Albanian j. agric. sci. 2013;12 (4): 735-740 *Agricultural University of Tirana*

RESEARCH ARTICLE **(Open Access)**

Protein balance and evaluation of velocity constant *k* (drained rate) on syneresis of milk

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Abstract:

The syneresis process is influenced by various factors such as milk pH, curd incubation temperature, fat content, heat treatment of milk, acidity, salt, curd dimension and gel firmness at cutting time. The aim of this study was to investigate balance of protein, the syneresis kinetic of whey drainage and evaluation of velocity constant ***k*** (drained rate) on curd incubation temperature (25 and 30°C) and heat treatment (at 70°C for 5 minutes). Milk was sampled from cow, sheep and goat breeds. The milk samples were analyzed for physical and chemical properties (pH, acidity, protein, casein, fat and lactose), coagulation parameters (R coagulation time in minutes, curd firmness measured in volt after 20 minutes (A20) or 30 minutes (A30) and the rate of firming K20 in minutes) as well as for whey volume drained after 30, 50, 70, 90, 110, 130 and 150 minutes. During this study it was observed that the curd incubation temperature is the major factor affecting syneresis. Velocity constant ***k*** value (drained rate) is increased with higher temperature, but can be decrease significantly at low temperature. The syneresis rate differs between breed's milk and is influenced by their coagulation properties. Regarding balance of protein, protein recovery and curd yield results to be higher at incubation temperature of 25°C, in spite of breed. Whey protein loss result to be higher for goat's milk on two incubation temperature (41.05–58.35%), while the whey loss on sheep's milk result to be lower (14.01–37.61%).

Key Words: Syneresis, protein balance, k value (drained rate), milk

1. Introduction

Syneresis is defined as the process of separation of whey and its components as a result of the contraction of the mass of curd and doesn't represent only a physical process. Matrices formed by enzymatic coagulation of casein rebuild their structure forming a compact mass of gel. Syneresis starts at the moment when the coagulum is cut and agitated. Syneresis is important because of the amount of whey expelled and its influence on the moisture content of the curd [**8**]. Moisture control of the cheese curd is important because moisture directly affects the specific sensory and functional properties of cheese. To standardize cheese quality, all variables affecting drainage rate must be controlled during processing. A number of studies are carried out about of the factors that affect the process of syneresis, such as temperature, pH, gel strength [**1**] and calcium chloride supplements [**3,5**]. Syneresis is closely related to the rheological and micro structural properties of the gel at the time of cutting. If the gel is cut too early, when it has lower firmness, the structure of the gel is disrupted more easily, partial destruction of gel may occur [**7**]. If the gel is cut too late, when it has higher firmness, the whey removal from the surface of the curd is immediate which leads to the formation of membrane which, on the other hand, may decrease or completely stop further removal of the whey from the center of the curd. PH values play a significant role in determining the rheological and microstructure characteristics of the gel [**6,16**]. Gels are grouped as particulate, fine-stranded, or mixed networks; and pH, solutes, and gelation kinetics determine the type of gel matrix formed. Protein molecules are charged at pH values significantly higher or lower than their isoelectric points (pI), and an appreciable electrostatic repulsion opposing intermolecular protein-protein interactions is present. The influence of parameters such as fat content, incubation temperature of curd (25, 30 and 45°C) and duration of the heat treatment of milk (70°C for 5 or 30 min) on the kinetics of whey drained were studied by [**1**], who concluded that the kinetic of syneresis is fitted to first order kinetic equation.

With regard to the market demand for good quality and safety dairy products, this study is carried out to evaluate factors that affects syneresis process of milk of some breeds grown up in Albania. The aim is to introduce a compatible method suitable for industrial application by Albanian dairy industry, taking into consideration the EU standards.

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(Accepted for publication 25 November 2013)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

Materials and Methods

2.1 Sampling

Milk was sampled from morning milking on volume 500 ml per sample. There were selected 5 samples for each different breed, raised on home conditions of:

a) Cow: xhersej, laramane, and third race bought from EU countries, from locality of Tirana (Laknas) and Devolli (Proger).
b) Sheep: rude, French rude, Shkodra red sheep and bardhok of tropoja, from locality of Tirana (Laknas, UBT) and Korça (Zemblak).
c) Goat: Red Alpine and White sona, from locality of Tirana (Laknas, UBT) and Devolli (Shyec).

Samples were taken in March to July 2012. Milk‘s samples were treated with 1 ml solution of 1% sodium azide (w/v) and were refrigerated at 2-4^{o}C, after that samples were sent to laboratory [**9**].

2.1.1. Milk sample preparation for syneresis

Sample pH was adjusted to pH 7 with 1 M

NaOH at 25°C, the milk was stirred carefully during the addition of acid to avoid localized coagulations. Some milk samples were skimmed by centrifugation before analysis. Whole milk was incubated in a water bath at 30°C for 20 min. After that, samples were centrifuged at 3000×g for 30 min at 5°C. Milks were then placed in an ice-water bath, and after 30 min the solidified fat was removed with a spatula. The skimmed milk was filtered through glass fiber pads (helped with a vacuum pump) to remove any residual fat. Milk was heated at 70°C in a water bath for 5 min in tightly sealed Pyrex glass tubes (16×162mm). Heated samples were immediately cooled in an ice water bath and kept under refrigeration until analysis [**10**].

2.2 Analyses

There were carried out analyses regarding to **Physical and chemical properties**, acidity (in Turner degrees), content of casein (according to Sorensen), content of non-fat solids, content of crude protein, content of fat, content of lactose and content of added water, with ultrasonic milk analyzer Lactoscan. **Coagulation properties of milk** (coagulation time R in minutes, curd firming after 20 and 30 minutes A20 and A30 in volt and rate of curd firming K20 in minutes) were performed by Optigraf, AMS, France [**4,11,12,13,14,15**]. **Curd syneresis** was evaluated according the method of [**17**]. Curd was formed in 100 ml beakers by the addition of 1 ml of rennet solution to 60 ml of milk and maintained at 30°C. The coagulation time was determined by the method of [**11**]. After 15 min of coagulation, the curd was cut crosswise and around the sides of the beaker with a spatula. After cutting, a plastic grid was placed on the curd surface. This retained the curd while allowing the whey to be poured off. Syneresis was measured by weighing the quantity of whey removed at various intervals after cutting. Experiments were performed by incubating the cut coagulum at 25 or 35°C to study the influence of the incubation temperature. The syneresis evaluation of each experiment was performed by duplicate. Whey loss protein was determined by ultrasonic milk analyzer Lactoscan.

2.3. Protein balance calculations

Abbreviations:
DQ – Density of milk, g/cm^3
MQ – Mass of milk, gr.
PQ – Protein of milk, %
VH – Whey volume, cm^3 (ml)
MH – Mass of whey, gr.
PH – Protein of Whey, %
MB – Mass of curd, gr
PB – Protein of curd, %
ReB – Yield of curd, in % considered milk volume
RPB – Protein recovery on curd, in % considering total protein of milk
HPH – Whey protein loss, in % considering total protein of milk

Balance calculation was based on low of mass conservation for two cases:

Product mass balance:

$$MQ = MB + MH$$

Protein mass balance:

$$MQxPQ/100=MBxPB/100 + MHxPH/100$$

Since parameters measured during laboratory experiments are volumes of milk and whey, the respective mass were calculated based on the measured values of the corresponding density [**18**].

In this way the calculation of the balance of the system resulting from the solution of the equations:

$$VQ \times DQ = MB + VH \times DH$$

$$VQ \times DQ \times PQ = MB \times PB + VH \times DH \times PH$$

From which unknown values of MB and PB were defined.

Further indicators were calculated following:

ReB– yield of curd:

$$ReB = (MB/MQ) \times 100$$

Protein recovery on curd:

$$RPB = (MB \times PB/MQ \times PQ) \times 100$$

Whey protein loss: $HPH = 100 – RPB$

*2.4 Velocity constant **k**value calculations*

2.4 Velocity constant k value calculations

The rate of syneresis was fitted to a first-order kinetic equation:

$\ln (C_0/C) = k\,t$

where C_0 is the original milk volume and C is the curd volume (milk volume minus whey volume) after time t_o. The ***k*** value is the velocity constant of the reaction.

3. Results and Discussion

3.1 Protein recovery on curd and loss on whey

Protein loss during drainage has a significant impact on cheese manufacturing industry. Whey proteins are quantified for total volume of each sample.

Data's from Figure 1 shown that one of the factors that affect recovery of protein on curd or protein loss on whey is the species of cattle. Sheep's milk lost less protein, while goat's lost more, considering total protein of milk.

On table 1 are shown mean values of protein balance and protein loss on whey for milks sheep, cow and goat on curd incubation temperature 25 and 30^{o}C. In spite of species, it seems clearly that recovery of protein and yield of curd result to be higher on incubation temperature 25^{o}C, but content of protein in curd decrease due to the increase of its mass and volume. Whey protein loss result to be higher for goat's milk on two incubation temperature (41.05–58.35 %), while the whey loss on sheep's milk result to be lower (14.01–37.61%).

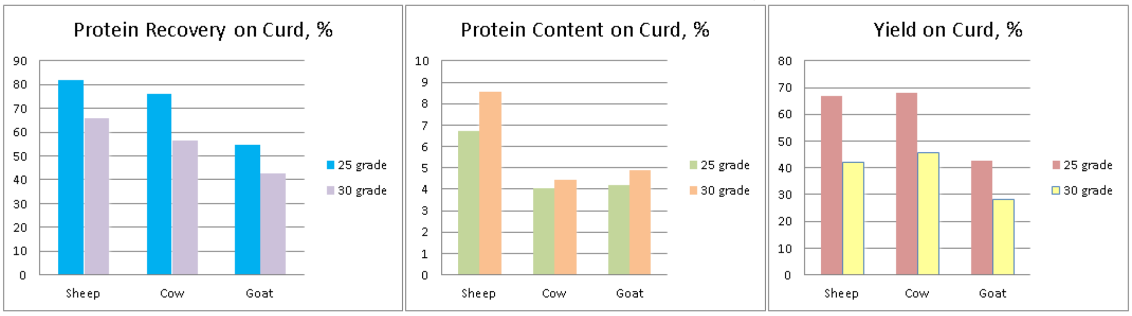

Figure 1. Protein recovery on curd, content of protein on curd and yield on curd for sheep, cow and goat breeds.

Table 1. Protein balance calculation of syneresis for sheep's, cow's and goat's milk.

	DQ, gr/cm3	PQ, %	VH, cm3	PH, %	PB, %	ReB, %	RPB, %	HPH, %
				Sheep's milk				
				Curd incubation temperature 30 'C				
D.Rude	1.035	5.72	43.15	3.06	11.98	29.79	62.39	37.61
D.French Rude	1.034	4.89	32.66	3.04	6.95	46.84	65.54	34.46
D.Shkodrane	1.033	5.48	33.00	3.06	8.30	46.22	69.97	30.03
D.Tropojane	1.032	5.89	36.30	2.94	10.17	40.80	70.45	29.55
				Curd incubation temperature 25 'C				
D.Rude	1.035	5.72	27.65	3.06	7.93	55.02	75.83	24.17
D.French Rude	1.034	4.89	18.30	2.96	6.18	70.21	82.93	17.07
D.Shkodrane	1.033	5.48	18.30	2.89	6.58	70.18	84.27	15.73
D.Tropojane	1.032	5.89	18.40	2.75	7.24	69.99	85.99	14.01
				Cow's milk				
				Curd incubation temperature 30 'C				
KE	1.030	3.33	35.50	2.71	4.19	41.98	52.78	47.22
Xhersej	1.030	3.43	35.50	2.76	4.56	42.01	53.64	46.36
Laramane	1.032	3.59	30.13	2.80	4.34	50.83	61.24	38.76
				Curd incubation temperature 25 'C				
KE	1.030	3.33	3.50	2.68	3.37	94.28	95.40	4.60
Xhersej	1.030	3.43	21.00	2.69	4.07	65.69	73.69	26.31
Laramane	1.032	3.59	21.58	2.68	4.24	64.78	74.04	25.96
				Goat's milk				
				Curd incubation temperature 30 'C				
Red Alpina	1.029	3.28	44.65	2.62	5.15	26.96	41.65	58.35
White Sona	1.027	2.97	41.30	2.39	4.23	32.31	45.42	54.58
				Curd incubation temperature 25 'C				
Red Alpina	1.029	3.28	36.25	2.59	4.37	40.70	52.92	47.08
White Sona	1.027	2.97	31.60	2.35	3.69	48.21	58.95	41.05

3.2 Evaluation of velocity constant k (drained rate) of syneresis

Calculated values of velocity constant k of curd formed from sheep, cow and goat milk, fits to logarithmic functions with a correlation coefficients R^2=97.6%-99.7%. The calculated mean values of velocity constant (k) according to the first order kinetic equation are shown on table 2, 3 and 4, where the whey drainage volume is expressed as a function of the incubation time (in minutes) of curd formed of the sheep's milk breed, cow's breed and goats breed respectively.

Drainage rate between four breeds of sheep taken under study shown significant variance for k value (drained rate) calculated on incubation temperature 30°C. While on incubation temperature 25°C variances are shown only for rude sheep, whereas milk from the other breeds show the same behavior, represented by similar values of velocity constant k (figure 2). For the same breed of sheep values of velocity constant k result to be 2 times higher on incubation temperature 30°C that means that drainage rate diminished 2 times when incubation temperature is pulling down from 30°C to 25°C.

Drainage rate of the curd formed from milk of cow's breed (KE, Laramane and Xhersej) show variance only for incubation temperature 30°C, where calculated values of velocity constant k result to be significantly different (table 3). While, on curd incubation temperature 25°C, milk from cow's breed shown similar behavior except KE breeds. For four segment of time selected for calculation of k value, drainage rate of milks from Xhersej and Laramane breed have the similar behavior, except milks from KE breed that have some lockage. Milk from Xhersej cow breeds show higher drainage rate, in spite of curd incubation temperature (figure 3).

Differences on drainage rate are founded for curd formed from two goats breed Red Alpina and White Sona, in spite of curd incubation temperature. Velocity constant k is about 1,5 time higher on incubation temperature 30°C compared to 25°C. Changes on drainage rate results to be higher for milks from goats breed Red Alpina compared to White Sona$k_{RA}>k_{WS}$.

Table 2. Calculated values of velocity constant k (drained rate) of milk syneresis of sheep breeds on curd incubation time 30°C and 25°C.

Breed	t = 30 min		t = 70 min		t = 110 min		t = 150 min	
	Ln (Vq/Vb)	K	Ln (Vq/Vb)	K	Ln (Vq/Vb)	K	Ln (Vq/Vb)	K
Curd incubation temperature 30 'C								
D.Rude	0.25	$8.3*10^{-3}$	0.6	$8.5*10^{-3}$	0.89	$8.1*10^{-3}$	1.27	$8.5*10^{-3}$
D.Frech Rude	0.11	$3.6*10^{-3}$	0.28	$3.9*10^{-3}$	0.52	$4.7*10^{-3}$	0.79	$5.2*10^{-3}$
D.Shkodrane	0.16	$5.3*10^{-3}$	0.44	$6.3*10^{-3}$	0.64	$5.9*10^{-3}$	0.8	$5.3*10^{-3}$
D.Tropojane	0.16	$5.4*10^{-3}$	0.47	$6.7*10^{-3}$	0.74	$6.7*10^{-3}$	0.93	$6.2*10^{-3}$
Curd inubation temperatura 25 'C								
D.Rude	0.07	$2.2*10^{-3}$	0.25	$3.6*10^{-3}$	0.42	$3.8*10^{-3}$	0.64	$4.2*10^{-3}$
D.Frech Rude	0.04	$1.3*10^{-3}$	0.11	$1.5*10^{-3}$	0.25	$2.2*10^{-3}$	0.42	$2.6*10^{-3}$
D.Shkodrane	0	0	0.14	$2*10^{-3}$	0.29	$2.6*10^{-3}$	0.36	$2.4*10^{-3}$
D.Tropojane	0	0	0.15	$2.1*10^{-3}$	0.3	$2.7*10^{-3}$	0.37	$2.4*10^{-3}$

Table 3.Calculated values of velocity constant k (drained rate) of milk syneresis of cow's breeds on curd incubation time 30oC and 25oC.

Breed	t = 30 min		t = 70 min		t = 110 min		t = 150 min	
	Ln (Vq/Vb)	K	Ln (Vq/Vb)	K	Ln (Vq/Vb)	K	Ln (Vq/Vb)	K
Curd incubation temperature 30 'C								
KE	0.03	$8.4*10^{-4}$	0.14	$2*10^{-3}$	0.31	$2.8*10^{-3}$	0.90	$5.9*10^{-3}$
Laramane	0.06	$2*10^{-3}$	0.20	$2.9*10^{-3}$	0.35	$3.2*10^{-3}$	0.61	$4.1*10^{-3}$
Xhersej	0.08	$2.6*10^{-3}$	0.26	$3.7*10^{-3}$	0.53	$4.8*10^{-3}$	0.85	$5.7*10^{-3}$
Curd inubation temperatura 25 'C								
KE	0	0	0.00	0	0.00	0	0.06	$4*10^{-4}$
Laramane	0.01	$3.3*10^{-4}$	0.08	$1.2*10^{-3}$	0.18	$1.6*10^{-3}$	0.32	$2.1*10^{-3}$
Xhersej	0.02	$5.6*10^{-4}$	0.08	$1.1*10^{-3}$	0.21	$1.9*10^{-3}$	0.36	$2.4*10^{-3}$

Table 4. Calculated values of velocity constant k (drained rate) of milk syneresis of goat's breeds on curd incubation time 30°C and 25°C.

Breed	t = 30 min		t = 70 min		t = 110 min		t = 150 min	
	Ln (Vq/Vb)	K	Ln (Vq/Vb)	K	Ln (Vq/Vb)	K	Ln (Vq/Vb)	K
Curd incubation temperature 30 'C								
Red Alpina	0.21	$7.1*10^{-3}$	0.60	$8.5*10^{-3}$	1.04	$9.5*10^{-3}$	1.37	$9.2*10^{-3}$
White Sona	0.19	$6.2*10^{-3}$	0.57	$8.1*10^{-3}$	1.00	$9.1*10^{-3}$	1.19	$7.9*10^{-3}$
Curd inubation temperatura 25 'C								
Red Alpina	0.11	$3.7*10^{-3}$	0.36	$5.1*10^{-3}$	0.68	$6.2*10^{-3}$	0.96	$6.4*10^{-3}$
White Sona	0.13	$4.4*10^{-3}$	0.35	$5.1*10^{-3}$	0.62	$5.6*10^{-3}$	0.78	$5.1*10^{-3}$

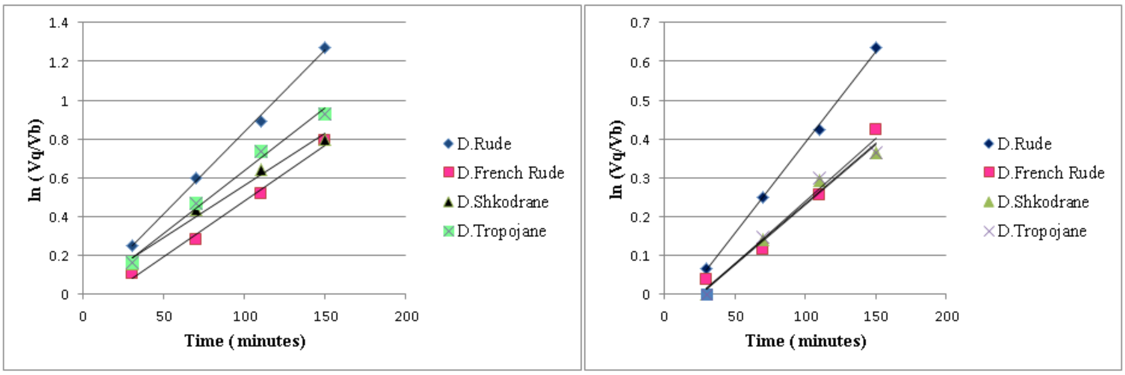

Figure 2. Velocity of changes of drainage rate of syneresis for sheep's milk

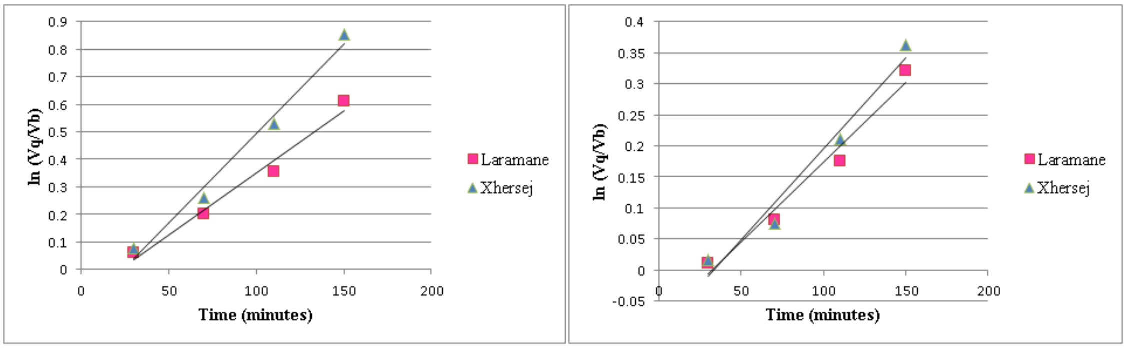

Figure 3. Velocity of changes of drainage rate of syneresis for cow's milk

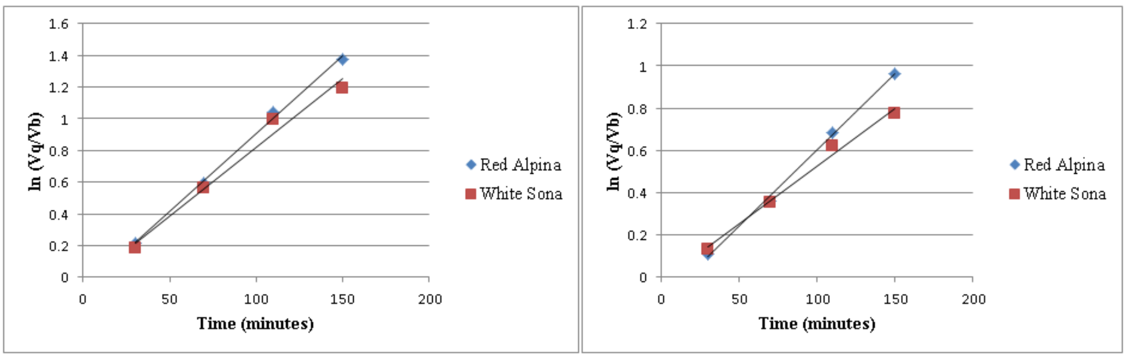

Figure 4. Velocity of changes of drainage rate of syneresis for goat's milk

4. Conclusions

Whey protein loss result to be higher for goat's milk on two incubation temperature (41.05–58.35 %), while the whey loss on sheep's milk result to be lower (14.01–37.61%). Calculated values of velocity constant k of curd formed from sheep, cow and goat milk, fits to logarithmic functions with a correlation coefficients

R^2=97.6%-99.7%. For the same breed of sheep, values of velocity constant k result to be 2 times higher on incubation temperature 30°C that means that drainage rate diminished 2 times when incubation temperature is pulling down from 30°C to 25°C. Drainage rate of the curd formed from milk of cows breed (KE, Laramane and Xhersej) shown variance only for incubation temperature 30oC, where calculated values of velocity constant k result to be significantly different. Milk from Xhersej cow breeds show higher drainage rate, in spite of curd incubation temperature Changes on drainage rate results to be higher for milks from goats breed Red Alpina compared to White SonakRA>kWS.

5. References

1. Calvo M.M. and Balcones E: **Some Factors Influencing the Syneresis of Bovine, Ovine, and Caprine Milks**. J.DairySci 2000, 83: 1733–1739.
2. Johnson M.E, Chen C. M and Jaeggi J. J: **Effect of rennet coagulation time on composition, yield, and quality of reduced fat cheddar cheese**. J.Dairy Sci. 2001, 84: 1027–1033.
3. Marshall R: **An improved method for measurement of the syneresis of curd formed by rennet action on milk**. J.Dairy Res 1982. 49: 329–336.
4. McMahon D.J, Brown R.J, Richardson G.H and Ernstrom C.A: **Effects of calcium, phosphate and bulk culture media on milk coagulation properties.** J.DairySci 1984, 67: 930–938.
5. Lucey J.A and Fox P.F: **Importance of calcium and phosphate in cheese manufacture.** A review. J.Dairy Sci. 1993, 79: 1714–1724.
6. Lucey J.A: **Formation and physical properties of milk protein gels**. J. Dairy Sci. 2002, 85: 281–294.
7. Mishra R, Govindasamy-Lucey S and Lucey J.A: **Rheological properties of rennet-induced gels during the coagulation and cutting process: Impact of processing conditions**. J.Texture Stud. 2005, 36: 190–212.
8. Lawrence A.J: **Syneresis of rennet curd. Part 1. Effect of time and temperature**. Aust. J. Dairy Technol. 1959, 14: 166–168.
9. Martins A.P, Belo A.T, Vasconcelos M.M., Fontes A.L., Pereira E.A., and Belo C.C, **Characterization of production system of Niza cheese (PDO): Effect of sheep breed on milk composition and coagulation properties**. Options Méditerranéennes, A. 2009, no. 91: 221-225
10. Alves S.M.P, Martins A.P.L, Mourato M.P, Vasconcelos M.M and Fontes A.M.L: **Effect of clotting agent on coagulation properties of sheep milk.** In: Proceedings of the International Symposium "The Future of the Sheep and Goat Dairy Sectors", FIL/IDF-CIHEAM, Zaragoza (Spain), 28-30 October 2004, p. 278-279
11. McMahon D.J. and Brown R.J: **Evaluation of Formagraph for comparing rennet solutions**. J.Dairy Sci. 1982, 65: 1639-1642.
12. Pellegrini O, Remeuf F and Rivemale M: **Évolution des caractéristiques physico-chimiques et des paramètres de coagulation du lait de brebis collecté dans la région de Roquefort.** Lait, 1994, 74: 425-442.
13. De Marchi M, Dal Zooto R, Cassandro M, and Bittante.G: **Milk coagulation ability of five dairy cattle breeds.** American dairy Science Association, April 15, 2007, p. 3986-3992.
14. Delacroix-Buchet A, Barilet F, and Lagrifoul G: **Caracterisation de l'aptodude fromagere des laits de brebis Lacaune a l'aide d'un Formagraph.** Lait, 1994, p. 173-186.
15. Toffanin V, De Marchi M, Penasa M, Pretto M, Cassandro M: **Characterization of milk coagulation ability in bulk milk samples.** 20th Int. Symp. "Animal Science Days", Kranjskagora, Slovenia, Sept. 19th–21st, 2012.
16. Jovanović S, Maćejl O and Denin-Djurdjević J: **Influence of selected factors on induced syneresis**. Journal of Agricultural Sciences 2004, 49/2: 205-217
17. Marshall R. J: **An improved method for measurement of syneresis of curd formed by rennet action on milk**. J.Dairy Res. 1982, 44: 329
18. Hoxha M, Zoga P and Bode A: **Material Balance Adjustment in Raw Material Processing**. International Journal of Business and Technology 2012, 1/1: 336.