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Evaluation of physico-chemical characteristics of fresh, refrigerated and frozen Lacaune ewes' milk

[Avaliação das características físico-químicas de leite fresco, resfriado e congelado de ovelhas Lacaune]

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ABSTRACT

The production of ewe milk is seasonal and milk yield per animal is low, even in specialized animals. This study aimed to verify the possibility of preserving bulk tank milk for seven days under cooling (5°C) and freezing (-5°C), verify the influence of cooling treatments and of the months of the year on the physical and chemical characteristics of the product. The chemical composition of milk, including the fat, protein, lactose and total solids contents, was not altered by cooling and freezing. Protein and lactose contents varied according to the months of the year. The average percentage and standard deviation of fat, protein, lactose and total solids was 8.10 ± 1.30 , 5.22 ± 0.37 , 4.43 ± 0.23 and 19.34 ± 1.54 , respectively. The density, pH, titratable acidity, as well as alcohol and heat stability tests were significantly influenced by the treatments used (P < 0.05), but no differences were found between fresh and frozen milk. Prolonged refrigeration caused an increase in acidity and decrease in pH, with a consequent reduction in the stability of milk. These results demonstrated that freezing does not affect the chemical composition and physical characteristics of milk in nature and it could be a solution for the producer and the sheep milk industry.

Keywords: ewe's milk, physical and chemical characteristics, storage

RESUMO

O leite ovino, mesmo no caso de raças especializadas, apresenta sazonalidade de produção e baixa produtividade por fêmea. Em função disso, o objetivo do presente estudo foi verificar a possibilidade de conservação do leite fresco, por um período de sete dias, sob refrigeração e congelamento, verificando a influência dos tratamentos aplicados, e dos meses do ano, sobre as características físico-químicas do produto. A composição química, incluindo gordura, proteína, lactose e sólidos totais, não sofreu alterações com o resfriamento e congelamento do leite, contudo os teores de proteína e lactose variaram durante os meses do ano. O percentual médio de gordura, proteína, lactose e sólidos totais foi de 8,10±1,30, 5,22±0,37, 4,43±0,23 e 19,34±1,54, respectivamente. A densidade, o pH, a acidez titulável e as provas de estabilidade ao álcool e ao calor sofreram influência significativa dos tratamentos aplicados (P<0,05), não havendo diferenças entre o leite fresco e o leite congelado. O resfriamento prolongado causou aumento da acidez e diminuição do pH, com a consequente diminuição da estabilidade do leite. Os resultados do presente estudo demonstram que o congelamento não afeta a composição química e as características físicas do leite in natura, podendo ser uma solução para o produtor e para a indústria de leite ovino.

Palavras-chave: leite ovino, características físico-químicas, congelamento, estocagem, sazonalidade

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INTRODUCTION

The world production of ewe's milk in 2010 was above ten million tons, with China being the largest milk producer, also having the world's greatest number of sheep. Greece is the world's second largest sheep milk producer; however, the size of its flock holds the 34th position (FAOSTAT, 2010).

Ewe's milk differs from milk of other species mostly due to its high solid contents (Assenat, 1991); however, it is rarely consumed *in natura*, being mainly used in the production of cheeses and yoghurts (Ramos and Juarez, 2011). The Lacaune breed selection has been directed for meat and dairy production (Barrilet *et al.*, 2001), thus allowing higher production values. However, the seasonality in the ewe's milk production and the low productivity per animal make it difficult to achieve a uniform distribution of milk throughout the year. A commonly used solution to gather sufficient quantities of milk for processing purposes is the freezing of raw milk for several weeks or months (Wendorff, 2001).

The majority of dairy sheep producers often need to freeze bulk milk. However, the method which has been used can affect the quality of the raw material and final product. It is recommended to previously cool milk to temperatures between 4 and 6° C and then quickly perform the freezing at around -25°C. Slow freezing at around -12°C may cause protein degradation after a storage period of two or three months and this degradation impairs the formation of curds in cheese production (Berger, 2001).

Previous researches have been carried out in order to assess the effect of freezing on the physical and chemical characteristics of ewe's milk (Wendorff, 2001; Zhang *et al.*, 2006); however, milk of Lacaune ewes has not been studied for such purpose. Considering this fact and the importance of preserving fresh ewe's milk for longer periods until a sufficient volume of milk has been obtained for processing purposes, this study aimed to evaluate low temperature storage during seven days on the quality of milk, by taking milk samples for twelve months.

MATERIAL AND METHODS

Bulk tank milk samples were collected monthly between January and December 2011 from four dairy sheep farms in the mountainous region of the State of Rio Grande do Sul, Brazil. The selection criteria for these farms were based on the fact that these are the most important milk producer regions in Rio Grande do Sul.

The average size of the properties was 20 hectares and milk production was the main source of income for the producers. Additionally, three properties also cultivated grapes for wine production. Only one of the properties used expansion tanks to cool milk and the others used immersion tanks. Daily milk production was 1.5L.ewe ⁻¹ by milking twice a day.

Only one of the farms did not discard the first squirts of milk before the actual milking. Predipping was not performed in any farm, but post-dipping was performed in three, with iodine being the active ingredient common to all of them. After milking, all ewes were fed in order to keep them in standing position until teat sphincter closure. All farms performed machine milking.

Mixed milk samples were collected from each farm, directly from the storage tank. In order to collect the samples, milk was previously homogenized and stored in identified plastic bottles. The 900 mL volume obtained was stored and transported in isothermal boxes containing ice to the Dairy Laboratory.

During February and March one of the farms did not produce milk for industrialization due to grape harvest. A total of 138 samples were analyzed.

Each sample was divided into three 300mL subsamples: The first sample was analyzed on the same day (denominated fresh milk - F) and the remaining ones were analyzed within seven days, one of which under refrigeration (at 5°C, denominated refrigerated milk - R) and the other one under domestic refrigerator freezing (at > -5°C, denominated frozen milk - Fr). The frozen milk sample was slowly thawed for 24 hours under refrigeration. These three subsamples were now called samples.

The samples were submitted to density determination analyses 20°C at thermolactodensimeter, pH test, titratable acidity by Dornic method, as well as to ethanol stability and thermal stability (boiling) tests (Brasil, 2006). Ethanol stability test was performed by mixing 2mL of ethanol (in different concentrations) and 2mL of milk in a Petry dish. The result of the alcohol stability test was defined as the highest alcohol graduation in which milk did not precipitate as curds. Thermal stability test was performed by putting 10mL of milk in a tube and submitting to boiling. Stable milk does not coagulate after boiling. These tests were conducted in triplicate.

Approximately 40mL of each sample were taken from the samples of each different treatment (F, R and Fr), stored in bottles containing Bronopol® and then sent, under refrigeration, to the milk quality accredited laboratory at Embrapa Clima Temperado (Pelotas, RS, Brazil), in order to have their chemical composition determined by midinfrared wavebands in a BENTLEY 2000 equipment.

Descriptive statistics were performed for each of the milk treatments and evaluation months. A correlation analysis was performed among the dependent variables. A non-parametric test (Kruskal-Wallis) was used only for the thermal stability test, which is a categorical variable for curd formation (positive and negative). The remaining data were analyzed through the multivariate ANOVA test (MANOVA), in order to assess the effect of the treatments used, the months of study and the interaction between these two with the results obtained. For significant differences (P<0.05), Tukey's test was applied to identify groups with homogenous means. SPSS 19.0 software was used (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The monthly average of fat, protein, lactose and total solids (TS) contents are presented in Tab. 1. Milk composition did not show significant variations when the different treatments were compared (P>0.05). The protein and lactose contents varied significantly in the months studied (P<0.05), only in fresh milk. The interaction between the treatments used and the months of study did not influence significantly the milk composition.

Table 1. Means and standard deviations of fat, protein, lactose and total solids of Lacaune ewe's fresh milk from four dairy farms in Rio Grande do Sul state, Brazil, between January and December 2011

Month	Fat (%)	Protein (%)	Lactose (%)	TS (%)
Jan	8.18±1.87	5.15±0.57ab	4.28±0.14ab	19.17±2.48
Feb	8.94 ± 1.22	5.57 ± 0.57 bc	$4.17 \pm 0.20a$	20.38±1.35
Mar	7.59 ± 1.46	$5.79\pm0.36c$	4.18±0.33a	18.93 ± 1.62
Apr	8.01 ± 0.83	$5.16\pm0.52ab$	4.52±0.18bc	19.33±1.32
May	8.78 ± 1.88	$5.20\pm0.50ab$	4.40±0.38abc	20.08 ± 2.18
June	8.02 ± 0.37	$5.26\pm0.15ab$	4.49 ± 0.11 bc	19.45 ± 0.49
July	7.86 ± 2.38	$5.25\pm0.34ab$	$4.60\pm0.16c$	19.17±1.11
Aug	8.22 ± 0.98	$5.10\pm0.14ab$	4.58 ± 0.09 bc	19.50±1.11
Sept	8.14 ± 0.51	$5.20\pm0.15ab$	4.53±0.06bc	19.55±0.56
Oct	7.65 ± 0.86	$5.09\pm0.16ab$	4.47±0.17abc	18.88 ± 0.95
Nov	8.03 ± 0.71	$5.06\pm0.22a$	4.42±0.21abc	18.98 ± 0.84
Dec	7.81 ± 0.81	$5.08\pm0.14ab$	4.40±0.20abc	18.77±0.79

Means followed by different letters in the same row are different (Kruskal-Wallis P<0.05).

Fat is the milk component most affected by variations (Alais, 1985; Assenat, 1991). In this study, however, it did not present significant seasonal variations nor was it influenced by the treatments used. The average fat percentage found was 8.10 ± 1.30 , which is higher than the values found by Brito *et al.* (2006), in ewes of

same breed and from the same region. In a comparative study done with 1/2 Lacaune and 1/2 East Friesian ewes, Lacaune-cross ewes produced a higher percentage of milk fat; nonetheless, the values found were below those observed in this study (Thomas *et al.*, 2001).

Despite lactose being the milk component less subject to variation (Alais, 1985), its contents, as well as the milk protein contents, were affected by seasonal variation, with a significant increase in lactose in March and April (fall) and a reduction in the protein contents in the same period. The inverse variation of these components may be explained by the osmotic function of lactose in milk production, associated with the dilution effect of the remaining milk elements (Stelwagen, 2011). However, these results differ from those obtained by the study of cow's milk performed in California, in which the highest lactose concentrations were found in April (summer) and the lowest protein concentrations were found between May and August (spring - summer) (Bruhn and Franke, 1977).

The total dry extract contents did not vary significantly with the treatments used and the

months of study (P > 0.05), showing an average of 19.34 $\%\pm1.54$. Similar contents were found by Casanova *et al.* (1999), who observed a daily average percentage of 19.6 ±0.09 in the total solid contents of the milk of Manchega ewes in Spain. In Nigeria, studies performed with Yankasa ewes showed variations in the EST contents, since Adewumi and Olorunnisomo (2009) found an average value of 19.9% and lower values were found by Malau-Aduli and Anlade (2002), with an average of 15.19% ±0.69 total solids.

The average values of density, pH, titratable acidity and alcohol stability tests, according to the treatments used, are shown in Tab. 2. These variables were significantly influenced by the treatments used and the months of the year, but only density and pH values were significantly affected by an interaction between these two factors (P<0.05).

Table 2. Means and standard deviations of density (g/mL⁻¹), titratable acidity (TA) and ethanol stability of fresh milk (F), refrigerated milk (R) and frozen milk (Fr) of Lacaune ewes from four dairy farms from Rio Grande do Sul state, Brazil, between January and December 2011.

	Density (g/mL)	рН	TA (°D)	Ethanol stability (°GL)
F	1.0380±0.0018 a	6.68±0.12a	24.11±1.46a	63.17±3.64a
R	1.0396±0.0016 b	$6.57 \pm 0.26b$	$28.61 \pm 4.11b$	53.91±12.51b
Fr	1.0382±0.0022 a	$6.71 \pm 0.20a$	24.30±1.58a	63.00±3.29a

Means followed by different letters in the same row are different (Kruskal-Wallis P<0.05).

The density of frozen milk samples was affected by seasonal variations, being increased in January and February (summer), as well as in May and June (fall). Although density decreases as the amount of fat increases (Tronco, 2010), these variables have a weak negative correlation (-0.25). Additionally, the amount of milk fat did not experience significant variations during the months of study. Differences were verified between the treatments used in February, October, November and December (P<0.05). Kanwal et al. (2004) found lower density values in Pakistan, where ewe's milk had a maximum density of 1,029 g/mL. The same authors found pH values similar to those of fresh milk observed in this study, with an average of 6,58. However, the titratable acidity average was 18°D, which was below all results of the treatments used in the current study. In a research carried out in Greece (Simos et al., 1996), the density and titratable acidity averages of the milk of Epirus Montain ewes was 1,0372g/mL and 22,5°D, which were similar to the results obtained with fresh and frozen milk in this study.

The interaction between the treatments used and the months of study did not influence significantly the pH of milk (P<0.05). In fresh, refrigerated and frozen milk samples, the pH had seasonal variations, with values increasing in March and April (fall) and then decreasing in May. Although the pH and titratable acidity of milk had a strong negative correlation (-0.69), in this research an inverse behavior of these variables during these months was not observed. There were differences between the treatments used in January, February, April, July, August and October. Pavić et al. (2002) also observed a strong negative correlation between the pH and titratable acidity of milk (-0.66), but the average pH found was higher, i.e. 6.77±0.01. The same pH values were observed in January for refrigerated and frozen milk samples and in December for fresh milk. There was a significant reduction in the pH of refrigerated milk samples.

The ethanol stability test showed a strong correlation with the titratable acidity (-0.90) and the pH (0.70) of milk. Regarding the seasonal variation, a significant reduction was observed in the alcohol degree used from May to November, which is possibly associated with the higher temperatures in that period, which is favorable for bacterial contamination. According to Walstra et al. (2006), the stability of milk protein decreases as acidity increases. To support this information, there was a significant reduction in milk stability during the alcohol test in refrigerated milk, due to the higher acidity of these samples. In the study performed by Guo et al. (1998), the alcohol stability test in ewe's milk obtained an average of 44%±3. According to the authors, this low stability, in comparison with cow's milk, is not associated with the microbiological quality, but rather with the milk's own characteristics. Similarly, the results of this study have demonstrated a lower stability of ewe's milk in the alcohol test, in comparison with cow's milk.

In the thermal stability test, the months of study did not influence the results. However, there were differences between the treatments used (P<0.05). There were no differences between fresh and frozen milk samples, but they showed significant differences in comparison to refrigerated milk samples. Of ten samples that developed curds upon boiling, nine were refrigerated milk samples, which demonstrates a lower thermal stability in this treatment. This can be explained by the higher acidity of these samples because the thermal stability of ewe's milk decreases as the pH decreases, according to Montilla and Calvo (1997), when they studied goat milk. However, Raynal-Ljutovac et al. (2007) stated that milk from small ruminants is usually not thermally stable at its natural pH which is 6.65, according to Assenat (1991).

When milk is frozen, its proteins (casein) loose their stability (Desai *et al.*, 1961). Casein denaturation, which occurs when milk is frozen, is more easily observed when the non-fat solid contents are increased, causing a progressive precipitation of proteins (Webb and Hall, 1935). Freezing and thawing of full fat milk may cause partial coalescence of milk fat globules due to the formation of ice crystals. In this case, membrane lipoproteins can be released (Walstra *et al.*, 2006). Although these alterations occur in

frozen milk, freezing did not affect the physical and chemical characteristics of ewe's milk in this study.

Similarly, Zhang *et al.* (2006) studied the effects of freezing and storage time in the milk of East Friesian x Lacaune ewes, observed that these factors did not affect the total solid, protein and lactose contents. However, the fat content was progressively reduced as the storage time increased, possibly due to the destruction of milk fat globules, enzymatic degradation of triglycerides and microbial activity. According to Curi and Bonassi (2007), the effects of freezing and thawing in ewe's milk are less pronounced than in cow's milk and, therefore, this process can be used to produce cheese without altering the sensorial characteristics of the final product.

Nevertheless, frozen milk may undergo alterations in its composition that may affect cheese yield (Jaeggi et al., 2004). Milk refrigeration causes changes in the salt solubility, with consequent reduction in the quantity of micellar calcium phosphate. There is loss of milk fat globule membrane components, as well as structural alterations, thus increasing the creaming rate (Walstra et al., 2006). Possibly due to these alterations, milk refrigeration during the seven day period has influenced the results of the physical analyzes of milk. In the study performed by Guinot-Thomas et al. (1995), samples of cow's milk were observed at 4°C during 6 days, with a consequent reduction of the colloidal calcium phosphate, which resulted in milk instability, possibly due to the degradation of casein micelles by microbial proteases. The same authors observed a reduction in milk pH after 4 days of refrigeration, which was associated with action of microbial lipases psychrotrophic bacteria. Similarly, in this study, refrigeration led to a decrease in milk stability and pH and to an increase in the titratable acidity. The density increase observed may be explained by the alteration in the milk fat globules structure.

CONCLUSIONS

The results of this study demonstrate that the refrigeration of ewe's milk for seven days affects the physical characteristics of the product. Nevertheless, when milk is frozen for the same period, its physical and chemical quality is not

affected. This may be the solution for the seasonality of milk production and its low productivity, which are typical for the species.

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