The Effect of Long-term Freezing on Renneting Properties of Sarda Sheep Milk

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Summary

Cold storage is a well-known strategy to limit concerns about seasonality for sheep dairy productions. The aim of this study was to investigate the effect of long-term freezing on milk renneting properties from the Sarda sheep, an autochthonous breed from Italy. Two-hundred milk samples from 50 pluriparous Sarda ewes were collected at monthly intervals throughout the lactation from April to July. Each sample of fresh milk was analysed for composition and subsamples were obtained and frozen for one, three and five months. Renneting properties, both from the fresh and frozen subsamples, were achieved using the Formagraph instrument and results were submitted to a mixed model statistical analysis. The storage effect significantly affected ($P<0.01$) the renneting parameters. A large amount of non coagulating subsamples was registered after a long-term frozen storage. Furthermore, milk clotting time was longer in frozen subsamples and curd firmness diminished after a freezing period of five months. In conclusion, the remarkable decreasing of sheep milk renneting characteristics after frozen storage can predict a worse yield and quality of cheese-making and suggests that freezing of Sarda raw milk should be limited to shorter periods.

Key words

sheep, milk, renneting properties, freezing

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Aim

There has been a remarkable rise of 22% in sheep milk production in the period 2000-2010 (FAOSTAT, 2013) and even if sheep dairy products are going to be produced almost worldwide, the biggest share is still mostly concentrated in some Mediterranean countries of the European Union. In these areas sheep breeding is frequently conducted in a traditional way by means of semi-extensive methods (Boyazoglu and Morand-Fehr, 2001), and dairy production and yield is significantly influenced by seasonality of reproduction. A well-known example of this type of sheep breeding is present in Sardinia (Carcangiu et al., 2011), a region of Italy, where productions from the autochthonous breed, the Sarda, are mostly influenced by reproductive performance and pasture availability, which is inadequate during the summer. This represents a primary limit in order to achieve a favourable exploitation of milk all over the year and finally improve the economic gain of sheep farms.

Cold and freezing storage are two strategies used to overcome the concern regarding seasonality of food of animal origin and improve handling, preservation and marketing of agricultural productions (Hundy et al., 2008). These techniques are especially exploited for meat (Muela et al., 2010) and dairy products (Wendorff 2001) which are exclusively available in particular and limited periods of the year, as like those from sheep species. Many researches have been carried out about the effects of freezing on composition of sheep milk (Wendorff 2001), cheese (Zhang et al., 2006) and curds (Picon et al., 2010) nevertheless no study has investigated the effect on renneting properties; these traits are used to evaluate the ability of milk to cheese-making and are measured by both mechanical and infrared methods (Pazzola et al., 2011; Pretto et al., 2011; Cipolat-Gotet et al., 2012). This study aimed to investigate the effect of long-term freezing on renneting properties of individual milk samples of the Sarda breed.

Material and methods

Fifty pluriparous ewes of Sarda breed were randomly selected. All the ewes were at third parturition, lambed in the month of February 2012 and belonged to a farm located in the Province of Sassari, Sardinia, Italy.

The animals were fed at pasture and received a supplementation of 300g/head of a commercial concentrate feed (15.5% crude protein) during the milking procedures. They were milked using a milking machine twice a day (6:00 AM and 16:00 PM).

Individual milk samples (100 mL) were collected from all the 50 ewes, during the morning milking, at monthly intervals from April (45 days in milking, DIM) to July (135 days in milking). On the whole, each of the 50 ewes was sampled four times, with a total amount of 200 samples. For each sampling session, individual milk yield of the morning was also recorded. Samples were transported at +4° C to the laboratory within 1 h after collection and analysed for milk composition and renneting properties. Four subsamples of about 15 mL were obtained pouring the individual samples in four plastic volumetric conical tube. The first subsample was used to achieve analysis from fresh milk and the other three after freezing.

Milk composition was evaluated only on fresh milk. Fat, total protein, casein contents and pH were obtained according to IDF 141C:2000 and using a mid-infrared spectrophotometer (Milko-Scan FT 6000; Foss Electric, DK-3400 Hillerød, Denmark); freezing point (FP) in Hortvet degrees (H°) according to IDF 108:2002 and by a thermistore cryoscope (Astor 4000/SE Double; Astori Tecnica, Poncarale BS, Italy); somatic cell count (SCC) using an automatic cell counter (Fossomatic 5000, Foss Electric, Hillerød, Denmark) according to IDF 148-2:2006 and total microbic mesophilic count (TMC) using a Bacto-Scan FC 150 (Foss Electric, Hillerød, Denmark) (IDF 358:2000).

As regards renneting properties, these were achieved both from fresh and frozen milk.

The first subsample (fresh milk, FM) was immediately refrigerated at +4° C and analysed within 12 hours collection. The others were frozen at -20° C and analysed after different periods of storage: the second one after one month of freezing (1M), the third after three months (3M) and the fourth after five months (5M).

Fresh and frozen milk subsamples were analysed following the same procedure; frozen subsamples were thawed at +4° C for about 12 hours. Ten mL of milk was kept at 35° C for 30 min before mixing with 200 µL of a 0.8/100 (v/v) rennet solution (Red Line Rennet, Clerici, Cadorago, Italy) and analysed using the Formagraph instrument (Foss Italia SPA, Padova, Italy) and the method by Mc Mahon & Brown (1982) to achieve milk rennet coagulation time (RCT) in minutes, curd firming time (k20) in minutes, and curd firmness (a30) in millimeters. Data regarding milk yield and composition of fresh milk were analysed in order to give a descriptive general overview of milk characteristics throughout the lactation. A repeated measures one-way analysis of variance (ANOVA) was performed, with the month of the stage of lactation measured as days in milking (DIM) as fixed effect, and multiple comparison of the means by the Tukey's method. SCC and TMC were previously transformed into logarithmic form (log10) to normalize the distribution of the frequencies.

Statistics regarding renneting parameters was performed by means of a mixed effect model procedure, which considered as fixed effects S_i for the storage treatment (i = 4) and the month of the stage of lactation (j = 4) measured as DIM_j. Random effect of the individual animal was computed with animal nested within DIM. Orthogonal contrasts were performed to achieve the effect of freezing treatment, evaluating fresh versus frozen milk (FM vs 1,3,5M) and milk frozen one month versus frozen five months (1M vs 5M).

Analyses were carried out using SAS (version 9.1, SAS Institute Inc., Cary, NC).

Since the duration of the Formagraph analysis was set at 30 minutes, subsamples with a value of RCT higher than 30 min did not show any of the three renneting properties; these samples were named non coagulating (NC) and not included in the mixed model but, as previously described by Pazzola et al. (2012), analysed by means of the Fisher's exact test according to the stage of lactation and storage treatment. For all the statistical tests, differences were declared significant at $P<0.05$.

Results and discussion

Milk yield and composition, pH, FP, TMC (log10) and SCC (log10) of individual samples throughout the lactation are shown in Table 1. All the parameters, except pH and SCC, were affected (P<0.01) by the stage of lactation. Decrease of yield and the corresponding increase of protein and fat content in the late stages of lactation of dairy sheep have been already studied and described in the literature (Jaeggi et al., 2005), and results of the present study consisted with data published for the Sarda breed in previous papers (Morand-Fehr et al., 2007; Mura et al., 2012) and by the official standard (Asso.Na.Pa., 2013).

Table 2 shows results regarding milk samples with a value of milk clotting time equal and lower (coagulating, C) or higher (non coagulating, NC) than 30 min. The effect of the different stages of lactation was not significant, while storage treatment significantly influenced (P-value = 0.001) the number of NC samples, and the greatest amount was registered for subsamples frozen for five months. NC samples of fresh milk were always categorized as NC after freezing for one, three and five months, because also the corresponding frozen subsamples showed a r value greater than 30 min.

The results of statistical analysis and variation of least square means regarding renneting parameters according to the effects of the stage of lactation and storage treatments are respectively illustrated in Table 3 and Figure 1.

The effect of the stages of lactation significantly influenced rennet coagulation time and curd firmness at P<0.01 while curd firming time at P <0.05. Examination of least square means and standard deviation throughout the lactation showed a decrease both for RCT (21.60 ±0.19 min at 45 DIM and 19.43 ±0.19 at 135 DIM) and k20 (1.82 ±0.02 min at 45 DIM and 1.75 at 135 DIM), while tendency of a30 was fluctuating with the highest value at 135 DIM, 50.33 ±0.75 mm. Given that acidity of milk was stable throughout the stages of lactation, these results were probably

Table 1. Least square means of yield and composition, pH, freezing point (FP), total microbic count (TMC) and somatic cell count (SCC) of fresh milk throughout the lactation (n=50)

	DIM[1]				SEM[2]	P-value
	45	75	105	135		
Milk yield[3] (kg)	0.918^{A}	0.755^{B}	0.559^{C}	0.354^{D}	26.5	**
Fat (%)	5.53^{B}	6.01^{B}	6.70^{AB}	8.20^{A}	0.14	**
Protein (%)	5.64^{B}	5.53^{B}	5.60^{B}	5.90^{A}	0.06	**
Casein (%)	4.38^{BC}	4.28^{C}	4.49^{B}	4.75^{A}	0.05	**
pH	6.68	6.71	6.70	6.67	0.01	NS
FP (°H)	-0.575^{B}	-0.569^{A}	-0.582^{C}	-0.589^{D}	0.01	**
TMC (log10)	4.33^{AB}	3.97^{B}	4.66^{A}	3.85^{B}	0.14	**
SCC (log10)	5.38	5.32	5.49	5.50	0.09	NS

[1] Days in milking; [2] Standard error of the mean; [3] yield of the morning milking; ** = P<0.01; NS = not significant; ^{A,B,C,D} Means in the same row with different letters differ significantly (P<0.01) in month comparison.

Table 2. Coagulating (C, milk clotting time equal or lower than 30 min) and non coagulating milk samples (NC, higher than 30 min) according to the stage of lactation (DIM) and storage treatment.

	DIM[1]				Storage[2]			
	45	75	105	135	FM	1M	3M	5M
n	200	200	200	200	200	200	200	200
C	163	173	174	179	185	179	171	154
NC	37	27	26	21	15	21	29	46
P-value[3]	0.131				0.001			

[1] Days in milking; [2] FM = fresh milk, 1M = frozen for one month, 3M = frozen for three months; 5M = frozen for five months; [3] obtained by chi-square test.

Table 3. P-values of renneting parameters according to the effects of the stage of lactation (DIM) and storage treatment

Parameters[1]	Effects - P-values		Animal RMSE[3]	Storage[4] - P-values		Residual RMSE[5]
	DIM[2]	Storage		FM vs 1,3,5M	1M vs 5M	
RCT (min)	<0.01	<0.01	9.26	<0.01	<0.01	2.95
k20 (min)	<0.05	<0.01	0.06	<0.01	<0.01	0.03
a30 (mm)	<0.01	<0.01	45.32	<0.01	<0.01	72.36

[1] RCT = rennet coagulation time, k20 = curd firming time, a30 = curd firmness; [2] Days in milking; [3] root of the mean squared error due to the individual animal; [4] FM = fresh milk, 1M = milk frozen for one month, 3M = frozen for three months; 5M = frozen for five months; [5] root of the mean squared error of the residual.

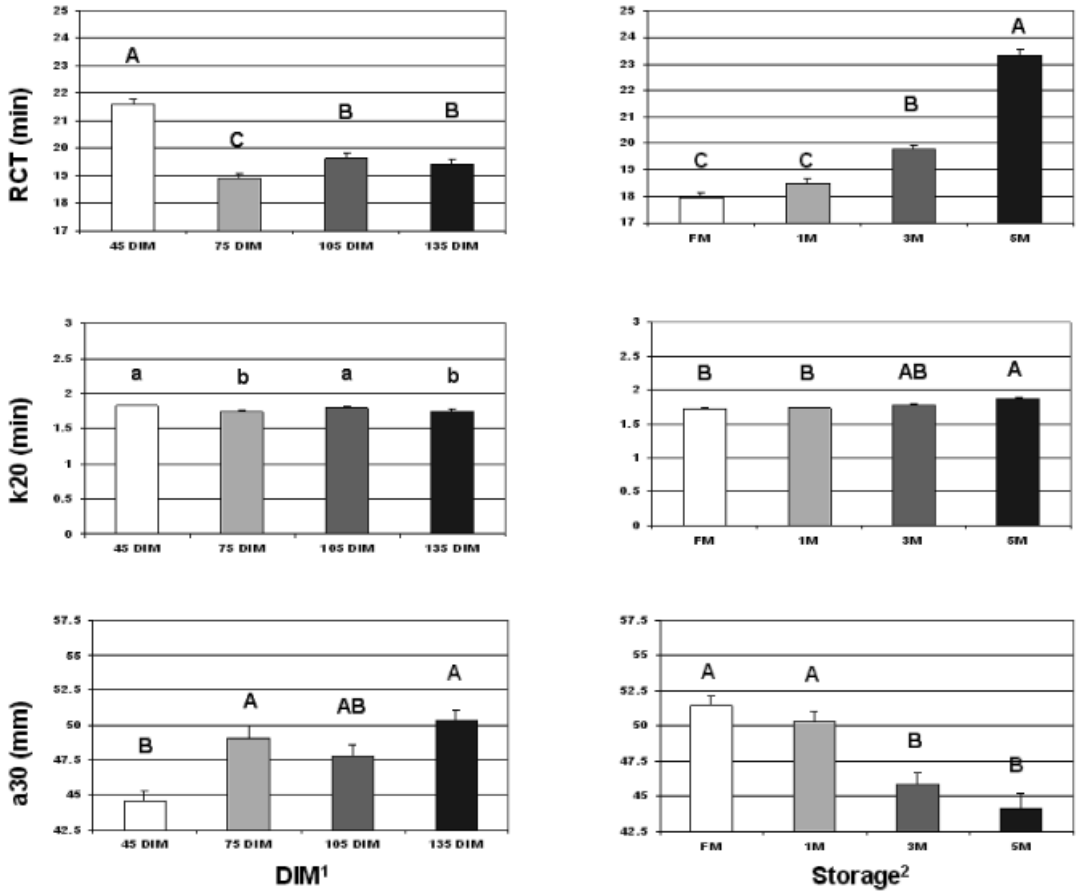

Figure 1. Least square means and standard deviations of renneting parameters according to the effects of the stage of lactation (DIM) and storage treatment; [1] Days in milking; [2] FM = fresh milk, 1M = frozen for one month, 3M = frozen for three months; 5M = frozen for five months. [A,B,C] Different capital letters in the same graphics differ at P<0.01; [a,b,c] different lower-case letters in the same graphics differ at P<0.05.

affected by the increase of casein content in the late stages of lactation, because the higher is the casein content the longer is milk clotting time and the stronger curd firmness (Dalgleish 1981). On the whole RCT and a30 were similar while k20 was lower than those parameters registered for other Italian dairy sheep breeds (Pugliese et al., 2000; Sevi et al., 2000). As regards the storage effect, the highest significance level was recorded for all the three renneting parameters. It can be noticed that rennet coagulation time of fresh milk was significantly shorter (17.97 ±0.16 min) than frozen subsamples, and values of subsamples frozen for one month were significantly lower than those frozen for five months (18.50 ±0.17 vs. 23.31 ±0.27 min); furthermore, as regards the curd firmness, data obtained from fresh milk (51.48 ±0.67 mm) significantly diminished after five months of frozen storage (44.14 ±1.12).

These results, summed up to the large amount of non coagulating subsamples which were registered after a freezing storage of five months, evidenced that long-term freezing caused a remarkable decreasing of sheep milk renneting characteristics and, on the whole, they can predict a worse yield and quality of cheese-making. The prediction of a lowered quality of cheese-making evidenced by the present study is partially in agreement with the study by Zhang et al. (2006), who record a limited reduction of yield, but no effect on fatty acids composition, in cheese produced from sheep milk frozen for six months. Acidity is one of the main source of variation for milk coagulation properties (Bittante et al., 2012), but as evidenced by Katsiari et al.(2001) acidity of sheep milk is not affected by frozen storage up to six months. Many other causes have been indicated as decisive for the reduction of the overall quality and yield of cheese made from frozen milk, like damaging of fat globules (Zhang et al., 2006) and protein destabilization (Wendorff 2001). The potential decrease of cheese yield from frozen milk is habitually overcome in the cheese making industry by means of different standardized techniques, such as the utilization of frozen curds; indeed, the effect of long-term freezing did not alter characteristics of frozen curds in the manufacture of sheep cheese (Picon et al, 2010).

Conclusions

Freezing storage of milk could be a suitable strategy to improve availability of some dairy products which are normally limited by concerns about seasonality. Indeed, utilization of milk rather than frozen curd for cheese-making could be favourable for future market expansion of dairy products like fresh cheese, ricotta-cheese and yogurt. These could be complementary alternatives to other traditional types of cheese, which are mainly available only in particular seasons and are obtained by means of longer ripening times. The present study evidenced that frozen storage up to five months significantly affected renneting properties of milk from the Sarda sheep. This predicts a decrease in cheese yield and quality and suggests that freezing of raw milk should be limited to shorter periods.

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