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# INFLUENCE OF VARIOUS COAGULATION FACTORS ON CHEMICAL COMPOSITION OF SERA GAINED BY CENTRIFUGATION FROM CASEIN GEL

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**Abstract:** Technological operations applied during curd processing influence syneresis and total solids content of cheese. Syneresis is not a simple physical process representing whey segregation due to curd contractions. Numerous factors can influence the process of syneresis.

The aim of this work was to investigate the influence of various parameters (pH, quantity of $CaCl_2$ added, temperature of coagulation and heat treatment) on induced syneresis. Reconstituted instant skim milk (control samples) and reconstituted instant skim milk heated at 87ºC for 10 min (experimental samples) were coagulated at 30ºC and 35ºC, and pH of 5.8 and 6.2 with 100, 200 and 400 mg/l of $CaCl_2$ added.

According to our results, these parameters had signifficant influence on nitrogen content of serum as well as on the distribution of nitrogen matter from gel into sera. Due to the formation of coaggregates, the best rheological properties of gel were obtained for experimental samples coagulated with 400 mg/l of $CaCl_2$ added at pH 5.8 and temperature of 35ºC.

**Key words:** milk, casein gel, coagulation factors, syneresis, sera.

## Introduction

Numerous factors have influence on syneresis and macro and micro-structure of cheese. The most important are pH, temperature, curd permeability, milk composition and degree of concentration, ionic strength, $Ca^{2+}$ and other ion concentration, agitation or mixing method used, method and the degree of curd

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pulverisation during cutting, milk homogenisation, milk heating and cooling, drying of curd particles, whey separation, molding, pressing and cheese salting (Fox and Cogan, 1990, Fox and Cogan, 2000, Green, 1987, Green and Grandison, 1993, Guinee et al., 1992, Pearse and Mackinlay, 1989, Pearse et al., 1986, Pudja and Maćej, 1996, van den Bijgaart, 1989, Walstra, 1993, Zoon et al., 1988).

According to van den Bijgaart (1989), chemical composition of milk, especially milk fat content, has direct effect on syneresis. Low casein content of milk induces low hardness curd formation and an increase of syneresis. Also, milk fat and proteins disappear to the greater extent (Pearse and Mackinlay, 1989, Pudja and Maćej, 1996). Due to the change of curd structure, total solid concentration of milk significantly reduces intensity of syneresis.

This method induces the formation of curd with roughness protein matrix and facilitates milk fat crossing to whey (Green, 1987, Green and Grandison, 1993, Guinee et al., 1992). Higher content of β-casein increases syneresis velocity, while β-casein dephosphorilation has the opposite effect. Dephosphorilisation of 30% of this protein caused the total absence of coagulation (Green and Grandison, 1993, Pearse and Mackinlay, 1989, Pearse et al., 1986, Walstra, 1993).

Milk homogenisation causes the formation of curd with fine structure. Due to the casein adsorbtion, milk fat drops have pseudo-protein nature and induce slower whey separation (Dozet et al., 1972, Green and Grandison, 1993, Green et al., 1983, Pearse and Mackinlay, 1989).

During syneresis, whey passes through the protein matrix, which can be explained by the law of Darcy. Thus Walstra (1993) reported that the gel permeability has significant influence on the intensity of syneresis. The permeability increases during coagulation. This parameter depends on several factors, such as the degree of crosslinking of curd, casein concentration and temperature. An increase of pH and concentration of casein decreases permeability and syneresis, while the increase of temperature has opposite effect (Green, 1987, Pearse and Mackinlay, 1989, Walstra, 1993, Zoon et al., 1988).

Available literature contains different data about effect of $CaCl_2$ on syneresis. Most authors suggest that the addition of low quantity of $CaCl_2$ (up to 10 mM) increases syneresis, while the others suggest that the influece of this parameter is low (Walstra, 1993). Van der Waarden (according to Walstra, 1993) considered that due to the decrease of pH, the addition of $CaCl_2$ increases the intensity of syneresis. However, if the pH value is kept constant, the addition of $CaCl_2$ will reduce syneresis. In contrast, the addition of $MgCl_2$ significantly increases syneresis (Walstra,1993).

Regarding the fact that syneresis significantly depends on β–casein content of micella, milk cooling treatment indirectly reduces syneresis of curd. To eliminate the effect of cooling, Pearse and Mackinlay (1989) suggested that milk had to be termostated for 30 minutes at 60ºC.

Heat treatments usually used in cheese making have low influence on curd syneresis. However, higher heat treatments of milk cause coaggregate formation and induce low hardness and more cross-linked curd formation. This curd is characterised by low capability of contraction and slower whey separation (Green and Grandison, 1993, Pearse and Mackinlay, 1989, Pearse et al., 1985, Pearse et al., 1986, Walstra, 1993).

At lower pH, the intensity of syneresis increases due to a higher capability of contraction (Green, 1987, Walstra, 1993). Lower pH affects residual quantity of rennet and Ca- ions. Thus, lower pH values influence structure of cheese. At lower pH, the dissociation of CCP-a is significantly higher as well as Ca-content of whey.

## Material and Method

In this work the effect of pH, $CaCl_2$, temperature of milk coagulation and heat treatment on induced syneresis was investigated. Reconstituted instant skim milk powder was used. To avoid the effect of variation of raw milk chemical composition, this milk is usually used in standard procedure for rennet activity determination. Total solid of reconstituted skim milk powder was adjusted to 9%.

Reconstituted skim milk was marked as Control sample, while reconstituted skim milk treated at 87ºC for 10 minutes was Experimental sample.

We used the following coagulation conditions:

- coagulation temperature (30ºC and 35ºC);
- milk pH value (6.2 and 5.8);
- amount of added $CaCl_2$ (100, 200 and 400 mg/l).

Lactic acid solution (10%) was used to adjust pH, while the level of $CaCl_2$ was adjusted with 20 % solution of this compound.

Liquid rennet from »Biopak» was used for coagulation. Rennet activity was 1: 5000. After gel formation samples were centrifuged for 5 min at 3000 rpm (*LC-320 Techtnica*, Slovenia). The following analyses of separated serum were performed:

-total solid content (%) by standard drying method at 102±1ºC (Carić et al.,2000)
-total nitrogen content (%) according to the method of Kjeldahl (Carić et al., 2000).
-total nitrogen content in total solid by calculating method;
-distribution of nitrogen matter from gel to sera by calculating method.

All experiments were repeated 6 times. Statistical analysis was performed. All data for the investigated parameters are shown as mean values. Also, the analyses of variance for all data were performed-standard deviation and coefficient of variation (Stanković et al., 1989).

## Results and Discussion

### Total solid content of sera

The results of these investigations are shown in Table 1.

From the results presented in Table 1. it can be seen that the total solid content of serum separated after centrifugation at 3.000 rpm was similar, regardless of the coagulation conditions. Total solids were in the range of 6.56% (control samples coagulated at pH 6.2, temperature 35ºC and with added 100 mg/l of $CaCl_2$) to 6.28% (control samples coagulated at pH 6.2, temperature 35ºC and with added 400 mg/l $CaCl_2$). Also, the coefficient of variation was low. This indicates that the results obtained from the series varied in low range. According to these results, it can be concluded that gels obtained from both types of samples under experimental conditions were completely formed.

### Nitrogen content of sera

The effect of different conditions of coagulation on nitrogen content of milk serum is shown in Table 2. and Fig. 1.

From data shown in Table 2. and Fig. 1 it can be seen under the same coagulation conditions, serum of all control samples, except in two cases, had higher nitrogen content than experimental samples. In the first case, this is obtained for the samples coagulated at 30ºC, pH 5.8 and with added 200 mg/l $CaCl_2$. The difference between these samples was 0.0015%. More exactly, if nitrogen content of experimental samples represents 100%, these samples will have by 1.09% higher nitrogen content than control ones. Also, in the case of the samples coagulated, in the presence of 400 mg/l $CaCl_2$, at the same temperature (30ºC) and pH 6.2., the difference between experimental and control samples was 0.0017%. Namely, serum of experimental samples had by 1.27% nitrogen than control ones.

If nitrogen content of control samples coagulated at pH 6.2, 30ºC and with added 100 mg/l $CaCl_2$ is designed as 100%, then nitrogen content of the serum of experimental samples coagulated at pH 6.2, 35ºC and with added 400 mg/l $CaCl_2$ was the minimum. Their average content was 0.1210%, and represented 81.87% of control sample marked as 100%. Slightly higher nitrogen content of the samples coagulated at pH 5.8 and at the same temperature and $CaCl_2$ content was observed. For these samples nitrogen content was 0.1240% and represents 85.63% of maximal values.

From data shown in Table 2. and Fig. 1 it can be seen that nitrogen content of control samples coagulated at 30ºC and 35ºC increased with the increase of added $CaCl_2$. Also, it is obvious that temperature caused the most intensive reduction of

T a b. 1. - Total solids content of sera obtained by gel centrifugation at centrifugal force of 3000 revolutions a minute

| Number r/min (τ=5min) | Coagulation temperature (°C) | pH value of milk | CONTROL SAMPLE Total solids (%) Amount of added $CaCl_2$ (mg/l) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 100 | | | 200 | | | 400 | | |
| | | | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) |
| 3000 r/min | 30 | 6.2 | 6.33 | 0.0873 | 1.38 | 6.50 | 0.0232 | 0.36 | 6.37 | 0.0915 | 1.44 |
| | 35 | | 6.56 | 0.0622 | 0.95 | 6.47 | 0.1042 | 1.61 | 6.29 | 0.0611 | 0.97 |
| | 30 | 5.8 | 6.49 | 0.0728 | 1.12 | 6.37 | 0.0622 | 0.97 | 6.51 | 0.0566 | 0.87 |
| | 35 | | 6.31 | 0.1402 | 2.22 | 6.38 | 0.0674 | 1.06 | 6.50 | 0.0615 | 0.95 |
| | Coagulation temperature (°C) | pH value of milk | EXPERIMENTAL SAMPLE Total solids (%) Amount of added $CaCl_2$ (mg/l) | | | | | | | | |
| | | | 100 | | | 200 | | | 400 | | |
| | | | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) |
| | 30 | 6.2 | 6.33 | 0.0789 | 1.24 | 6.37 | 0.0658 | 1.03 | 6.33 | 0.0789 | 1.24 |
| | 35 | | 6.34 | 0.0800 | 1.26 | 6.29 | 0.0186 | 0.30 | 6.28 | 0.0691 | 1.10 |
| | 30 | 5.8 | 6.45 | 0.0700 | 1.08 | 6.64 | 0.2101 | 3.16 | 6.36 | 0.0796 | 1.25 |
| | 35 | | 6.38 | 0.0711 | 1.11 | 6.42 | 0.1013 | 1.58 | 6.49 | 0.0958 | 1.48 |

Control sample- reconstituted skim milk
Experimental sample- reconstituted skim milk heat treated at 87°C/10 min.

T a b. 2. - Total nitrogen content in sera obtained by gel centrifugation at centrifugal force of 3000 revolutions a minute

| Number r/min (τ=5min) | Coagulation temperature (°C) | pH value of milk | CONTROL SAMPLE Total nitrogen (%) Amount of added $CaCl_2$ (mg/l) 100 x (n=6) | 100 Sd | 100 Cv (%) | 200 x (n=6) | 200 Sd | 200 Cv (%) | 400 x (n=6) | 400 Sd | 400 Cv (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3000 r/min | 30 | 6.2 | 0.1478 | 0.0052 | 3.51 | 0.1398 | 0.0049 | 3.51 | 0.1323 | 0.0022 | 1.63 |
| | 35 | | 0.1390 | 0.0019 | 1.36 | 0.1347 | 0.0023 | 1.74 | 0.1320 | 0.0017 | 1.27 |
| | 30 | 5.8 | 0.1448 | 0.0016 | 1.11 | 0.1365 | 0.0008 | 0.61 | 0.1382 | 0.0010 | 0.71 |
| | 35 | | 0.1397 | 0.0012 | 0.87 | 0.1345 | 0.0010 | 0.78 | 0.1372 | 0.0013 | 0.97 |
| | Coagulation temperature (°C) | pH value of milk | EXPERIMENTAL SAMPLE Total nitrogen (%) Amount of added $CaCl_2$ (mg/l) 100 x (n=6) | 100 Sd | 100 Cv (%) | 200 x (n=6) | 200 Sd | 200 Cv (%) | 400 x (n=6) | 400 Sd | 400 Cv (%) |
| | 30 | 6.2 | 0.1340 | 0.0017 | 1.25 | 0.1328 | 0.0022 | 1.68 | 0.1340 | 0.0017 | 1.25 |
| | 35 | | 0.1298 | 0.0031 | 2.41 | 0.1298 | 0.0031 | 2.41 | 0.1210 | 0.0011 | 0.90 |
| | 30 | 5.8 | 0.1322 | 0.0015 | 1.11 | 0.1380 | 0.0012 | 0.89 | 0.1255 | 0.0021 | 1.65 |
| | 35 | | 0.1253 | 0.0012 | 0.97 | 0.1255 | 0.0005 | 0.44 | 0.1240 | 0.0014 | 1.14 |

Control sample- reconstituted skim milk
Experimental sample- reconstituted skim milk heat treated at 87°C/10 min.

nitrogen content in control samples coagulated at 100 mg/l $CaCl_2$, while practically had no influence on samples coagulated at the same pH and with added 400 mg/l $CaCl_2$.

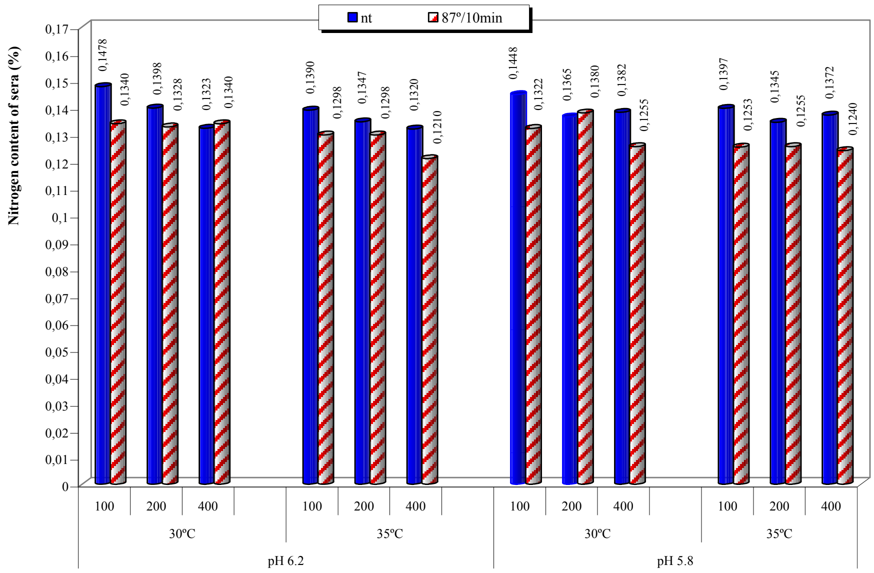


Fig. 1. – Nitrogen content of sera obtained by gel centrifugal force of 3000 revolutions a minute

The increase of added $CaCl_2$ from 100 to 200 mg/l for control samples coagulated at pH 5.8 was similar, irrespectively of the temperature used. However, serum of samples treated with added 400 mg/l of $CaCl_2$ at the temperature of 35ºC had slightly higher nitrogen content than those with added 200 mg/ml of $CaCl_2$, treated at 30ºC. This implies that these conditions, $Ca^{2+}$ concentration has no significant influence on nitrogen content. Also, it can be seen that serum of control samples treated with added 400 mg/l of $CaCl_2$ and at pH 5.8 had more nitrogen than samples treated with the same quantity of $CaCl_2$ at pH 6.2 and both temperatures. This may be explained with higher degree of contractions and higher degree of distribution of soluble nitrogen compounds from gel to serum.

Serum of the experimental samples, except for two previosly mentioned cases, had lower nitrogen content than control samples.

The reasons for this are complex:

- as a result of heat treatment (87ºC/10 min) chemical complex between casein and serum proteins is formed. Thus, opposite to control sample, it seems that nitrogen content of serum of experimental samples was mostly non-proteinaceous. This is in agreement with the data reported in literature. About 4-10 % of total milk nitrogen content is non-proteinaceous nitrogen (Alekseeva et al., 1986).

- coagulation factors, such as relatively low pH (5.8), temperature and the quantity of $CaCl_2$ added (especially 200 mg/l and 400 mg/l) represent optimal conditions for preparing gels with better rheological properties. These gels are characterized by improved water holding capacity and more cross-linked structure.

**Nitrogen content of total solids of sera**

If the results of nitrogen content are expressed relative to total solids content of sera, the situation will be something different. From this point of view, the effect of heat treatment used for coaggregates formation on the higher degree of milk proteins utilization, their higher retention in gel and their lower diffusion in sera are more clearly visible. This indirectly characterizes rheological properties of gels. The results of these investigations are presented in Table 3 and Figure 2.

According to our results, nitrogen content of total solids of serum obtained from control samples in all cases is higher related to the experimental samples. This confirms the fact that the higher content is the result of heat-induced coaggregates formation (87ºC/10 min). Serum of experimental samples coagulated at pH 5.8, at 35ºC and with added 400 mg/l of $CaCl_2$ had minimum nitrogen. Its average content was 1.91%. Coagulation at pH 6.2 and identical other conditions reduced nitrogen content to 1.93%.

If the highest content of nitrogen registered in total solids (samples coagulated at pH 6.2, 30ºC and added 100 mg/l of $CaCl_2$) is expressed as 100%, then values obtained for experimental samples coagulated at pH 5.8, 30ºC and added 400 mg/l $CaCl_2$ will be 81.62%. On the other side, if control samples coagulated at pH 5.8 and at 35ºC with added 400 mg/l $CaCl_2$ marked as maximum (100%), then experimental samples treated under the same conditions will have lower nitrogen content by 9.48%. These data confirm our previous conclusion about higher degree of utilization of proteins during experimental samples preparation, mostly due to the utilisation of serum proteins.

**Distribution of nitrogen matter into sera**

The results of these investigations are presented in Table 4 and Figure 3.

From these results, it could be seen that during centrifugation, under the same conditions, the higher quantity of nitrogen was distributed in serum of control samples (Table 2, Figure 1). From the results presented in Tab. 4. and Fig. 3. it can be seen that the lowest distribution of nitrogen matter from gel to sera was for experimental samples coagulated at pH 5.8, temperature of 30ºC and $CaCl_2$ added at concentration of 200 mg/l. The degree of distribution of nitrogen matter for experimental samples coagulated with 100 mg/l $CaCl_2$ added was 8.14%, while these values for samples treated with 200 mg/l and 400 mg/l of $CaCl_2$ added were 7.13% and 8.26%, respectively.

T a b. 3. - Total nitrogen content in total solids of sera gained by gel centrifugation at centrifugal force of 3000 revolutions a minute

| Number r/min (τ=5min) | Coagulation temperature (°C) | pH value of milk | CONTROL SAMPLE<br>Total nitrogen in total solids (%)<br>Amount of added $CaCl_2$ (mg/l)<br>100 | | | 200 | | | 400 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | $C_v$ (%) | x (n=6) | Sd | Cv (%) |
| 3000 r/min | 30 | 6.2 | 2.34 | 0.0848 | 3.63 | 2.15 | 0.0759 | 3.53 | 2.08 | 0.0609 | 2.93 |
| | 35 | | 2.12 | 0.0468 | 2.21 | 2.08 | 0.0157 | 0.75 | 2.10 | 0.0446 | 2.12 |
| | 30 | 5.8 | 2.23 | 0.0434 | 1.94 | 2.14 | 0.0279 | 1.30 | 2.12 | 0.0262 | 1.23 |
| | 35 | | 2.21 | 0.0544 | 2.46 | 2.11 | 0.0319 | 1.51 | 2.11 | 0.0158 | 0.75 |
| | Coagulation temperature (°C) | pH value of milk | EXPERIMENTAL SAMPLE<br>Total nitrogen in total solids (%)<br>Amount of added $CaCl_2$ (mg/l)<br>100 | | | 200 | | | 400 | | |
| | | | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) |
| | 30 | 6.2 | 2.11 | 0.0241 | 1.14 | 2.08 | 0.0287 | 1.38 | 2.11 | 0.0241 | 1.14 |
| | 35 | | 2.05 | 0.0617 | 3.01 | 2.06 | 0.0440 | 2.13 | 1.93 | 0.0219 | 1.14 |
| | 30 | 5.8 | 2.05 | 0.0331 | 1.61 | 2.12 | 0.0364 | 1.72 | 1.97 | 0.0377 | 1.91 |
| | 35 | | 1.96 | 0.0285 | 1.45 | 1.96 | 0.0353 | 1.80 | 1.91 | 0.0410 | 2.14 |

Control sample- reconstituted skim milk
Experimental sample- reconstituted skim milk heat treated at 87°C/10 min.

T a b 4. - Nitrogen matter distribution in sera gained by gel centrifugation at centrifugal force of 3000 revolutions a minute

| Number r/min (τ=5min) | Coagulation temperature ($^oC$) | pH value of milk | CONTROL SAMPLE Nitrogen matter distribution (%) Amount of added $CaCl_2$ (mg/l) 100 | | | 200 | | | 400 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) |
| 3000 r/min | 30 | 6.2 | 17.99 | 0.8949 | 4.97 | 15.85 | 0.3796 | 2.39 | 16.35 | 0.2319 | 1.42 |
| | 35 | | 16.71 | 0.3607 | 2.16 | 17.84 | 0.6350 | 3.56 | 16.72 | 1.4232 | 8.51 |
| | 30 | 5.8 | 17.28 | 0.7475 | 4.32 | 17.38 | 0.6030 | 3.47 | 16.06 | 0.9220 | 5.74 |
| | 35 | | 15.88 | 1.3228 | 8.33 | 17.79 | 0.1081 | 0.61 | 17.41 | 1.0904 | 6.26 |
| | Coagulation temperature ($^oC$) | pH value of milk | EXPERIMENTAL SAMPLE Nitrogen matter distribution (%) Amount of added $CaCl_2$ (mg/l) 100 | | | 200 | | | 400 | | |
| | | | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) | x (n=6) | Sd | Cv (%) |
| | 30 | 6.2 | 15.40 | 0.6885 | 4.47 | 14.01 | 1.8589 | 13.27 | 15.40 | 0.6885 | 4.47 |
| | 35 | | 15.53 | 0.6113 | 3.94 | 14.42 | 1.8419 | 12.77 | 12.43 | 0.8126 | 6.53 |
| | 30 | 5.8 | 8.14 | 2.3669 | 29.08 | 7.13 | 0.4734 | 6.63 | 8.26 | 1.9567 | 23.69 |
| | 35 | | 14.46 | 0.8528 | 5. 90 | 15.67 | 0.5916 | 3.77 | 12.79 | 1.5409 | 12.05 |

Control sample- reconstituted skim milk
Experimental sample- reconstituted skim milk heat treated at $87^oC$/10 min.

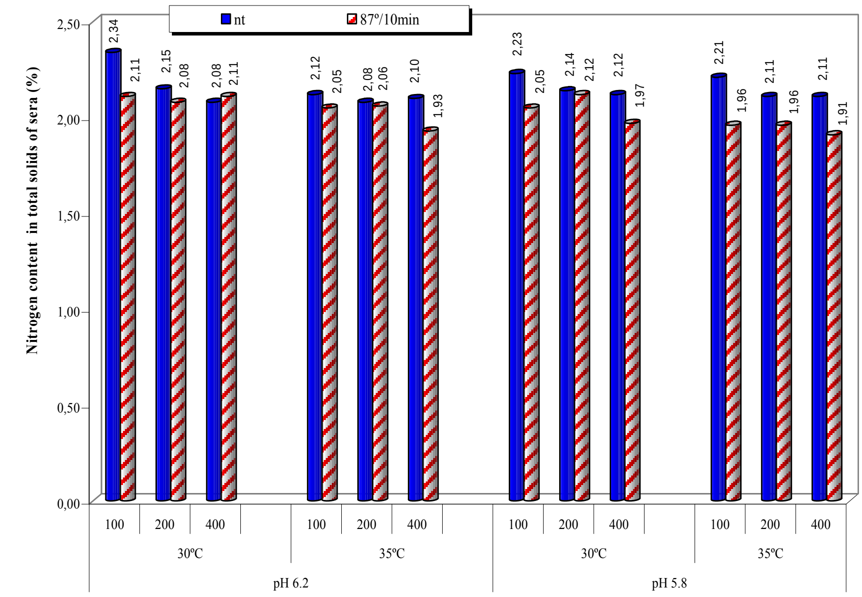


Fig. 2. – Nitrogen content in total solids of sera obtained by gel centrifugation at centrifugal force of 3000 revolutions a minute

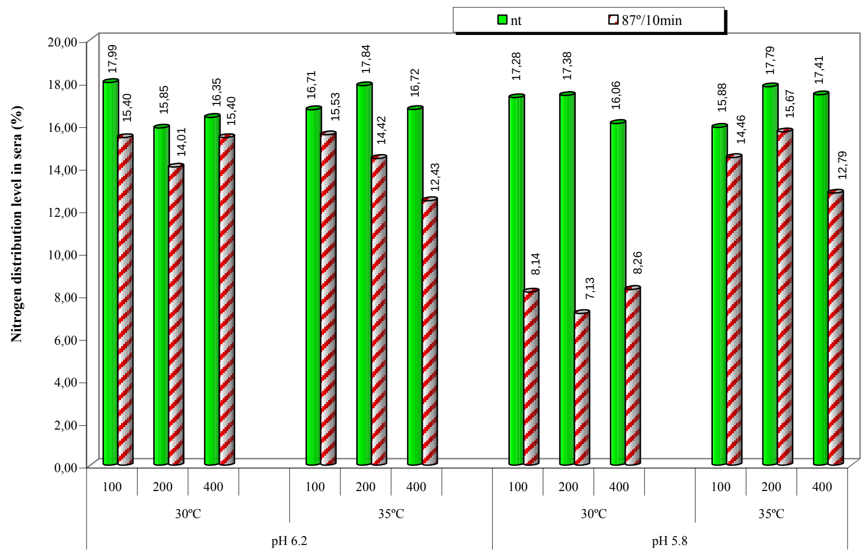


Fig. 3. – Nitrogen distribution in sera obtained by gel centrifugation at centrifugal force of 3000 revolutions a minute

## Conclusion

The investigated factors of coagulation (pH, temperature, concentration of $CaCl_2$ and thermal treatmen used) had intensive influence on serum nitrogen content of control and experimental samples, the degree of nitrogen distribution from gel to sera.

Gel of experimental samples obtained at pH 5.8, 35ºC with added 400 mg/l of $CaCl_2$ had best rheological properties.

Based on the results of nitrogen content in serum and in total solids, it could be concluded that gels formed under coaggregate formation conditions had better rheological properties with fine cross/linked structure. Under the same coagulation conditions, during centrifugation more nitrogen proceeds to serum of control than experimental samples.

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## UTICAJ ODABRANIH FAKTORA KOAGULACIJE NA HEMIJSKI SASTAV SERUMA DOBIJENOG CENTRIFUGIRANJEM SLATKOG KAZEINSKOG GELA

**Snežana Jovanović,[1] O, Maćej[1] i M. Barać[1]**

R e z i m e

Tehnološke operacije koje se primenjuju za vreme obrade gruša, utiču na brzinu sinerezisa i na sadržaj suve materije sira. Sinerezis predstavlja izdvajanje surutke usled kontrakcija gruša i ne predstavlja jednostavan fizički proces. Na brzinu izdvajanja surutke odnosno sinerezis utiče veći broj faktora.

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U ovom radu je ispitivan uticaj različitih faktora: primenjenog termičkog tretmana mleka, pH, količine dodatog $CaCl_2$ i tempearature koagulacije na količinu izdvojenog seruma odnosno sinerezis. Rekonstituisano obrano mleko (kontrolni uzorak) i rekonstituisano obrano mleko termički tretirano na 87ºC/10 minuta (ogledni uzorak) je koagulisalo pri različitim temperaturama 30ºC i 35ºC, pH vrednostima 5.8 i 6.2, kao i pri dodatku 100, 200 i 400 mg/l $CaCl_2$.

Na osnovu dobijenih rezultata može se zaključiti da ispitivani faktori koagulacije utiču na sadržaj azotnih materija u izdvojenom serumu. Kod oglednog uzorka u kojem je došlo do obrazovanja koagregata proteina mleka najbolje reološke osobine slatkog kazeinskog gela su dobijene pri sledećim uslovima koagulacije: 35ºC, pH 5.8 i dodatku 400 mg/l $CaCl_2$.

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