

## PHYSICO-CHEMICAL AND TECHNOLOGICAL CHARACTERISTICS OF LACAUNE EWE'S MILK

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### Abstract

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The chemical composition of the Lacaune ewe's milk and its basic technological properties in production of Bulgarian sour milk and Bulgarian white brined cheese were studied during March-August 2016. Studied Lacaune ewe's milk exhibited high content of total solids, fat and protein. The average percentage of these milk components was 18.84, 7.21 and 6.19%, respectively. The established renneting time (495.0 s - first appearance of small visual flocs) in Lacaune ewe's milk was in the normal limits for cheese-making. Lacaune ewe's milk is a good and suitable medium for the growth of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*. The studied Bulgarian sour milk had a dense, smooth, uniform coagulum, surface without syneresis and pleasantly sour taste. Matured Bulgarian white brined cheese had 50.22 % moisture, 54.66 % fat in dry matter, 8.33 % salt in the moisture and 69.06 % moisture in the non-fat substance. The titratable acidity reached average value 251.9 °T. The cheese yield was very similar to that established in studies on the milk of other Bulgarian sheep breeds.

**Keywords:** Lacaune; yogurt; renneting; syneresis; cheese

**Abbreviations:** DM – dry matter, M – moisture, F – fat, FDM – fat in dry matter, P – protein, P/F – protein to fat ratio, RIG – rennet induced gel, LAIG - lactic acid induced gel, MNFS – moisture-in-non-fat-substance, S/M – salt in the moisture

### Introduction

Sheep milk has a high nutritional value and high concentrations of proteins, fats, minerals, and vitamins, as compared to the milks of other domestic species. Sheep milk is mainly used for the production of fine cheese varieties, yogurt, and whey cheeses (Haenlein and Wendorff, 2006; Balthazar et al., 2017). Due to its specific composition - the high levels of protein, fat, and calcium by casein the sheep milk is an excellent raw material for cheese production, yogurt and other dairy products (Haenlein and Wendorff, 2006; Barłowska et al., 2011; Balthazar et al., 2017).

In France most ewes' milk is produced in the Roquefort area in the mountainous Massif Central plateau by the native Lacaune breed, and it is mainly processed into Roquefort blue cheese. The dairy Lacaune breed is the main French

dairy sheep breed. It is widely distributed in many countries and ranked among one of the most important dairy sheep breed in the world (Pellegrini et al., 1997; Barillet et al., 2001; Thomas, 2001; Thomas et al., 2001).

White-brined cheeses (also known as white-pickled cheeses) are the most popular varieties of cheeses manufactured in the Balkans and North-East Mediterranean area. They are manufactured from ovine, buffalo, bovine and/or caprine milk or from mixtures of these milks (Bintsis and Papademas, 2002).

The composition and technological properties of the milk in several dairy ewe's breed in Bulgaria are well studied (Pechovski et al., 1988; Slavov et al., 1990; Djorbineva et al., 1992; Kafedjiev et al., 1998; Stankov et al., 1998; Boikovski et al., 2003; Raicheva et al., 2004; Panayotov et al., 2011; Stancheva et al., 2011). The Lacaune sheep breed is one of

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the new to Bulgaria and there is no information regarding the technological properties of this breed' milk.

The main objectives of the present study were to establish technological properties of the Lacaune ewe's milk in Bulgarian sour milk and Bulgarian white brined cheese production.

## Material and methods

### Milk

The study was performed with milk, obtained from purebred Lacaune ewes from on first lactation, reared in the flock of the private farm in Vodenichane village, district Yambol. Milk samples were obtained in the morning and the evening, proportionally to the milk yield, according to rules for milk sampling. The milk composition was established by Milko-Skan 104 (A/S Foss Elektrik, Denmark).

The phosphorous content was assayed spectrophotometrically by the molybdate/vanadate technique – BSS ISO 11263. The other elements were assayed by atomic absorption spectrophotometer Perkin-Elmer 380, model Analyst 800 AA spectrometer.

Renneting time of the milk was measured visually in milk samples as the time from rennet addition until the formation of the first visible flocks (Chomakov et al., 2000).

### Bulgarian sour milk production

The milk (5 l) was pasteurized (95°C/30 min), cooled to 45°C and inoculated with 1.5% yogurt culture consisting of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* (Lactina 17, produced by Bankya, Bulgaria). The samples were then cultivated at 42°C until coagulation, then cooled and stored in a refrigerator at 4-6°C.

The titratable acidity of milk was determined on the 1st h and 2nd h after milk culturing, at the time of coagulation, and after storage in a refrigerator for 24 h and 72 h. The syneresis of Bulgarian sour milk was studied by a curd drainage method (Shidlovskaya, 1979) immediately after coagulation and after the storage of milk at 4-6°C for 24 h and 72 hours. The amount of fermented lactose was calculated by Gorbatova (2004).

### Bulgarian white brined cheese

The milk – 20 l was processed into Bulgarian white brined cheese under laboratory conditions without standardization as described in our earlier study (Peichevski et al., 1988). Commercial pepsin liquid rennet “Slivenska” (Biokom Trendafilov, Sliven, Bulgaria) for renneting was used. The rennet strength was determined by Chomakov et al. (2000).

Dry matter, fat and total protein content of the cheese was determined according to the Bulgarian state standard,

ISO standards and International Dairy Federation standards (IDF). The content of dry matter by oven drying to constant weight at 102°C ± 2°C (BSS 1109:1989); fat - BSS ISO 3433/IDF 222; total protein was determined by Kjeldal method according to BSS EN ISO 8968-1 (2001); sodium chloride (NaCl) - BSS 8274-82 (1982); titratable acidity by Thorner method (BSS 1111-80,1980), and expressed in Thorner degrees (°T).

Fat in dry matter (FDM), moisture in non-fat substance (MNFS) and salt in moisture (S/M) were calculated according to Lawrence and Gilles (1980).

Trials were conducted in triplicate over a 6-month period.

For statistical analysis, ANOVA was performed on the corresponding replicates, using statistical software (Statistica 6.0).

## Results and discussion

The physico-chemical characteristics of the milk used for the production of Bulgarian sour milk and Bulgarian white brined cheese are presented in Table 1.

**Table 1**  
Lacaune ewe's milk composition and properties (n=3)

Studied parameters	$\bar{x}$	$S\bar{x}$
Total solids (TS), %	18.84	0.737
Solids non-fat (SNF), %	11.63	0.141
Fat (F), %	7.21	0.723
Protein (P), %	6.19	0.085
Lactose (L), %	4.50	0.035
P/F ratio	0.87	0.087
Calcium, mg/100g	218.34	5.966
Phosphorous, mg/100g	133.00	3.00
Potassium, mg/100g	112.16	5.675
Sodium, mg/100g	46.88	1.010
Magnesium, mg/100g	19.36	0.404
Zinc, mg/100g	0.50	0.002
Iron, mg/100g	0.10	0.004
Copper, mg/100g	0.04	0.002
Density, g/mL	1.0358	0.0078
Titratable acidity, °T	22.6	2.463
Renneting time, s	495.0	61.785

Studied Lacaune ewe's milk exhibited high content of total solids, fat and protein. The average percentage and standard deviation of these milk components was 18.84, 7.21 and 6.19%, respectively.

Similar to some earlier studies on Lacaune ewe's milk composition (Alichanidis and Polychroniadou, 1996; Kaminarides and Anifantakis, 2004; Oravcová et al., 2007; Fava et al., 2014) our data for above components are higher than the established by Brito et al. (2006) for the same breed. The milk fat content in our study is lower than that determined by Fava et al., (2014) and higher than the results of Kaminarides and Anifantakis (2004), and Oravcová et al. (2007).

Milk protein showed higher content than the levels of the protein content cited by the above authors. High levels for the total solids, fat, protein and lactose in Lacaune ewe's milk are received during the 127-197 days of the lactation period (Kuchtík et al., 2017). The alteration of the protein to fat ratio (P/F) of cheese milk in the range 0.70 to 1.15 has marked effects on cheese composition, component recoveries, and cheese yield (Guinee et al., 2007).

Our results for the basic milk composition in this study are most similar to data of Pellegrini, et al. (1997) for the basic Lacaune sheep milk composition.

The average calcium content of Lacaune milk in our study confirms the data in earlier research (Pellegrini, et al., 1997; Kaminarides and Anifantakis, 2004) and it is significantly higher than some recently published results for this macroelement in the milk of the same breed (Kuchtík et al., 2017). This trend is also established in respect to the phosphorous content. Kuchtík et al. (2017) assume that the gradual increase of the content of Ca and P during lactation is mainly affected

by gradual increase of the total protein and casein contents during lactation.

Our data for the Ca, P, K, Na and Cu content of the Lacaune milk is very similar to the results in other breeds (Polychroniadou and Vafopoulou, 1985; Iliev et al., 2000; Gerchev and Mihaylova, 2012). The calcium and phosphorous content of Lacaune milk in this study is significantly higher than in the milk of Bulgarian Dairy Synthetic Population, the East-Friesian and the Awassi breeds (Ivanova et al., 2011).

The zinc and iron contents in Lacaune milk are very close to data by Balthazar et al. (2017). The copper content of Lacaune milk corresponds to the lowest level of its varying range – 0.03-0.10 mg/100g (Haenlein and Wendorff, 2006). Similar low cooper content levels in sheep milk were reported by Ivanova et al. (2011).

The milk density and titratable acidity have lower values compared to the results of Fava et al. (2014), but are close to the data of Kaminarides and Anifantakis (2004) for the same breed.

The established in our study renneting time in Lacaune ewe's milk was longer than the results of Kuchtík et al. (2017) for the same breed and for the milk of local Stara Zagora sheep (Peichevski et al., 1988; Iliev et al., 2000) and Bulgarian Dairy Synthetic Population (Stancheva et al., 2011), but it was in the normal limits for cheese-making. These differences could be attributed to the milk composition and the type of applied rennets (Kowalchuk and Olson, 1979; Pearse and Mackinlay, 1989; Fox and McSweeney, 1997; Uniacke-Lowe and Fox, 2017). The rennet coagulum syneresis in this study is much slower in comparison with the results of Peichevski et al. (1988) and Iliev et al. (2000) (Figure 1).

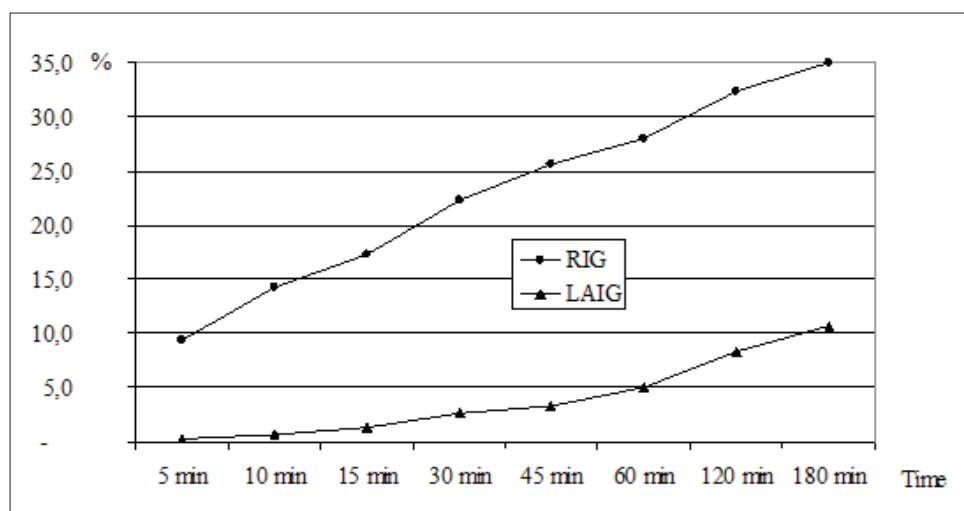


Fig. 1. Syneresis of the rennet (RIG) and lactic acid (LAIG) induced gels in Lacaune ewe's milk

O'Keeffe et al. (1982) found a poor relationship between rennet coagulation time and syneresis, which shows that there are factors other than the rate of rennet coagulation which determine the rate of syneresis

The main differences in the syneresis between lactic acid- and enzymic-induced milk gels could be briefly explained with unchangeable permeability of the acid-induced gel during the first 24 h after gelation. On the other hand the enzymic-induced gel is more robust than an acid-induced gel and increases continuously its permeability during the same period. In addition heat-induced  $\beta$ Lg/ $\kappa$ -casein interactions at yogurt production and possibly involvement of –SS linkages, and various salts, for example calcium phosphate, inhibit micellar coalescence, so giving rise to firmer curds with reduced tendencies to syneresis (Tamime and Robinson, 2007).

The quality of milk as a raw material for the dairy industry is determined not only by its chemical composition and properties, but also by its quality as a growth medium for the starter's lactic acid bacteria (Chomakov, 1978; Kondratenko et al., 1985; Chomakov and Boycheva, 1996).

After the milk culturing, the titratable acidity reached to levels of 35.7; 50.6; and 96.3 °T at the 1-st, 2-nd h and coagulation point, respectively (Table 2).

At 24 h and 72 h the values of the titratable acidity were 121.6 and 158.2 °T, respectively. The cultured milk from Lacaune ewe's breed coagulated after 155.7 min. These results show similarly technological quality to Stara-Zagora sheep breed milk processed to Bulgarian sour milk (Djorbineva et al., 1992). The Bulgarian sour milk titratable acidity in this study is insignificantly higher than acidity of the yogurt made by the milk of the same breed (Kaminarides and Anifantakis, 2004). Compared to Bulgarian Dairy Synthetic

Population breed Lacaune ewe's breed showed better technological values of the milk processed into Bulgarian sour milk (Stancheva et al., 2011).

Lacaune ewe's milk is a good and suitable medium for the growth of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*. The microscopy observations did not established differences in morphology of the lactic acid bacteria after coagulation of the milk.

The studied Bulgarian sour milk had a dense, smooth, uniform coagulum, surface without syneresis and pleasantly sour taste, corresponding exactly to BDS 12:2010.

Our calculated data for the fermented lactose in cultured milk showed reduction from 4.50% in the raw milk (Table 1) to 4.39; 4.26, 3.87% at 1h, 2 h after lactic acid starter inoculation and coagulation point, respectively (Table 2). The final product at 24 h showed lactose reducing to 3.65% and 3.34%, in 72 h storage at 4–6°C.

Compared to data of Kaminarides and Anifantakis (2004) related to initial milk and produced yogurt acidity, the calculations show that our results for fermented lactose amount are slightly higher – 0.846g (lactose reducing from 4.50 to 3.65%) vs. 0.723 g (4.86 to 4.14%).

The Bulgarian sour milk has a different titratable acidity, often varying in the range 100–160°T, depending on the raw material, applied starters, technology and the regime of cooling. Calculated in lactic acid, it is equal to 0.85–1.44%. The residual amount of unfermented lactose is within the range of 3.70–4.35%. The higher degree of fermentation inhibits the growth of lactic acid bacteria (Kondratenko et al., 1985).

Goodenough and Kleyn (1976) reported that in several brands of commercial yogurt, lactose content ranged from 3.31 to 4.74%, galactose – 1.48 to 2.50%, and glucose - only a trace in all samples.

Whey or serum separation (syneresis), which is also called wheying-off, is the appearance of whey on the surface of a gel, and it is a common defect during storage of fermented milk products like yogurts. Manufacturers try to prevent whey separation by increasing the total solids content of milk, subjecting the milk to a severe heat treatment or by adding stabilizers (Lucey, 2002).

Bulgarian sour milk produced by Lacaune ewe's milk demonstrated very low values of syneresis at all stages of studying (Figure 2).

Our results showed better serum holding capacity of the cultured Lacaune ewe's milk than Bulgarian sour milk produced by Stara Zagora sheep breed's milk (Djorbineva et al., 1992). There was a slow increase in the syneresis during 72 h storage period, which confirms the standpoint that rate of syneresis is directly related to the acidity (Panesar and Shinde, 2011).

**Table 2**  
**Dynamic of the titratable acidity and lactose content reduction in Bulgarian sour milk from Lacaune ewe's milk**

Inoculation and storage time	Titratable acidity, °T	Remained lactose, g
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
1 h	35.7 ± 1.725	4.39 ± 0.053
2 h	50.6 ± 6.301	4.26 ± 0.009
At coagulation	96.3 ± 8.105	3.87 ± 0.029
24 h	121.6 ± 8.322	3.65 ± 0.045
72 h	158.2 ± 22.926	3.34 ± 0.210
Coagulation time, min	155.7 ± 2.333	

The cheese composition and properties data are presented in Table 3.

The moisture content in the fresh (one day old) cheese ( $55.26 \pm 0.839\%$ ) was higher than established in the cheese produced from the Stara Zagora ewe's milk (Peichevski et al., 1988). The reasons for the higher moisture content are various: milk acidity, e.g. retention of denatured whey protein of high water-holding capacity, occlusion effect of insoluble whey protein, curd acidity etc. (Dilanyan, 1984; O'Keeffe, 1984).

Cheese is commonly considered to be composed of three components - fat (F), moisture (M), respectively dry matter (DM) and non-fatty solids (NFS - mainly casein). Cheese quality is more closely related to the MNFS, than to the moisture of the cheese. The MNFS also influences cheese body both directly and through its effect on the rate of protein breakdown (Gilles and Lawrence, 1973; Pearce, 1978).

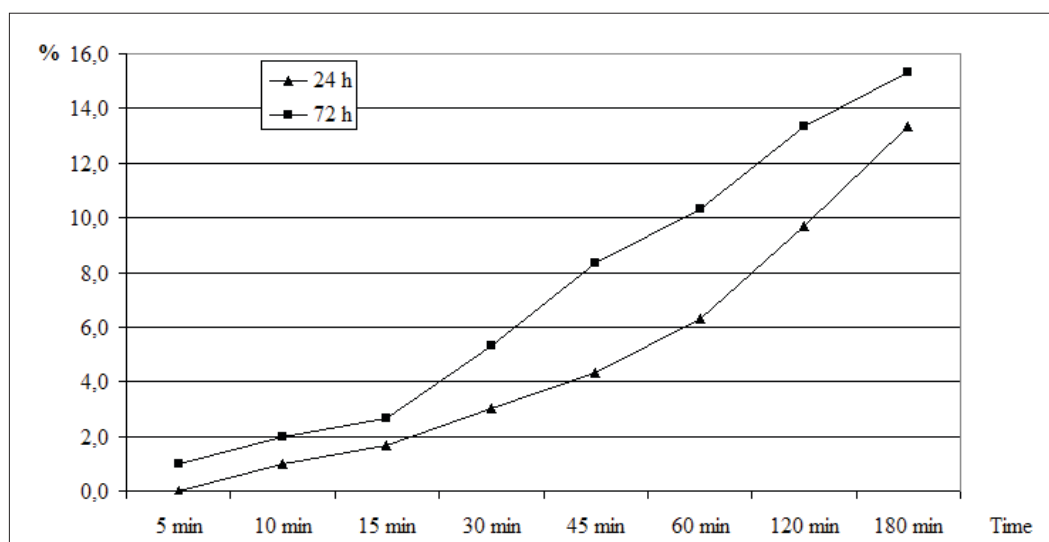
MNFS and S/M are important factors for correct cheese maturation and its quality (Thomas and Pearce, 1981; Kestenova and Chomakov, 1984). After 14th days these parameters of Bulgarian white brined cheese remain constant. An increase of the one day old cheese S/M above 4.43% sharply reduces its quality. The sensory evaluation of the product on the 14th day might serve as an indication of the quality of cheese on the 30th, 60th, 90th and 120th day of age (Kestenova and Chomakov, 1984).

The established titratable acidity ( $173.7^{\circ}\text{T}$ ) showed that the fresh cheese (24 h after production) is a good medium for the growth of starter's lactic acid bacteria.

The values for moisture (50.22%), fat (27.25%), protein (17.94%), moisture in nonfat substances (69.06%), salt in

**Table 3**  
**Composition of the Bulgarian white brined cheese made from Lacaune ewe's milk**

Composition and acidity	One day old cheese	Matured cheese
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Moisture (M), %	$55.26 \pm 0.839$	$50.22 \pm 1.112$
Fat (F), %	$24.17 \pm 0.601$	$27.25 \pm 1.893$
Fat in the Dry Matter (FDM), %	$54.10 \pm 2.328$	$54.66 \pm 2.928$
Protein (P), %	$16.37 \pm 1.312$	$17.94 \pm 1.295$
Salt-in-the Moisture (S/M), %	$3.45 \pm 0.083$	$8.33 \pm 0.251$
Moisture in the non-fat substance (MNSF), %	$72.89 \pm 1.674$	$69.06 \pm 1.048$
Titratable acidity, $^{\circ}\text{T}$	$173.7 \pm 20.309$	$251.9 \pm 37.195$
Cheese yields, kg		
In actual cheese moisture and real milk fat content		$25.983 \pm 1.425$
In 50% adjusted cheese moisture and real milk fat content		$25.957 \pm 1.033$
In actual cheese moisture and 6.5% fat corrected milk		$23.643 \pm 1.062$
In 50% adjusted cheese moisture and 6.5% fat corrected milk		$23.519 \pm 0.646$
Milk amount per 1 kg cheese at 50% adjusted cheese moisture, l		
Fat uncorrected milk		$3.897 \pm 0.284$
6.5% fat corrected milk		$4.258 \pm 0.115$



**Fig. 2.** Syneresis in Bulgarian sour milk



the moisture (8.33%), and titratable acidity (251.9°T) of the matured cheese were similar to those found in other earlier studies on Bulgarian white brined cheese (Kestenova and Chomakov, 1984; Peichevski et al., 1988; Naydenova et al., 2013).

Cheese yield of the processed Lacaune milk was very close to those established for milk of Stara Zagora sheep breed and its crossbreds with East-Friesian rams (Peichevski et al., 1988) and South-Bulgarian Corriedale breed (Stankov et al., 1998), and higher, compared to Bulgarian Dairy Synthetic Population (Stancheva et al., 2011).

## Conclusions

The milk of Lacaune ewe's breed is very suitable raw material for production of Bulgarian sour milk and Bulgarian white brined cheese. Lacaune ewe's milk is a good and suitable medium for the growth of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*. Bulgarian sour milk saves its good technological properties during storage period. The Bulgarian white brined cheese produced from the milk of Lacaune ewe's breed meet the requirements of the BSS and its compositional and yields parameters are very typical for this type of cheese.

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