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# THE INFLUENCE OF DIFFERENT HEAT TREATMENTS ON THE CONTENT AND DISTRIBUTION OF NITROGEN MATTER FROM MILK TO SERA OBTAINED BY ACID COAGULATION OF MILK WITH GLUCONO-δ-LACTONE (GDL)

**S. Fetahagić, O. Maćej, Jelena Denin Djurdjević and Snežana Jovanović***

**Abstract:** Skim milk with 9.15% DM was heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min, respectively. Untreated milk was used as control. Milk samples were coagulated by glucono-δ-lactone (GDL) at the temperature of 45°C until pH 4.60 was reached. Untreated milk had the longest time of acidification.

Nitrogen matter content in sera obtained from untreated milk was 79.40 mg%. Sera samples obtained from milk heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min had 47.80 mg%, 45.00 mg% and 43.50 mg% of nitrogen matter, respectively.

Distribution of nitrogen matter from untreated milk to milk sera was 12.70%, while distribution of nitrogen matter from milk heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min to sera samples, were 6.95%, 6.57% and 6.22%, respectively.

**Key words:** acid coagulation, GDL, nitrogen matter, distribution, heat treatment.

* Safet Fetahagić, B.Sc., PKB IMLEK, Zrenjaninski put bb, 11213 Padinska Skela, FR Yugoslavia

Dr Ognjen Maćej, Professor, Jelena Denin Djurdjević, B.Sc., Postgraduate student, and Dr Snežana Jovanović, Assistant, Department of Food Technology and Biochemistry, Faculty of Agriculture, Nemanjina 6, 11081 Belgrade-Zemun, FR Yugoslavia

## Introduction

Significant changes occur in milk components when milk has been subjected to heat treatment. The most important changes occur in proteins, because their functional properties are altered. Numerous investigations show that chemical complexes between casein and whey proteins are formed during heat treatment of milk. In literature these complexes are known as coaggregates of milk proteins. A prerequisite for the formation of coaggregates is heating of milk to the temperature more than 80°C. Numerous complexes could be formed during heat treatment of milk, e.g. a complex between α-lactalbumin and ß-lactoglobulin; α-lactalbumin and æ-casein; ß-lactoglobulin and æ-casein; as well as a complex between α-lactalbumin, ß-lactoglobulin and æ-caseins. The most recent are known as milk protein coaggregates (Elfagm and Wheelock, 1977, 1978a, 1978b, Havea et al., 1998, Lyster, 1970, Maćej, 1983, 1989, Maćej and Jovanović, 2000, Maćej et al., 2000a, 2000b, Melo and Hanse, 1978).

Heating of globular proteins leads to unfolding of polypeptide chains and exposition of –SH groups which are buried (hidden) in proteins interior. Not only disulphide interchange, but also calcium bridges and hydrophobic aggregation participate in protein-protein interaction (Paulsson and Dejmek, 1990). The formation of complex occurs due to sulphydryl-disulphide interchange between free thiol group of ß-lactoglobulin and disulphide links of α-lactalbumin (Elfagm and Wheelock, 1977, 1978a, 1978b, Havea et al., 1998, Lyster, 1970, Melo and Hanse, 1978), but other proteins that possess disulphide links could participate in the formation of those aggregates (Lyster, 1970). Elfagm and Wheelock, 1978a, 1978b, assumed that primary aggregation of ß-lactoglobulin is the first step in the formation of aggregates. Newly formed and exposed –SH groups, located on protein surface, became enough reactive to interact with æ-casein and formed a new protein complex, namely milk protein coaggregates (Long et al., 1963, Maćej, 1983, 1989, McKenzie et al., 1971, Singh and Fox, 1987). Based on the experiment with whole milk, Paulsson and Dejmek, 1990, and Shalabi and Wheelock, 1976, concluded that the heat induced changes on æ-casein occur due to interaction of disulphide links of æ-casein and sulphydryl groups of other proteins (e.g. whey proteins). The principal interaction mechanism involves hydrophobic interactions and sulphydryl-disulphide interchange. Doi et al., 1983, and Purkaystha et al., 1967, considered that the disulphide interaction is the most important chemical link involved in the reaction, while Haque and Kinsella, 1988, indicated that the hydrophobic interactions predominate in the first phase. According to Corredig and Dalgleish, 1996, the rate and the degree of whey protein denaturation, as well as interaction with casein micelle (protein-protein interaction) depend on both heat transfer conditions (processing conditions) and temperature of heat treatment. The degree of interaction between whey protein and casein micelle increases when time and temperature (from 75°C to 90°C) of heat treatment are increased. The amount of whey proteins associated with casein micelles arise to the finite, maximum value during forewarming, while higher temperature influence faster protein-protein interactions. The amount of whey proteins present in milk is another factor that influences the quantity of formed complex (Corredig and Dalgleish, 1999).

Co-precipitates could be obtained by precipitation of formed complexes. For the production of co-precipitates, different inorganic (HCl, $H_2SO_4$) and organic acid (acetic acid and lactic acid) and $CaCl_2$ could be used during acid coagulation of milk with stirring (Maćej, 1983, Maćej et al. 2000a, 2000b, 2001a). For slow acid coagulation LAB (Maćej et al. 2001) and glucono-δ-lactone (GDL) could be used. GDL is often used for the investigation of structural changes in casein micelle during acid coagulation of milk, since it slowly hydrolyzes in gluconic acid in milk (Harwalkar and Kalab, 1980, Hashizume and Sato, 1988, Lucey et al., 1998, 1999).

The aim of these investigations was to investigate influence of different milk heat treatment on the content of nitrogen matter in sera, as well as the distribution of nitrogen matter from milk to sera obtained after slow acid coagulation of milk by GDL.

## Materials and Methods

The reconstituted skim milk powder with 9.15% DM was used for this investigation.

Milk samples were heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min, while untreated milk was used as control.

The following analyses were performed in milk samples:

- Dry matter content determination - AOAC method 16.032
- Determination of fat content – standard method according to Gerber - FIL/IDF 105:1981
- Determination of total nitrogen content by Kjeldahl method - FIL/IDF 20B: 1993

Milk was coagulated by the addition of 1% GDL, at the temperature of 45°C. Acidification was stopped when pH 4.60 was reached. Gained gel was cut into small cubes to simplify whey (sera) separation. Coagula were self-pressed during 10 min, and whey was collected.

The following analyses were performed in milk sera samples gained after self-pressing of coagula:

- Dry matter content determination - AOAC method 16.032
- Determination of total nitrogen content by Kjeldahl method - FIL/IDF 20B: 1993
- Distribution of nitrogen matter from milk to sera and coagula was calculated

All experiments were repeated five times. Statistical analysis was performed. All data for the investigated parameters are shown as mean values. Analyses of variance for all data were performed (standard deviation and coefficient of variation).

## Results and Discussion

Investigated quality parameters are shown in table 1.

Tab. 1. - Milk quality parameters

| | Investigated parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DM (%) | | | MF (%) | | | NFDM (%) | | | N % | | |
| HT | Calculated parameters | | | | | | | | | | | |
| | $\bar{x}$ | Sd | Cv | $\bar{x}$ | Sd | Cv | $\bar{x}$ | Sd | Cv | $\bar{x}$ | Sd | Cv |
| untreated | 9.15 | 0.1663 | 1.82 | 0.05 | 0.0000 | 0.00 | 9.10 | 0.1663 | 1.83 | 0.4596 | 0.0022 | 0.48 |
| 85°C/10' | 9.30 | 0.0251 | 0.27 | 0.05 | 0.0000 | 0.00 | 9.25 | 0.0251 | 0.27 | 0.4591 | 0.0075 | 1.64 |
| 90°C/10' | 9.38 | 0.0630 | 0.67 | 0.05 | 0.0000 | 0.00 | 9.33 | 0.0630 | 0.68 | 0.4553 | 0.0073 | 1.61 |
| 95°C/10' | 9.55 | 0.0660 | 0.69 | 0.05 | 0.0000 | 0.00 | 9.50 | 0.0660 | 0.69 | 0.4539 | 0.0099 | 2.19 |

DM - dry matter
MF - milk fat
NFDM - non-fat dry matter
HT - heat treatment

Results shown in table 1. indicate that dry matter content of milk increased as a result of evaporation during heat treatment. On the other hand, milk nitrogen matter content decreased as shown in table 1. and figure 1.

The less pronounced decrease of milk nitrogen matter is observed for milk sample heat treated at 85°C/10 min. Obtained results for the reduction of milk nitrogen matter during heat treatment agree well with the results of Denin Djurdjević, 2001, Jovanović, 2001, Maćej, 1983, 1989, and Maćej and Jovanović, 1998, 2000.

GDL is white, crystal substance, of sweet taste, which could be well resolved in water. GDL is frequently used for slow acidification in peace, because it slowly hydrolyzes giving gluconic acid, which decreases milk pH. There are numerous investigations related to the use of GDL in acid coagulation of milk (Guinee et al., 1993, Harwalkar and Kalab, 1980, Hashizume and Sato, 1988, Kim and Kinsella, 1989).

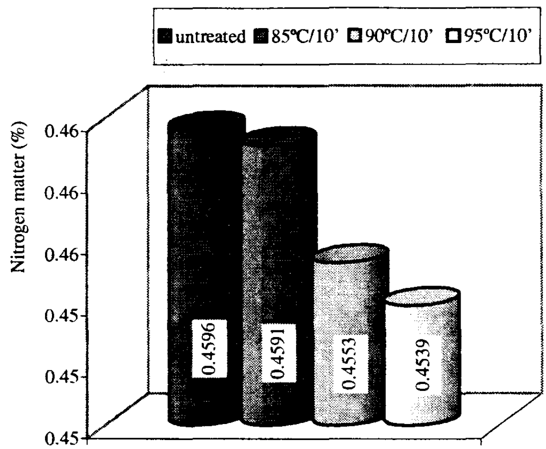


Fig. 1. - Influence of heat treatment on the change of nitrogen matter content of milk

Figure 2. shows reduction of milk pH during acidification.

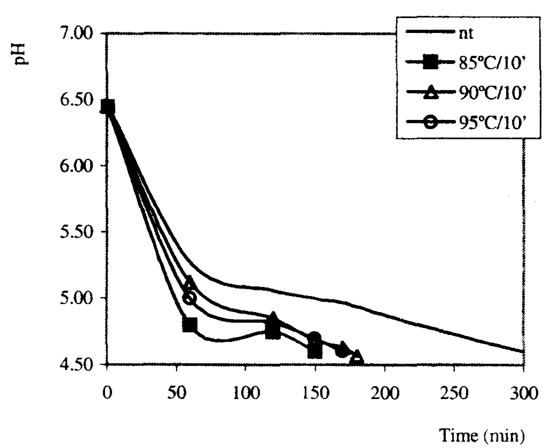


Fig. 2. - Influence of milk heat treatment on pH drop during acidification with GDL

Untreated milk samples had the longest acidification time as could be seen from fig. 1.

The results related to chemical composition of milk sera are shown in table 2.

Tab. 2. - Composition of milk sera

| | Investigated parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DM (%) | | | N (mg%) | | | | |
| HT | Calculated parameters | | | | | | | |
| | $\bar{x}$ | Sd | Cv | $\bar{x}$ | Sd | Cv | N in DM (%) | I (%) |
| untreated | 8.35 | 0.0536 | 0.64 | 79.40 | 0.0013 | 1.64 | 0.95 | 100 |
| 85°C/10' | 7.60 | 0.3932 | 5.17 | 47.80 | 0.0004 | 0.80 | 0.63 | 60.20 |
| 90°C/10' | 7.94 | 0.0870 | 1.10 | 45.00 | 0.0011 | 2.45 | 0.57 | 56.68 |
| 95°C/10' | 8.16 | 0.1424 | 1.74 | 43.50 | 0.0005 | 1.21 | 0.53 | 54.79 |

DM - dry matter
HT - heat treatment
I – index of sera nitrogen matter content; as 100% was used nitrogen matter content of sera produced from untreated milk

Milk sera obtained after self-pressing of coagula formed from milk samples was transparent and light yellow regardless of used heat treatment.

As indicated in fig. 2, dry matter content of sera gained from heat treated milk samples was smaller than dry matter content of sera obtained from untreated milk. These results agree with the results of Maćej, 1983, and Denin Djurdjević, 2001, for sera gained after acid coagulation of milk and Maćej and Jovanović, 1998, and Jovanović, 2001, for sera obtained from rennet-coagulated milk.

Nitrogen matter content of sera gained from untreated milk was the greatest (79.4 mg%). When results for nitrogen matter content in milk and sera are compared (table 1 and 2), it could be concluded that about 17.28% of nitrogen matter remained in sera after acid coagulation of milk and removing of coagula.

The results for nitrogen matter content in sera samples obtained after acid coagulation of heat treated milk are shown in figure 3.

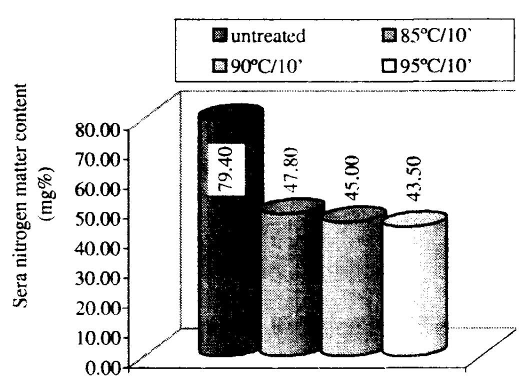


Fig. 3. - Influence of milk heat treatment on the sera nitrogen matter content

It could be assumed that the smallest amount of nitrogen matter remained in sera from milk samples heat treated at 95°C/10 min, while the greatest amount remained in sera from milk heat treated at 85°C/10 min.

Sera samples gained from milk heat treated at 85°/10 min, 90°C/10 min and 95°C/10 min, contain 10.41%, 9.88% and 9.58% nitrogen matter, respectively. Also, nitrogen matter content in sera dry matter is decreased, as could be seen from table 2. and figure 4.

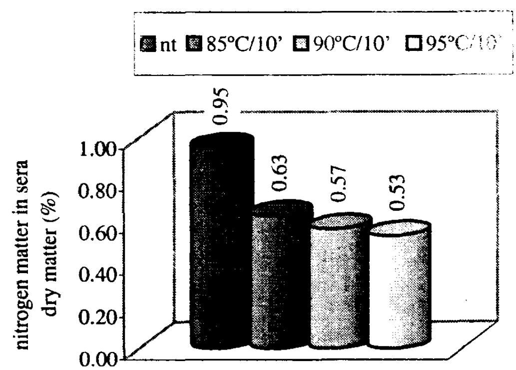


Fig. 4. - Influence of milk heat treatment on nitrogen matter content in dry matter content of sera

From aforementioned results, it could be concluded that the greatest influence on the coaggregate formation had heat treatment at 95°C/10 min, which is in accordance with the results of Corredig and Dalgleish, 1996, Corredig and Dalgleish, 1999, Maćej, 1983, and Maćej et al. 2000a. On the other hand, some investigations showed that the longer time of heating at 85°C had similar influence on the formation of coaggregates, as heating at 95°C/10 min (Corredig and Dalgleish, 1996, Denin Djurdjević, 2001, Dalgleish, 1990). Guha and Roy, 1973, showed linear relationship between undenatured whey protein content and both time and temperature of heat treatment of milk. According to Elfagm and Wheelock, 1978a, 1978b, and Mullvihill and Donovan, 1987, the rate of primary aggregates forms of ß-lactoglobulin formation, achieves maximum in the temperature range between 80-85°C, which is a prerequisite for the secondary phase of aggregation and formation of coaggregates. Corredig and Dalgleish, 1999, concluded that ß-lactoglobulin could be linked to only certain number of positions in casein micelles, because the addition of ß-lactoglobulin in milk didn't increase quantity of formed coaggregates, while the addition of α-lactalbumin increased quantity of formed complex with casein micelle.

The results for distribution of nitrogen matter from milk to sera are presented in Table 3.

T a b . 3. - The distribution of nitrogen matter from milk to milk sera and coagula

| | Investigated parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $m_{milk}$ (g) | | | $m_{sera}$ (g) | | | Distribution to coagula (%) | | | Distribution to sera (%) | | |
| HT | $\bar{x}$ | Sd | Cv | $\bar{x}$ | Sd | Cv | $\bar{x}$ | Sd | Cv | $\bar{x}$ | Sd | Cv |
| untreated | 50.08 | 0.0152 | 0.03 | 36.79 | 0.3844 | 1.04 | 87.30 | 0.3281 | 0.38 | 12.71 | 0.3281 | 2.58 |
| 85°C/10' | 50.02 | 0.0351 | 0.07 | 33.34 | 0.8883 | 2.66 | 93.05 | 0.2920 | 0.31 | 6.95 | 0.2920 | 4.20 |
| 90°C/10' | 50.05 | 0.0297 | 0.06 | 33.23 | 0.3496 | 1.05 | 93.43 | 0.2530 | 0.27 | 6.57 | 0.2530 | 3.85 |
| 95°C/10' | 50.05 | 0.0324 | 0.06 | 32.45 | 0.7474 | 2.30 | 93.78 | 0.2218 | 0.24 | 6.22 | 0.2218 | 3.57 |

HT - heat treatment

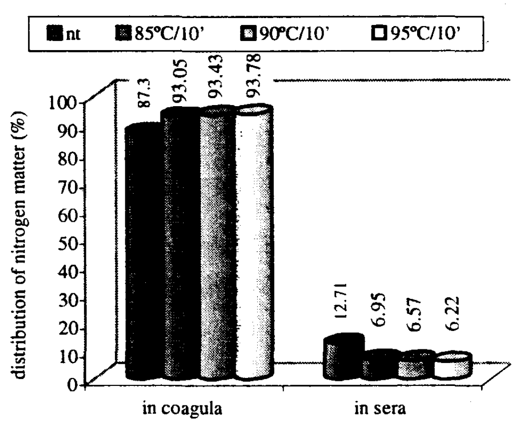


Fig. 5. - Distribution of nitrogen matter as influenced by milk heat treatment

As figure 5. shows, the smallest distribution of milk nitrogen matter into coagula was for the sample of untreated milk. The distribution of nitrogen matter from heat treated milk samples into coagula is over 93%. The greatest distribution of nitrogen matter from milk into coagula (93.78%) is noticed when milk was heat treated at 95°C/10 min.

The results gained for the distribution agree with the results of M a ć e j, 1983, M a ć e j et al., 2000a, 2000b, and D e n i n D j u r d j e v i ć, 2001.

## Conclusion

According to all aforementioned, it could be concluded:

1. Heat treatments used in these investigations had significant influence on the decrease of nitrogen matter in milk sera gained after acid coagulation of milk with GDL. Nitrogen matter content in sera obtained from untreated milk was 79.40 mg%. Sera samples obtained from milk heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min had 47.80 mg%, 45.00 mg% and 43.50 mg% of nitrogen matter, respectively.
2. Distribution of nitrogen matter from untreated milk into sera was the greatest (12.70%), while distribution of nitrogen matter from milk heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min into sera samples were 6.95%, 6.57% and 6.22%, respectively.
3. Untreated milk had the longest time of acidification with GDL.

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## UTICAJ RAZLIČITIH TERMIČKIH TRETMANA NA SADRŽAJ I DISTRIBUCIJU AZOTA IZ MLEKA U MLEČNI SERUM DOBIJEN KOAGULACIJOM MLEKA POMOĆU GLUKONO-δ-LAKTONA (GDL)

**S. Fetahagić, O. Maćej, Jelena Denin Djurdjević i Snežana Jovanović***

### Rezime

Obrano mleko sa 9.15% SM je termički tretirano na 85°C/10 min, 90°C/10 min and 95°C/10 min. Kao kontrolni uzorak korišćeno je termički netretirano mleko. Uzorci mleka su koagulisani pomoću glukono-δ-laktona (GDL) na

* Safet Fetahagić, dipl. ing., PKB IMLEK, Zrenjaninski put bb, 11213 Padinska Skela, SR Jugoslavija

Dr Ognjen Maćej, redovni profesor, Jelena Denin Djurdjević, dipl. ing., student poslediplomac, dr Snežana Jovanović, asistent, Institut za prehrambenu tehnologiju i biohemiju, Poljoprivredni fakultet, Nemanjina 6, 11081 Beograd - Zemun, SR Jugoslavija

temperaturi od 45°C do postizanja pH 4.60. Uzorci termički netretiranog mleka su imali najduže vreme acidifikacije pomoću GDL.

Kod termički netretiranog mleka sadržaj azota u serumu je u proseku iznosio 79.40 mg%. Kod uzoraka mlečnog seruma dobijenih od mleka termički tretiranih na 85°C/10 min, 90°C/10 min and 95°C/10 min, sadržaj azota je bio znatno manji i u proseku je iznosio 47.80 mg%, 45.00 mg% i 43.50 mg%, respektivno.

Distribucija azota iz mleka u mlečni serum kod uzoraka dobijenih od termički netretiranog mleka prosečno je iznosila 12.70%, a kod uzoraka dobijenih od mleka termički tretiranih na 85°C/10 min, 90°C/10 min i 95°C/10 min, iznosila je 6.95%, 6.57% i 6.22%, respektivno.

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