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THE INFLUENCE OF VARIOUS FACTORS ON MILK CLOTTING TIME

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Abstract: The influence of pH (6.5 and 5.8), amount of added $CaCl_2$ (0, 200 and 400 mg/l)), coagulation temperature (30ºC and 35ºC) and heat treatment of milk (65ºC/30 min and 87ºC/10 min) on the rate of rennet induced milk coagulation (s) were investigated.

The time (s) from rennet addition to onset of gelation (as indicated by the first visible floccules) was measured.

The milk samples heat-treated at 87ºC/10 min, with 400 mg/l added $CaCl_2$, which were coagulated at 35ºC and pH 5.8, coagulated 23.28-fold faster than the same samples without added $CaCl_2$, which were coagulated at 30ºC and pH 6.5.

The results of investigations related to the influence of particular coagulation factors on the coagulation rate of heat-treated milk showed that at pH 6.5 the most pronounced influence was demonstrated by the amount of Ca^{2+} and temperature of coagulation. At pH 5.8, different amounts of Ca^{2+} and used temperatures of coagulation did not influence coagulation rate regardless of the used heat treatment of milk.

The influence of used heat treatment of milk was particularly pronounced during coagulation of samples without added $CaCl_2$ that coagulated at 30ºC and pH 6.5. The used heat treatment of milk practically did not influence the milk coagulation rate at pH 5.8.

The greatest influence on milk coagulation rate was showed by pH. This influence was the most marked in coagulation of samples in which the coaggregates were formed, regardless of the amount of added Ca^{2+} and used coagulation temperatures.

Key words: milk, clotting time, coagulation rate, $CaCl_2$, pH value, coagulation temperature, heat treatment.

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Introduction

Milk coagulation and formation of rennet-induced gel (coagulum) is the most important and the most sensitive process in the production of the rennet curd cheese varieties. It was principally manifested through the action of numerous factors, which, on the other hand, control both biochemical and physico-chemical processes during coagulation and directly influence rheological characteristics of rennet-induced casein gel.

Milk coagulation induced by proteolytic enzymes was the oldest technological process in the cheesemaking (Abd El-Salam et al., 1993, Dalgleish, 1979, 1986, 1990, Djordjević, 1987, Djordjević and Carić, 1970, 1974, Ernstrom and Wong, 1974, Green and Morant, 1981, Hill et al., 1974, Maćej et al., 1998, Mehaia and Cheryan, 1983, Mulvihill and Fox, 1979, Pejić, 1956, Pudja et al., 1995, Shalabi and Fox, 1982).

It is well known that milk coagulates with the extract of the stomach of young milk-fed calves, kids or lambs, which is usually called rennet.

Milk coagulation could be presented as two-stage process that involves primary phase, i.e. limited hydrolysis of κ-casein, and secondary phase when hydrolyzed casein micelles cross-link in the presence of Ca^{2+} to form gel (Dalgleish, 1983, 1986, 1993, Djordjević, 1987, Gavarić, 1988, Scott, 1986).

Numerous factors influence primary and secondary coagulation phase as well as rheological properties of formed gels. The most essential factors are: casein concentration, milk pH value, type and concentration of enzymes, concentration of Ca^{2+} and coagulation temperature (Banks and Muir, 1984, Dalgleish, 1983, 1993, Fox, 1987, Gavarić et al., 1989, Lopez et al., 1998, Pudja et al., 1996).

Nevertheless, milk coagulation rate, activity of enzymes used for coagulation in cheesemaking as well as rheological properties of rennet-induced casein gel greatly depend on previously applied heat treatment of milk (Dalgleish, 1990, Guinee, et al., 1997, Maćej et al., 1996, Marshall, 1986, Singh and Fox, 1988).

Investigations of numerous authors showed that longer milk heating above 70°C leads to the formation of chemical complex between κ-casein and whey proteins (milk proteins coaggregates) (Djordjević et al., 1987, Elfagm and Wheelock, 1978a,b, Euber and Brunner, 1982, Haque and Kinsella, 1988, Hartman and Swanson, 1965, Jang and Swaisgood, 1990, Kirchmeier et al., 1985, Long et al., 1963, Maćej, 1983, 1989, Maćej and Jovanović, 1998, McKenzie et al., 1971, Morr et al., 1962, Pudja et al., 1995, Purkayastha et al., 1967, Singh and Fox, 1987, Smits and van Brouwershaven, 1980).

It was also concluded that coaggregates formation leads to a lesser sensitivity of casein to proteolytic enzymes even if the concentration of Ca^{2+} is greater than in raw milk.

This problem requires additional investigations, which could solve numerous problems associated with coagulation of milk in which coaggregates were formed, with the aim to create optimum coagulation conditions for the use of heat treated milk in cheese production. Also, this is a way to increase cheese yield due to a greater utilization of whey proteins which are lost with whey in traditional cheese production.

The aims of this investigation were to investigate the influence of pH, amount of added $CaCl_2$, coagulation temperature and applied heat treatment on the clotting time, as well as to maintain optimum conditions for the production of gel with good rheological properties from milk in which coaggregates were formed.

Material and Method

Raw milk gained from Dairy Beograd "AD IMLEK" Padinska Skela was used for investigations. The amount of 200 ml per sample was used. The experiments were performed at the Department of Dairy Technology – Faculty of Agriculture, Belgrade.

Clotting time (s) indicated as time from rennet addition to the formation of the first visible floccules were measured visually. The influence of the following factors were investigated:

- Milk pH value (6.5 and 5.8)
- Amount of added $CaCl_2$ (0, 200 and 400 mg/l)
- Applied heat treatment (65ºC/30 min and 87ºC/10 min)
- Coagulation temperature (30ºC and 35ºC)

Milk heat treated at 65ºC/30 min was designated as *Control sample,* while milk heat treated at 87ºC/10 min was designated as *Experimental sample*.

The 10% lactic acid was used to modulate milk pH value, while 20% solution of $CaCl_2$ was used for modulation of $CaCl_2$ amount. Liquid rennet "Biopak", declared activity 1:5000, was used for coagulation.

The following analyses of raw milk were performed:

a) Determination of total solids by standard drying method at 102±2ºC, (IDF Standard 4A:1982)
b) Determination of total nitrogen matter by Kjeldahl method, (IDF standard 20A:1986)
c) Titratable acidity determination according to Soxlet-Henkel, (Carić et al., 2000)
d) Determination of pH with pH-meter Sentron 1001
e) For density determination lactodensimeter was used, (Carić et al., 2000)

All experiments were repeated 5 times.

Statistical analysis was performed. All data for the investigated parameters are shown as mean values. Also, analyses of variance for all data were performed (Standard deviation and coefficient of variation).

Results and Discussion

Results of investigation are shown in Tables 1. and 2.

The quality parameters of milk used for investigations are shown in Table 1.

Tab. 1. - Investigated milk quality parameters

Calculated parameters	Investigated parameters					
	Total solid (%)	Nitrogen (%)	Proteins (%)	pH	Titratable acidity (ºSH)	Density (ρ)
min.	11.66	0.4198	2.68	6.53	6.60	1.0300
max.	12.30	0.4977	3.17	6.68	6.80	1.0315
□ (n=5)	12.01	0.4749	3.03	6.61	6.72	1.0309
Sd	0.2318	0.0322	0.2055	0.0550	0.1095	0.0006
Cv (%)	1.93	6.78	6.78	0.83	1.63	0.05

The results gained for rennet clotting time (s) as influenced by coagulation factors (pH value, coagulation temperature, amount of added $CaCl_2$ and applied heat treatment) are shown in Table 2.

Tab. 2. - The influence of investigated factors on milk clotting time

			Amount of added $CaCl_2$ (mg/l)								
			0			200			400		
			Clotting time (s)								
	T (ºC)	pH	□ (n=5)	Sd	Cv (%)	□ (n=5)	Sd	Cv (%)	□ (n=5)	Sd	Cv (%)
Control samples	30	6.5	2393.00	278.2553	11.63	1501.80	175.1187	11.66	1094.00	123.3272	11.27
		5.8	426.00	48.3032	11.34	354.20	30.7076	8.67	314.60	30.6372	9.74
	35	6.5	2245.20	286.1709	12.74	1324.40	173.8604	13.13	927.20	87.4949	9.44
		5.8	344.40	32.0287	9.30	282.20	24.9030	8.82	238.40	19.1478	8.03
Experimental samples	30	6.5	5205.00	295.9284	5.68	2537.60	157.1096	6.19	1567.20	76.7734	4.90
		5.8	416.00	39.2428	9.43	325.80	17.4057	5.34	301.60	18.5860	6.16
	35	6.5	3599.60	609.0220	16.92	1829.00	283.7443	15.51	1142.80	167.7062	14.67
		5.8	319.80	15.6895	4.91	251.00	8.9443	3.56	223.60	16.1567	7.22

According to the results presented in Table 2. it can be seen that control samples, coagulated at 30ºC and pH 6.5, without added $CaCl_2$ had clotting time

2393 s. Control samples with added 200 and 400 mg/l of $CaCl_2$, respectively, coagulated 1.59-fold and 2.19-fold faster, which indicate that clotting times were reduced by 891.20 s and 1299 s, respectively.

Conversely, experimental samples had lengthened clotting time. Experimental samples without added $CaCl_2$ had clotting time 5205 s that was 2.17-fold longer than in control samples. It can also be concluded that addition of $CaCl_2$ decreased clotting time, but even then clotting times were longer than clotting time of control samples.

Clotting time of experimental samples with added 200 mg/l of $CaCl_2$ was 2537.60 s and was 2.05-fold shorter than for samples without added $CaCl_2$. On the other hand, clotting time was 1.69-fold and 1.06-fold longer than for control samples with added 200 mg/l of $CaCl_2$ and without added $CaCl_2$, respectively.

Clotting time of experimental samples with added 400 mg/l of $CaCl_2$ was 1567.20 s and was 2.05-fold and 1.62-fold shorter, respectively, than for samples without added $CaCl_2$ and samples with added 200 mg/l of $CaCl_2$.

It can be concluded that milk in which coaggregates were formed when 400 mg/l $CaCl_2$ was added coagulate 1.53-fold faster than control samples without added $CaCl_2$. In contrast, clotting time was 1.04-fold and 1.43-fold longer compared with control samples with added 200 and 400 mg/l of $CaCl_2$, respectively.

At the pH 6.5 clotting times of both control and experimental samples decreased by raising coagulation temperature to 35ºC.

Control samples without added $CaCl_2$ had clotting time 2245.20 s. Control samples with added 200 mg/l of $CaCl_2$ had clotting time 1324.40 s that was 1.69-fold shorter. Control samples with added 400 mg/l of $CaCl_2$ had clotting time 927.20 s which was 2.42-fold and 1.43-fold shorter, respectively, than for samples without and with added 200 mg/l of $CaCl_2$.

The influence of coagulation temperature was very pronounced, because the samples without added $CaCl_2$ that coagulated at 30ºC had 1.06-fold longer clotting time than samples that coagulated at 35ºC. Control samples with added 200 and 400 mg/l of $CaCl_2$ that coagulated at 30ºC had 1.13-fold and 1.18-fold longer clotting time, respectively, than the same samples that coagulated at 35ºC.

The increase of coagulation temperature to 35ºC influenced decreasing of clotting time of experimental samples, which was still longer than in control samples, regardless of the concentration of Ca^{2+}.

Clotting time of experimental samples without added $CaCl_2$ was 3599.60 s. Clotting time of samples with added 200 and 400 mg/l $CaCl_2$ was 1829 s and 1142.80 s, respectively, and was 1.97-fold and 3.15-fold shorter than for samples without added $CaCl_2$.

Clotting time of experimental samples without and with added 200 and 400 mg/l $CaCl_2$, respectively, was 1.60-fold, 1.38-fold and 1.23-fold longer than for control samples with the same concentration of Ca^{2+}.

Decreasing of pH value from 6.5 to 5.8 had important influence on milk clotting time. The influence of pH value and other factors on milk clotting time is shown in Fig. 1.

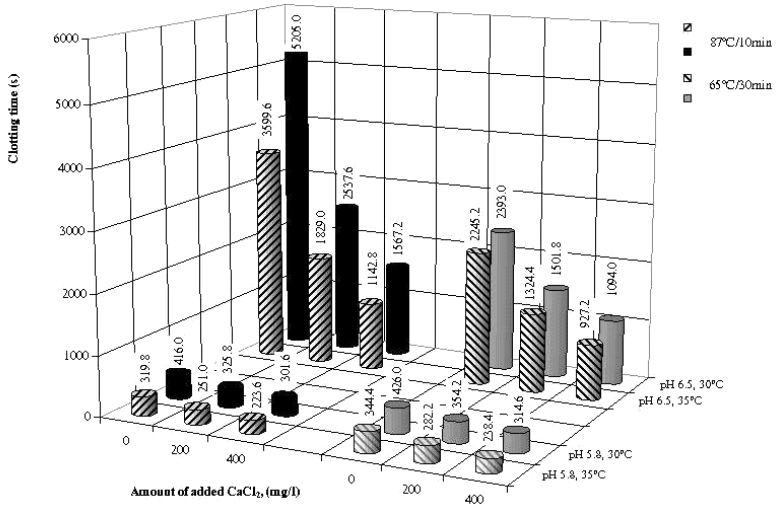

Fig. 1. - Influence of investigated factors on the rennet clotting time (s)

Clotting time of control samples without and with added 200 and 400 mg/l of $CaCl_2$ that coagulated at 30ºC was 426 s, 354.20 s and 314.60 s, respectively.

At pH 5.8 and 30ºC control samples without and with added 200 and 400 mg/l of $CaCl_2$, respectively, coagulated 5.62-fold, 4.24-fold and 3.48-fold faster than the same samples at pH 6.5.

Unexpectedly, experimental samples had shorter clotting time than control samples, regardless of the concentration of Ca^{2+}. Clotting time of experimental samples without added $CaCl_2$ was 416 s and was 1.02-fold shorter than clotting time of control samples. Samples with added 200 and 400 mg/l of $CaCl_2$ had 1.09-fold and 1.04-fold shorter clotting time.

Similar trend was observed when coagulation temperature was increased to 35ºC. Milk samples in which coaggregates were formed showed greater sensibility to rennet than control samples, apart from the amount of added $CaCl_2$.

Experimental samples without added $CaCl_2$ coagulated 1.08-fold faster than control samples, while samples with added 200 and 400 mg/l of $CaCl_2$, respectively, coagulated 1.12-fold and 1.07-fold faster than control samples with the same amount of added $CaCl_2$.

All aforementioned showed that clotting time was more or less influenced by the investigated coagulation factors. The influence of these factors was more

pronounced for samples of milk in which coaggregates were formed. This is confirmed by the fact that clotting time of milk samples heat-treated at 87ºC/10 min, with added 400 mg/l of $CaCl_2$ that coagulated at pH 5.8 and 35ºC, was 23.28-fold shorter compared with the same samples without added $CaCl_2$ that coagulated at pH 6.5 and 30ºC. Clotting time of control samples, on the other hand, with added 400 mg/l of $CaCl_2$ that coagulated at 35ºC and pH 5.8 was 10.04-fold shorter compared with control samples without added $CaCl_2$, which coagulated at 30ºC and pH 6.5.

The above discussed facts opened new questions, which could be classified as follows:

1) Which of the investigated factors have the greatest influence on clotting time;
2) To which extent is interaction among factors significant;
3) Interaction between which factors provides the most pronounced effect on clotting time;
4) How to explain lesser susceptibility of casein from heat-treated milk to rennet;
5) Is it possible to find out an optimal combination of factors to form casein gel with good rheological characteristics?

Tables 3., 4., 5., 6. and 7. give analysis of results with drawn conclusions that answer the above posed questions.

Results shown in Table 2. and Fig. 1. categorically indicate cumulative effect of pH, concentration of Ca^{2+}, coagulation temperature and applied heat treatment on clotting time.

The influence of investigated parameters on *index of milk clotting time* (IMCT) is shown in Table 3., where the longest clotting time is designated as 100%.

T a b . 3. - Index of milk clotting time as influenced by investigated coagulation factors

T (ºC)	pH	Heat treatment					
		65ºC/30 min			87ºC/10 min		
		Added $CaCl_2$ (mg/l)					
		0	200	400	0	200	400
		Index of milk clotting time (%)					
30	6.5	45.97	28.85	21.02	100.00	48.75	30.11
	5.8	8.18	6.80	6.04	7.99	6.26	5.79
35	6.5	43.13	25.44	17.81	69.16	35.14	21.96
	5.8	6.62	5.42	4.58	6.14	4.82	4.30

The longest clotting time is designated as 100%

Clotting time of samples heat-treated at 87ºC/10 min without added $CaCl_2$ that coagulated at 30ºC and pH 6.5 was designated by index 100%.

From the results shown in Table 3. we can see cumulative effect and interaction among investigated parameters on IMCT. IMCT of control samples was smaller by 54.03% than IMCT of experimental samples that coagulated under the same conditions.

The differences were less pronounced for both milk in which coaggregates were formed and control milk when concentration of Ca^{2+} was increased. IMCT of experimental samples with added 400 mg/l of $CaCl_2$ that coagulated at 30ºC and pH 6.5 was 30.11%, while control samples under the same coagulation conditions had IMCT 21.02%. Also, it is clearly evident from results that temperature increase induced IMCT decrease. Experimental samples with 400 mg/l added $CaCl_2$ that coagulated at 35ºC and pH 6.5 had IMCT 21.96%, while control samples under the same conditions had IMCT 17.81%.

As can be seen from Table 3., the decrease of pH value from 6.5 to 5.8 leads to the more pronounced decrease of IMCT. Also, experimental samples, regardless of the amount of added $CaCl_2$ and applied coagulation temperature, had smaller IMCT than control samples. It can be concluded that casein from heat-treated milk showed increased susceptibility to rennet under these coagulation conditions. At pH 5.8, the influence of Ca^{2+} and coagulation temperature on the IMCT was smaller than at pH 6.5.

Table 4. shows the influence of Ca^{2+} on IMCT.

Tab. 4. - Index of milk clotting time as influenced by concentration of Ca^{2+}

T (ºC)	pH	Heat treatment					
		65ºC/30 min			87ºC/10 min		
		Added $CaCl_2$ (mg/l)					
		0	200	400	0	200	400
		Index of milk clotting time (%)					
30	6.5	100.00	62.76	45.72	100.00	48.75	30.11
35		100.00	58.99	41.30	100.00	50.81	31.75
30	5.8	100.00	83.14	73.85	100.00	78.32	72.50
35		100.00	81.94	69.22	100.00	78.49	69.92

Samples without added $CaCl_2$ are designated as 100%

As can be seen in table 4., the influence of Ca^{2+} was more pronounced at pH 6.5 and coagulation temperature of 30ºC for milk samples in which coaggregates were formed, therefore IMCT decreased to 48.75% and 30.11%, respectively, when 200 and 400 mg/l of $CaCl_2$ was added. Also, the influence of added Ca^{2+} is evident when milk samples in which coaggregates were formed coagulated at 35ºC, so IMCT was 50.81% and 31.75%, respectively, when 200 and 400 mg/l of $CaCl_2$ was added.

The influence of Ca^{2+} was less pronounced for control samples than for experimental samples, regardless of the used coagulation temperature. Compared

with control samples without added $CaCl_2$, IMCT of control samples with 200 and 400 mg/l of added $CaCl_2$ was 62.76% and 45.72%, respectively. However, at pH 5.8, Ca^{2+} had only insignificant influence. To be precise, at pH 5.8, clotting time was the shortest, while concentration of Ca^{2+}, coagulation temperature and applied heat treatment play insignificant role. This is confirmed by the results shown in Tables 2. and 3., which show that clotting time and IMCT are manifold shorter at pH 5.8.

The influence of coagulation temperature on IMCT is shown in Table 5.

Tab. 5. - Index of milk clotting time as influenced by coagulation temperature

T (ºC)	pH	Heat treatment					
		65ºC/30 min			87ºC/10 min		
		Added $CaCl_2$ (mg/l)					
		0	200	400	0	200	400
		Index of milk clotting time (%)					
30	6.5	100.00	100.00	100.00	100.00	100.00	100.00
35		93.82	88.19	84.75	69.16	72.07	72.92
30	5.8	100.00	100.00	100.00	100.00	100.00	100.00
35		80.84	79.67	75.78	76.87	77.04	74.14

Samples that coagulated at 30ºC are designated as 100%

As can be seen from the results, at pH 6.5 the influence of coagulation temperature was noticeably less pronounced on control than on experimental samples. At 35ºC, IMCT of control samples without added $CaCl_2$ was only by 6.18% smaller than IMCT of control samples that coagulated at 30ºC. Under the same conditions, IMCT of experimental samples was even by 30.84% smaller. The influence of coagulation temperature on the IMCT of control samples increased with increase of Ca^{2+}. For example, IMCT decreased by 11.81% and 15.25%, respectively, with the addition of 200 and 400 mg/l of $CaCl_2$. The influence of coagulation temperature, on the other hand, was less obvious for samples heat-treated at 87ºC/10 min with added 400 mg/l of $CaCl_2$. Experimental samples without added $CaCl_2$ had smaller IMCT by 30.84%, while at 35ºC IMCT of experimental samples with added 200 and 400 mg/l of $CaCl_2$, respectively, was by 27.93% and 27.08% smaller. It can be concluded that coagulation temperature has insignificant influence on IMCT of control samples with added 200 and 400 mg/l of $CaCl_2$, while coagulation temperature has more pronounced influence on IMCT of milk samples heat-treated at 87ºC/10 min.

Also, the influence of coagulation temperature on control samples was increased when pH decreased from 6.5 to 5.8 in spite of added $CaCl_2$. At 35ºC, control samples without added $CaCl_2$ had smaller IMCT by 19.16% than the same samples that coagulated at 30ºC. Control samples with added 200 and 400 mg/l of $CaCl_2$ had smaller IMCT by 20.33% and 24.22%, respectively, which indicates more pronounced decrease of IMCT at pH 5.8 than at 6.5.

The influence of coagulation temperature on IMCT was greater for milk samples in which coaggregates were formed, while different concentrations of Ca^{2+} practically did not have any influence on IMCT.

The results show that for milk samples heat-treated at 87ºC/10 min, the influence of coagulation temperature was less pronounced at pH 5.8 than at pH 6.5.

The results in Table 6. show the influence of heat treatment on IMCT.

T a b . 6. - Index of milk clotting time as influenced by heat treatment of milk

T (ºC)	pH	Heat treatment					
		65ºC/30 min			87ºC/10 min		
		Added $CaCl_2$ (mg/l)					
		0	200	400	0	200	400
		Index of milk clotting time (%)					
30	6.5	45.97	59.18	69.81	100.00	100.00	100.00
35		62.37	72.41	81.13	100.00	100.00	100.00
30	5.8	102.40	108.72	104.31	100.00	100.00	100.00
35		107.69	112.43	106.62	100.00	100.00	100.00

Milk samples heat treated at 87ºC/10 min are designated as 100%

The results demonstrate that at pH 6.5, IMCT was greatly influenced by the applied heat treatment. IMCT of control samples without added $CaCl_2$ that coagulated at 30ºC was by 54.03% smaller than that of experimental samples under the same conditions. The influence of heat treatment was less pronounced when 200 and 400 mg/l of $CaCl_2$ was added, although differences were still significant, so IMCT of control samples was by 40.82% and 30.19% smaller.

The differences among IMCT of control and experimental samples were significantly reduced when coagulation temperature was increased from 30ºC to 35ºC. Control samples without added $CaCl_2$ had smaller IMCT by 37.63% than experimental samples under the same coagulation conditions. Smaller differences are (27.59% and 18.87%, respectively) when 200 and 400 mg/l of $CaCl_2$ was added.

The pH reduction from 6.5 to 5.8 did not influence IMCT of control and experimental samples at 30º. The difference between IMCT of control and experimental samples was 2.40%, 8.72% and 4.31%, respectively, for samples without and with added 200 and 400 mg/l of $CaCl_2$. Under these conditions, heat-treated milk samples had smaller IMCT than pasteurized samples. The differences are greater when coagulation temperature was increased from 30ºC to 35ºC.

All aforementioned indicate that under these coagulation conditions susceptibility of casein from heat-treated milk to rennet was regenerated.

Table 7. and Figures 2a. and 2b. show the influence of pH on the alteration of IMCT.

The results show that decrease of pH from 6.5 to 5.8 leads to manifold reduction of IMCT, but this influence is more significant for the heat-treated milk samples.

Tab. 7. - Index of milk clotting time as influenced by pH

T (ºC)	pH	Heat treatment					
		65ºC/30 min			87ºC/10 min		
		Added $CaCl_2$ (mg/l)					
		0	200	400	0	200	400
		Index of milk clotting time (%)					
30	6.5	100.00	100.00	100.00	100.00	100.00	100.00
	5.8	17.80	23.58	28.76	7.99	12.84	19.24
35	6.5	100.00	100.00	100.00	100.00	100.00	100.00
	5.8	15.34	21.31	25.71	8.88	13.72	19.56

Samples that coagulated at pH 6.5 are designated as 100%

At pH 5.8 control samples without added $CaCl_2$ that coagulated at 30ºC had smaller IMCT by 82.20% than at 6.5. The reduction of IMCT was 76.42% and 71.24%, respectively, for control samples with added 200 and 400 mg/l of $CaCl_2$ (Fig. 2a).

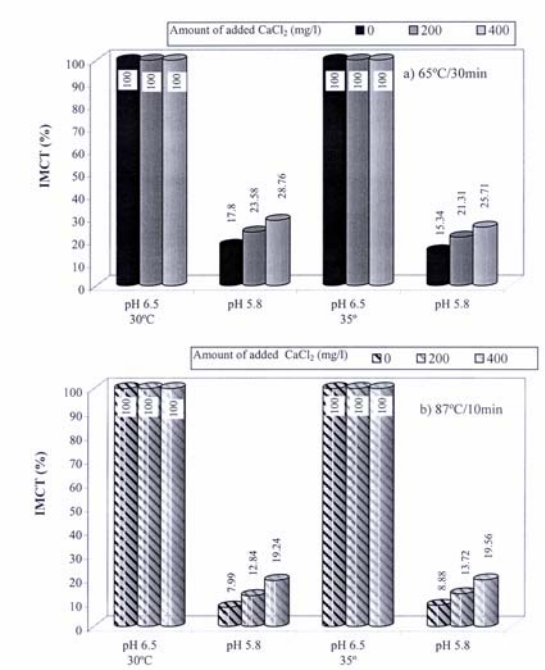

Fig. 2. – Index of milk clotting time (IMCT) as influenced by pH value

The influence of pH on IMCT of samples in which coaggregates were formed is more pronounced as Fig. 2b. shows. At pH 5.8, IMCT of experimental samples

without added $CaCl_2$ that coagulated at 30ºC was by 92.01% smaller than at 6.5. The reduction of IMCT was 87.16% and 80.76%, respectively, when 200 and 400 mg/l of $CaCl_2$ was added. However, when milk samples coagulated at 35ºC, decrease of pH from 6.5 to 5.8 induced faster coagulation, i.e. IMCT of control samples decreased in spite of Ca^{2+} concentration.

According to the less pronounced differences of experimental samples, it can be concluded that higher coagulation temperature did not influence the change of IMCT.

The results of our investigation agree well with the results of other authors. Guinee et al., (1997) assumed that milk heat treatment impaired rennet coagulation properties of milk, increased clotting time and decreased curd-firming rate. Maćej (1989) concluded that heat treatment at 87ºC/10 min multiplies coagulation time. Maćej et al., (1997) investigated the influence of heat treatment on the milk coagulation rate and detected lesser sensitivity of casein to rennet even when 200 and 400 mg/l $CaCl_2$ was added to heat-treated milk, which, on the other hand, increased Ca^{2+} concentration to the level greater than in raw milk. When milk heat-treated at 95ºC/20 min and raw milk are mixed in the ratio of 1:1, susceptibility of casein to rennet was regenerated and clotting time of this mixture was the same as clotting time of milk pasteurized at 65ºC/30 min.

Marshall (1986) and Singh and Fox (1988) considered that severe heat treatments of milk have more influence on the secondary than on the primary phase of coagulation. In contrast, Fox (1986) assumed that both the primary and secondary phases of coagulation are retarded in the milk heat-treated to the temperatures that influence whey protein denaturation and coaggregate formation via thiol-disulphide interchange, which makes Phe-Met bond (rennet-susceptible bond) less susceptible to rennet. The same opinion has Dalgleish (1990) who found out a linear increase of milk clotting time with heating of milk to 85ºC and 90ºC.

Maćej et al., (1989) investigated the influence of various factors on milk coagulation rate at different coagulation temperatures and concluded that pH had the greatest influence on the coagulation rate and after that concentration of Ca^{2+}, while coagulation temperature had the smallest influence. Pudja (1992) and Guinee et al. (1992) found that protein concentration and pH had the greatest influence on the coagulation rate of milk in which coaggregates were formed. Pudja (1992) found that milk heat-treated at 100ºC/2 min did not coagulate during 2 hours, but when UF milk (concentrated to the level 5) was added to increase protein concentration, coagulation completed in 20 min, which agree well with the clotting time of a control sample.

According to van Hooydonk et al. (1987) lowering of pH of heat-treated milk to 6.0 decreased clotting time. By the addition of 4mM $CaCl_2$ at constant pH value, clotting time of heat-treated milk was decreased but coagulation rate of untreated milk is unattainable. Bringe and Kinsella (1986) regarded

concentration of Ca^{2+} as a significant factor of coagulation rate and found out that coagulation begins at a lower level of κ-casein hydrolysis (30% instead of 90%) if concentration of Ca^{2+} was increased from 3 mM to 60 mM. Singh et al. (1988) concluded that heat-treated milk, after acidification and subsequent neutralization to pH 6.6, had shorter clotting time than untreated milk.

Conclusion

According to the aforementioned, it could be concluded that:

Cumulative effects of investigated parameters had the greatest influence on milk clotting time. Experimental samples with added 400 mg/l of $CaCl_2$ that coagulated at 35ºC and pH 5.8 had 23.28-fold shorter clotting time or had lower index of milk clotting time by 95.70% than the same samples without added $CaCl_2$ that coagulated at 30ºC and pH 6.5. This indicates a significant cumulative interaction among investigated parameters.

The results of investigations related to the influence of tested factors on clotting time showed that at pH 6.5 both Ca^{2+} and coagulation temperature had particular influence on clotting time of milk in which coaggregates were formed. At pH 5.8 different concentrations of Ca^{2+} and applied coagulation temperatures did not have great influence on the reduction of IMCT, regardless of the used heat treatment of milk.

The influence of applied heat treatment of milk was particularly marked when samples without added $CaCl_2$ coagulated at 30ºC and pH 6.5. The variations were lesser, but still obvious, when concentration of Ca^{2+} and coagulation temperature were increased. However, at pH 5.8, applied heat treatment did not have any influence on IMCT. According to this fact, it could be concluded that casein became more sensitive to rennet under these coagulation conditions.

Finally, it could be concluded that pH had the greatest influence on milk clotting time, particularly on clotting time of milk in which coaggregates were formed at each concentration of Ca^{2+} and applied coagulation temperature.

REFERENCES

1. Abd El-Salam, M.H., Alichanidis, E., Zerfiridis, G.K. (1993): Domiati and Feta Type cheeses in Cheese: chemistry, physics and microbiology. Volume 2. Major cheese groups. Second edition. Chapter 11, 301-355. Ed. by Fox, P. F., Chapman &Hall, London and New York.
2. Banks, J.M., Muir, D.D. (1984): Coagulum strength and cheese yield. Dairy Ind. Internat. 49 (9), 17-19, cont. on pages 21, 36.
3. Bringe, N.A., Kinsella, J.E. (1986): Influence of calcium chloride on chymosin- initiated coagulation of casein micelles. J. Dairy Res. 53 (3), 371-379.
4. Carić, M., Milanović, S., Vucelja, D. (2000): Standardne metode analize mleka i mlečnih proizvoda. Prometej, Novi Sad.

5. Dalgleish, D.G. (1979): Proteolysis and aggregation of casein micelles treated with immobilized or soluble chymosin. J. Dairy Res. 46 (4), 653-661.
6. Dalgleish, D.G. (1983): Coagulation of renneted bovine casein micelles: Dependence of temperature, calcium ion concentration and ionic strength. J. Dairy Res. 50, 331-340.
7. Dalgleish, D.G. (1986):The enzymatic coagulation of milk in Developments in Dairy Chemistry-1. Chapter 5, 157-187. Ed. Fox, P. F., Elsevier Applied Science Publishers Ltd, London and New York.
8. Dalgleish, D.G. (1990): The effect of denaturation of β-lactoglobulin on renneting -a quantitative study. Milchwissenschaft 45 (8), 491-494.
9. Dalgleish, D.G. (1993): The enzymatic coagulation of milk in Cheese: chemistry, physics and microbiology. Volume 1, Chapter-3, 69-100. Edit by Fox, P. F., Chapman &Hall, London and New York.
10. Djordjević, J. (1987): Mleko. Naučna knjiga, Beograd.
11. Djordjević, J., Carić, M. (1970): Uticaj kalijumnatrijumtartarata na koagulaciju mleka himozinom. Zbornik radova Tehnološki fakultet Novi Sad, br.2, 153-161.
12. Djordjević, J., Carić, M. (1974): Koagulaciju kazeina himozinom pri raznim koncentracijma Ca^{2+} (Mg^{2+}) u model sistemima. Zbornik radova Tehnološki fakultet Novi Sad, br. 5, 43-51.
13. Djordjević, J., Maćej, O., Milčić, M. (1987): Uticaj termičke obrade na stepen iskorišćenja azotnih materija mleka. Mljekarstvo 37 (10), 305-309.
14. Elfagm, A.A., Wheelock, J.V. (1978a): Interaction of bovine α-lactalbumin and β-lactoglobulin during heating. J. Dairy Sci. 61 (1), 28-32.
15. Elfagm, A.A., Wheelock, J.V. (1978b): Heat interaction between α-lactalbumin, β-lactoglobulin and casein in bovine milk. J. Dairy Sci. 61 (2), 159-163.
16. Ernstrom, C.A., Wong, N.P. (1974): Milk clotting enzymes and cheese chemistry in Fundamentals of dairy chemistry. Chapter 12, 662-771. Ed. by Webb, B.H., Johnson, A.H. and Alford, J.A.. The AVI Publishing Co., Inc. Westport.
17. Euber, J.R., Brunner, J.R. (1982): Interaction of κ-casein with immobilized β-lactoglobulin. J. Dairy Sci. 65 (12), 2384-2387.
18. Fox, P.F. (1986): Coagulants and their action. Proceedings XXII International Dairy Congress "Milk the vital force", Hague, 61-73.
19. Fox, P.F. (1987): Cheese manufacture: chemical, biochemical and physical aspects. Dairy Ind. Internat. 52 (7), 11-13.
20. Gavarić, D. (1988): Uticaj koncentrisanja mleka ultrafiltracijom na koagulaciju proteolitičkim enzimima. Doktorska disertacija. Tehnološki fakultet, Novi Sad.
21. Gavarić, D.DJ., Carić, M., Kalab, M. (1989): Effects of protein concentration in ultrafiltration milk retentates and the type of protease used for coagulation on the microstructure of resulting gels. Food microstructure 8, 53-66.
22. Green, M.L., Morant, S.V. (1981): Mechanism of aggregation of casein micelles in rennet-treated milk. J. Dairy Res. 48 (1), 57-63.
23. Guinee, T.P., Pudja, D.P., Mulholland, E.O., Reville, W.J. (1992): Ultrafiltration in cheesemaking, 3rd Cheese Symposium. Ed. by Cogan, T.M., Moorepark, 49-59.
24. Guinee, T.P., Gorry, C.B., O'Callaghan, D.J., O'Kennedy, B.T., O'Brien, N., Fenelon, M.A. (1997): The effects of composition and some processing treatments on the rennet coagulation properties of milk. Int. J. Dairy Tech. 50 (3), 99-106.
25. Haque, Z., Kinsella, J.E. (1988): Interaction between heated κ-casein and β-lactoglobulin: predominance of hydrophobic interactions in the initial stages of complex formation. J. Dairy Res. 55 (1), 67-80.
26. Hartman, G. H. Jr., Swanson, A. M. (1965): Changes in mixtures of whey protein and κ-casein due to heat treatments. J. Dairy Sci. 48 (9), 1161-1167.

27. Hill, R.D., Lahav, E., Givol, D. (1974): A renin-sensitive bond in α_{s1}-B-casein. J. Dairy Res. 41 (1), 147-153.
28. International Dairy Federation (I D F) (1982): Cheese and processed cheese. Determination of the total solids content. IDF Standard 4A.
29. International Dairy Federation (IDF) (1986): Milk. Determination of nitrogen content (Kjeldahl method) and calculation of crude protein content. IDF Standard 20A.
30. Jang, D.H., Swaisgood, H.E. (1990): Disulfide bond formation between thermally denaturated β-lactoglobulin and κ-casein micelles. J. Dairy Sci. 73 (4), 900-904.
31. Kirchmeier, O., Kamal, N.M., Klostermeyer, H. (1985): Heat treatment of milk and development of SH-groups. II. Milchwissenschaft 40 (12), 722-723.
32. Long, J. E., van Winkle, Q., Gould, I. A. (1963): Heat-induced interaction between crude κ-casein and β- lactoglobulin. J. Dairy Sci. 46 (12), 1329-1334.
33. Lopez, M.B., Lomholt, S.B., Qvist, K.B. (1998): Rheological properties and cutting time of rennet gels. Effect of pH and enzyme concentration. Int. Dairy J. 8 (4), 289-293.
34. Maćej, O. (1983): Prilog proučavanju koprecipitata radi potpunijeg iskorišćavanja belančevina mleka. Magistarski rad, Univerzitet u Beogradu.
35. Maćej, O. (1989): Proučavanje mogućnosti izrade mekih sireva na bazi koagregata belančevina mleka. Doktorska disertacija, Univerzitet u Beogradu.
36. Maćej, O., Jovanović, S. (1998): Uticaj različitih režima termičke obrade na iskorišćenje suve materije mleka. Prehramb. Ind. Mleko i mlečni proizvodi 9 (3-4), 46-50.
37. Maćej, O., Jovanović, S.T., Mikuljanac, A.M. (1998): Enzimska koagulacija mleka. Monografija "Sirevi parenog testa", Ur: Niketić, G., Pudja, P., Milanović, S., Sekulović, N. Beograd, 63-87.
38. Maćej, O., Mikuljanac, A., Petrović, D. (1989): Uticaj nekih faktora na vreme koagulacije mleka tretiranog na visokoj temperaturi. Arhiv za polj. nauke 50, 179 (3), 251-257.
39. Maćej, O., Jovanović, S., Mikuljanac, A., Niketić, G. (1997): Uticaj različitih režima termičke obrade na aktivnost sirišnog enzima i iskorišćenje azotnih materija. Prehramb. ind. Mleko i mlečni proizvodi 8 (3-4), 35-40.
40. Maćej, O., Dozet, N., Jovanović, S., Mikuljanac, A., Niketić, G. (1996): Uticaj različitih režima termičke obrade na brzinu koagulacije mleka pomoću sirišnog enzima. V Savjetovanje hemičara i tehnologa Republike Srpske. Banja Luka, 49-50.
41. Marshall, R.J. (1986): Increasing cheese yields by high heat treatment of milk. J. Dairy Res. 53, 313-322.
42. McKenzie, G.H., Norton, R.S., Sawyer, W.H. (1971): Heat-induced interaction of β-lactoglobulin and κ-casein in bovine milk. J. Dairy Res. 38 (3), 343-351.
43. Mehaia, M.A., Cheryan, M. (1983): The secondary phase of milk coagulation. Effect of calcium, pH and temperature on clotting activity. Milchwissenschaft 38, 137-140.
44. Morr, C.V., van Winkle, Q., Gould, I.A. (1962): Application of polarization of fluorescence technique to protein studies. II. The rotatory properties of κ-casein. J. Dairy Sci. 45, 817-822.
45. Mulvihill, D.M., Fox, P.F. (1979): Proteolytic specificity of chymosin on bovine α_{s1}-casein. J. Dairy Res. 46 (4), 641-651.
46. Pejić, O. (1956): Mlekarstvo II deo. Tehnologija mlečnih proizvoda. Naučna knjiga, Beograd.
47. Pudja, P. (1992): Karakteristike tvrdih sireva izradjenih od mleka koncentrovanog ultrafiltracijom u zavisnosti od termičke obrade mleka. Doktorska disertacija, Univerzitet u Beogradu.
48. Pudja, P., Maćej, O., Dozet, N., Jovanović, S., Mikuljanac, A. (1996): Uticaj sadržaja proteina mleka na reološke karakteristike sireva. Biotehnologija u stočarstvu 12 (1-2), 37-44.

49. Pudja, P., Maćej, O., Jovanović, S., Milčić, M., Mikuljanac, A. (1995): Iskorišćenje proteina mlečnog seruma u proizvodnji sireva primenom visokih temperatura. Monografija "Osnovna istraživanja u prehrambenoj tehnologiji", Ur: Radovanović, R. M. Beograd, 192-215.
50. Purkayastha, R., Tessier, H., Rose, D. (1967): Thiol disulfide interchange in formation of β-lactoglobulin-κ-casein complex. J. Dairy Sci. 50 (5), 764-766.
51. Scott, R. (1986): Cheesemaking practice. Second edition. Elsevier Applied Science Publishers Ltd, London and New York.
52. Shalabi, S.I., Fox, P.F. (1982): Influence of pH on the rennet coagulation of milk. J. Dairy Res. 49 (1), 153-157.
53. Singh, H., Fox, P.F. (1987): Heat stability of milk: influence of modifying sulphydryl-disulphide interactions on the heat coagulation time-pH profile. J. Dairy Res. 54 (3), 347-359.
54. Singh, H., Fox, P.F. (1988): Heat-induced changes in casein. Chapter 4. IDF bulletin No. 238, 24-30.
55. Singh, H., Shalabi, S.I., Fox, P.F., Flynn, A., Barry, A. (1988): Rennet coagulation of heated milk: influence of pH adjustment before or after heating. J. Dairy Res. 55 (2), 205-215.
56. Smits, P., van Brouwershaven, J.H. (1980): Heat-induced association of β-lactoglobulin and casein micelles. J. Dairy Res. 47 (3), 313-325.
57. van Hooydonk, A.C.M., de Koster, P.G., Boerrigter, I. J. (1987): The renneting properties of heated milk. Neth. Milk Dairy J. 41 (1), 3-18.

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UTICAJ ODABRANIH FAKTORA NA BRZINU KOAGULACIJE MLEKA

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R e z i m e

U okviru ovih istraživanja ispitivana je brzina koagulacije mleka (s) u zavisnosti od pH mleka (6.5 i 5.8), količine dodatog $CaCl_2$ (0, 200 mg/l i 400 mg/l), temperature koagulacije (30ºC i 35ºC) i režima termičke obrade mleka (65ºC/30 min i 87ºC/10 min).

Kod svih ogleda odredjivano je vreme (s) od momenta dodavanja sirila do pojave prvih vidljivih pahuljica gruša.

Uzorci mleka termički tretirani na 87ºC/10 min kojima je dodato 400 mg/l $CaCl_2$, čija je temperatura koagulacije bila 35ºC, a pH 5.8 koagulisali su 23.28 puta brže u odnosu na iste uzorke kod kojih nije dodat $CaCl_2$, čiji je pH bio 6.5, a temperatura koagulacije 30ºC.

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Rezultati istraživanja koji se odnose na uticaj pojedinačnih faktora koagulacije pokazala da je uticaj Ca^{2+} s jedne strane i temperatura koagulacije s druge strane bio jače izražen kod mleka u kojem su obrazovani koagregati, pri čemu je ovaj uticaj bio naročito izražen pri pH 6.5. Pri pH 5.8 različite koncentracije Ca^{2+} i primenjene temperature koagulacije nisu imali veliki uticaj na brzinu koagulacije bez obzira na primenjeni režim termičke obrade.

Uticaj termičkog tretmana naročito je bio izražen kod uzoraka bez dodatog $CaCl_2$, čija je temperatura koagulacije bila 30ºC, a pH 6.5. Pri pH 5.8 brzina koagulacije praktično više nije zavisila od primenjenih režima termičke obrade mleka.

pH mleka imao je najveći uticaj na brzinu koagulacije mleka. Uticaj pH najviše je bio izražen kod uzoraka u kojima su prethodno obrazovani koagregati pri svim koncentracijama Ca^{2+} i primenjenih temperatura koagulacije.

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