHA) (2001). *Standard methods for the examination of waste and wastewater* (18th ed.). APHA/AWNA/WEF.

Beltrão, L. G. C., Cruz, G. R. B., Sousa, S., Santana, A. M. S., Fonseca, S. B., Salviano, G. O., Ribeiro, N. L., & Andrade, R. O. (2022). Physicochemical profile of milk and cheese of goat feed with flaxseed oil substituting the corn. *Food Science and Technology*, *42*, e94821. https://doi.org/10.1590/fst.94821

Bomfim, M., Santos, K. M. O., Queiroga, R. C. R. E., Cordeiro, P. C., & Oliveira, L. S. (2013). Produção e qualidade do leite de cabra no Brasil. In 23º Congresso Brasileiro de Zootecnia. *Zootecnia do futuro: Produção Animal Sustentável* (pp. 4711-4718). Universidade Estadual do Oeste do Paraná.

Brasil (2000). Ministério da Agricultura Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Regulamento Técnico de Produção, identidade e qualidade do leite de cabra. Instrução Normativa nº 37, de 31 de outubro de 2000. *Diário Oficial da União*.

Brasil (2001). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Regulamento Técnico sobre os padrões microbiológicos para alimentos (Resolução RDC n. 12, de 02 de janeiro de 2001). *Diário Oficial [da] República Federativa do Brasil*.

Brasil (2006). Ministério da Agricultura Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Regulamento Técnico de métodos analíticos oficiais físico-químicos para controle de leite e produtos lácteos. *Instrução Normativa nº 68, de 12 de dezembro de 2006*.

Curi, R. A., & Bonassi, I. A. (2007). Elaboração de um queijo coalho análogo ao Pecorino Romano produzido com leite de cabra e coalhada congelados. *Ciência e Agrotecnologia*, *31*(1), 171-176. https://doi.org/10.1590/S1413-70542007000100025

Dutra, C. M. C., Svierk, B., Ribeiro, M. E. R., Pinto, A. T., Zanela, M. B., & Schmidt, V. (2014). Parâmetros de qualidade do leite de cabra armazenado sob frio. *Arquivos do Instituto Biológico*, *81*(1), 36-42. https://doi.org/10.1590/S1808-16572014000100007

Faria, E. V., & Yotsuyanagi, K. (2002). *Técnicas de Análise Sensorial*. ITAL/LAFISE.

Freire, L. F. S., Cruz, G. R. B., Costa, R. G., Ribeiro, N. L., Guerra, R. R., Sousa, S., Lima, A. M., Silva, G. F., Santana, A. M. S., & Nascimento, G. V. (2022). Glycerin diet affects the size of the fat globule and the fatty acid profile of goat's milk. *Food Science and Technology*, *42*, e85821. https://doi.org/10.1590/fst.85821

Lima, A. M., Cruz, G. R. B., Costa, R. G., Ribeiro, N. L., Beltrão, E. M. F., Sousa, S., Justino, E. S., & Santos, D. G. (2021). Physical-chemical and microbiological quality of milk and cheese of goats fed with bidestilated glycerin. *Food Science and Technology*, *41*(1), 25-33. https://doi.org/10.1590/fst.27119

Pinto Júnior, W. R., Ferrão, S. P. B., Rodrigues, F. L., Fernandes, S. A. A., & Bonomo P. (2012). Efeito do congelamento sobre os parâmetros físico-químicos do leite de cabras da raça saanen. *Revista Caatinga*, *25*(3), 110-117.

Santos, B. M., Oliveira, M. E. G., Sousa, Y. R. F., Madureira, R. M. F. M., Pintado, M. M. E., Gomes, A. M. P., Souza, E. L., & Queiroga, R. C. R. E. (2011). Caracterização físico-química e sensorial de queijo de coalho produzido com mistura de leite de cabra e de leite de vaca. *Revista do Instituto Adolfo Lutz*, *70*(3), 302-310. https://doi.org/10.53393/rial.2011.v70.32537

Santos, W. F., Cruz, G. R. B., Costa, R. G., Ribeiro, N. L., Beltrão Filho, E. M., Sousa, S., Justino, E. S., & Santos, D. G. (2022). Production and quality of cheese and milk goats fed with guava agroindustrial waste (*Psidium guajava* L.). *Food Science and Technology*, *42*, e00521. https://doi.org/10.1590/fst.00521

Sobral, G. C., Oliveira, J. S., Saraiva, C. A. S., Santos, E. M., Vieira, D. S., Cruz, A. F., Torres Junior, P. C., Albuquerque, I. R. R., Araújo, A. O., & Ribeiro, N. L. (2023). Sensory analysis of goat cheese feed with sorghum silage levels in forage cactus based diets. *Food Science and Technology*, *43*, e90022. https://doi.org/10.1590/fst.90022

Souza, A. K., Fiorini, J. E., Moraes, A. L. L., Oliveira, N. D. M. S., Clareto, S. S., & Nascimento, L. C. (2013). Características microbiológicas e físico-químicas do leite de cabra submetido à pasteurização e ao congelamento, comercializado na cidade de Alfenas-MG. *Revista da Universidade Vale do Rio Verde*, *11*(1), 224-233. http://dx.doi.org/10.5892/946

Statistical Analyses System (SAS) (2012). *Statistical Analysis System user's guide. Version 9.2*. Statistical Analyses System Institute.

Teixeira, J. L. P. (2016). *Qualidade do queijo de leite caprino tipo coalho condimentado com cumaru (Amburana cearensis A. C. Smith)* (Master's thesis). Centro de Ciências Humanas, Sociais e Agrárias, Universidade Federal da Paraíba.

Zhang, R. H., Mustafa, A. F., Ng-Kwai-Hang, K. F., & Zhao, X. (2006). Effects of freezing on composition and fatty acid profiles of sheep milk and cheese. *Small Ruminant Research*, *64*(3), 203-210. https://doi.org/10.1016/j.smallrumres.2005.04.025