

THE INFLUENCE OF APPLIED HEAT TREATMENTS ON WHEY PROTEIN DENATURATION

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Abstract: Reconstituted skim milk with 8.01% DM was standardized with 3% skim milk powder and with 3% demineralized whey powder (DWP), respectively. Gained milk samples are named as 8%, 11% and 8%+3%DWP. All samples were 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.

Milk nitrogen matter content decreased during heat treatments, but linear relationship between applied heat treatments and nitrogen matter decreasing was not found. Nitrogen matter content of sera gained from both untreated and heat treated milk increased with the increase of milk dry matter content and with the addition of DWP. The higher temperature of applied heat treatment, the smaller sera nitrogen matter content. Nitrogen matter content in sera obtained from untreated milk were 64.90 mg%, 96.80 mg% and 117.3 mg% for milk 8%, 11% and 8%+3.0% DWP, respectively. Sera samples obtained from milk 8% heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min had 38.70 mg%, 38.30 mg% and 37.20 mg% of nitrogen matter, respectively. Sera samples obtained from milk 11% heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min had 55.90 mg%, 52.80 mg% and 51.30 mg% of nitrogen matter, respectively. Sera samples obtained from milk 8% heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min had 69.50 mg%, 66.20 mg% and 66.00 mg% of nitrogen matter, respectively.

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Distribution of nitrogen matter from untreated milk to milk sera were 12.01%, 11.14% and 17.69% for milk 8%, 11% and 8%+3.0% DWP, respectively. Distribution of nitrogen matter from milk 8% heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min to sera samples were 6.99%, 6.72% and 6.59%, respectively. Distribution of nitrogen matter from milk 11% heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min to sera samples, were 6.02%, 5.32% and 5.21%, respectively. Distribution of nitrogen matter from milk 8%+3%DWP heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min to sera samples were 9.64%, 8.66% and 8.67%, respectively.

Whey protein denaturation increased with increasing of the temperature of the applied heat treatment. Denaturation was the most significant for milk sample 11%.

Key words: acid coagulation, nitrogen matter distribution, GDL, heat treatment, whey protein denaturation.

Introduction

Several investigations showed that during heating of milk at temperatures above 70°C whey protein denatured. The mechanism of whey proteins denaturation involves unfolding of polypeptide chains and exposition of buried thiol groups. Newly formed thiol groups become reactive enough to react with α -casein and form complex, namely coaggregates of milk proteins via thiol/disulphide interaction and intermolecular disulphide bridges (Dalglish, 1990, Jang and Swaisgood, 1990, Maćej, 1983, 1989, Oldfield et al., 1998, Smits and van Brouwershaven, 1980, Tessier and Rose, 1964). During heating of milk at 90°C, one thiol group/mol polypeptide chain of β -lactoglobulin becomes exposed. However, at temperatures higher than 100°C thiol groups become either masked due to self-aggregation or transformed in intramolecular disulphide bridges formed by oxidation processes (Maćej, 1983, 1989, Oldfield et al., 1998). It is assumed that during heating of milk, the following complexes could be formed: complex between α -lactalbumin and β -lactoglobulin; α -lactalbumin and α -casein; β -lactoglobulin and α -casein; as well as complex between α -lactalbumin, β -lactoglobulin and α -casein (Elfagm and Wheelock, 1977, 1978a, 1978b, Hartman and Swanson, 1965, Havea et al., 1998, Lyster, 1970, Maćej, 1983, 1989, Maćej and Jovanović, 2000, Melo and Hanse, 1978, Zittle et al., 1962). Interaction occurs as a result of thiol-disulphide interchange between free thiol group of β -lactoglobulin and disulphide bridges of α -lactalbumin (Elfagm and Wheelock, 1977, 1978a, 1978b, Havea et al., 1998, Lyster, 1970, Maćej and Jovanović, 2000, Melo and Hanse, 1978, Purkaystha et al., 1967, Smits and van Brouwershaven, 1980), but thiol groups of β -lactoglobulin have higher

reactivity than disulphide bridges of α -lactalbumin (Melo and Hanse, 1978). It could be concluded that all proteins that contain cysteine disulphide bridges may participate in interaction (Corredig and Dalgleish, 1996, Jang and Swaisgood, 1990, Lyster, 1970). Except of thiol/disulphide interaction, in the formation of coaggregates protein-protein interaction participates via calcium bridges and hydrophobic reactions (Haque and Kinsella, 1988, Jang and Swaisgood, 1990, Oldfield et al., 1998, Parry, 1974, Paulsson and Dejmek, 1990, Smits and van Brouwershaven, 1980) as well as rearrangement of polypeptide chains structure (McKenzie et al., 1971). Calcium ions influence protein association through formation of calcium bridges with ionic protein groups, which results in decreasing of intermolecular repulsion and formation of intermolecular hydrophobic bonds (Smits and van Brouwershaven, 1980). Hydrophobic, noncovalent interactions have significant role at temperature of 70°C and during first few minutes of heating (Haque and Kinsella, 1988, Havea et al., 1998, Jang and Swaisgood, 1990, Smits and van Brouwershaven, 1980).

Earlier investigations showed that heat-induced complex of whey proteins exist either attached at micellar surface or inside of micelle. By comparing micellar surface in untreated and heat treated milk, it is noticeable that newes formed surface is ragged with numerous filaments that protrude to sera. According to Davies et al., 1978, casein and whey proteins do not form complex if milk has been heated to 90°C and then immediately cooled. The surface of micelle remains smooth and appears as surface of micelle in untreated milk (Davies et al., 1978, Kalab et al., 1983). The temperature and time required for exchanges to take place are identical to those required for whey protein denaturation. The filaments composed of denatured β -lactoglobulin are formed on the surface of casein micelle after heating of milk at 95°C during 10 min, or after autoclaving at 121.7°C during 15 min (Davies et al., 1978, Smits and van Brouwershaven, 1980).

The rate and degree of whey proteins denaturation and interaction with casein micelle depend on processing conditions and temperature of heat treatments (Corredig and Dalgleish, 1996). An increase of temperature or a decrease of milk pH lead to faster interaction between casein and whey proteins. At the temperature between 70-90°C, interaction kinetics of both β -lactoglobulin and α -lactalbumin with κ -casein are similar, which indicates that complex formed between β -lactoglobulin and α -lactalbumin participates in the formation of coaggregates (Corredig and Dalgleish, 1996). Degree of interaction of whey proteins and casein micelles increases with time and temperature in region between 75-90°C. The amount of whey proteins that reacts with casein micelle increases to the defined, maximal value, while higher temperatures induce faster

protein-protein interaction (Corredig and Dalgleish, 1999). According to the results of Oldfield et al., 1998, although the amount of whey protein associated with casein micelle increases during heating in region 70-140°C, the maximal level of β -lactoglobulin association attains ~ 55%. On the other hand, maximum of α -lactalbumin association with casein is ~ 55% at the temperature below 90°C and ~ 40% in temperature range 95-130°C.

The aim of this work was to investigate of the influence of milk dry matter content, added demineralized whey powder and applied heat treatments on nitrogen matter content of sera gained by acidification with GDL, distribution of nitrogen matter from milk to milk sera, as well as the degree of whey protein denaturation.

Material and Method

Skim milk powder was reconstituted to obtain milk A (with 8.01% TS). Milk A was standardized with 3% of skim milk powder and 3% of demineralized whey powder (DWP), respectively, to obtain milk B (with 11.15% TS) and milk C (with 11.10% TS).

Milk samples were heat treated at:

- a) 85°C/10 min,
- b) 90°C/10 min and
- c) 95°C/10 min.

Untreated milk was used as control.

Coagulation of milk was carried out by GDL (concentration of 1% w/w) at 45°C until pH 4.60 was reached. After coagulation, gained coagulum was cut and separated from sera by self-pressing during 10 min.

Analyses and measurements

Dry matter content determination - AOAC method 16.032

Determination of total nitrogen content by Kjeldahl method - FIL/IDF 20B: 1993

Fat content by Gerber method - FIL/IDF 105:1981

pH value by pH -meter Sentron 1001

Degree of nitrogen matter distribution from milk in milk sera and coagula was calculated on the basis of mass balance

Degree of whey protein denaturation was calculated according to loss of whey protein solubility at pH 4.60 (modified method, according to Savello and Dargan, 1997).

Results and Discussion

Quality parameters of milk and sera

Milk and sera quality parameters are shown in Table 1. and Table 2.

Tab. 1. - Milk quality parameters

Milk sample	Applied heat treatment	Quality parameters			
		DM %	MF %	N %	P%
8 %	untreated	8.01	0.05	0.4105	2.62
	85°C/10'	8.33	0.05	0.3967	2.53
	90°C/10'	8.56	0.05	0.3905	2.49
	95°C/10'	8.81	0.05	0.4021	2.57
11%	untreated	11.15	0.05	0.5605	3.58
	85°C/10'	11.34	0.05	0.5565	3.55
	90°C/10'	11.49	0.05	0.5535	3.53
	95°C/10'	11.49	0.05	0.5524	3.52
8% + 3.0%	untreated	11.10	0.05	0.4990	3.18
	85°C/10'	11.21	0.05	0.4646	2.96
	90°C/10'	11.23	0.05	0.4705	3.00
	95°C/10'	11.38	0.05	0.4703	3.00

Tab. 2. - Sera quality parameters

Milk sample	Applied heat treatment	Sera quality parameters		
		DM %	N %	N in DM %
8 %	untreated	7.06	0.0649	0.92
	85°C/10'	7.00	0.0387	0.55
	90°C/10'	6.76	0.0383	0.57
	95°C/10'	7.02	0.0372	0.53
11%	untreated	10.09	0.0968	0.96
	85°C/10'	9.94	0.0559	0.56
	90°C/10'	9.88	0.0528	0.53
	95°C/10'	9.69	0.0513	0.53
8% + 3.0%	untreated	10.56	0.1173	1.11
	85°C/10'	10.10	0.0695	0.69
	90°C/10'	9.89	0.0662	0.67
	95°C/10'	10.27	0.0660	0.64

The influence of applied heat treatment on nitrogen matter content in milk is shown in Fig. 1.

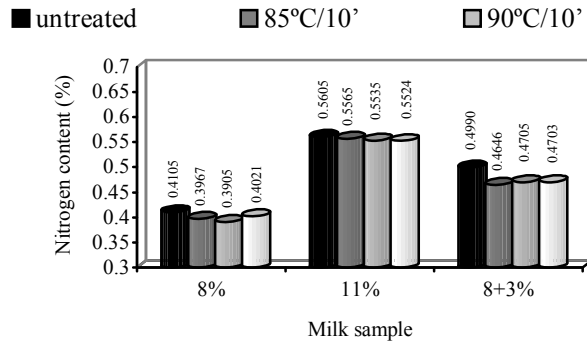


Fig. 1. - The influence of dry matter content and applied heat treatment on nitrogen content of milk

The influence of applied heat treatment on nitrogen matter content in sera is shown in Fig. 2.

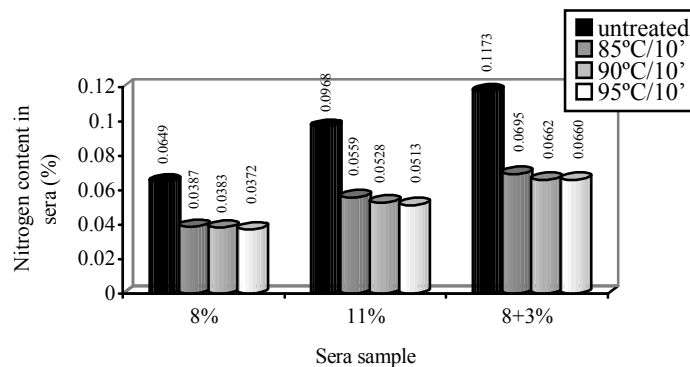


Fig. 2. - The influence of dry matter content and applied heat treatment on sera nitrogen content

As can be seen from the results shown in Table 1., nitrogen matter content increases with increase of dry matter. When skim-milk powder was used for dry matter standardization, an increase of nitrogen matter content was greater than when demineralized whey powder was used. On the other hand, as can be seen from Table 2., nitrogen matter content in sera was greater when demineralized whey powder was used for standardization. Gained results agree with those of Denin-Djurdjević, 2001, and Fetahagić et al., 2002.

The results presented in Table 1. and Fig. 1. show that during heat treatment nitrogen matter content of milk decreased, which agrees with the results of

Denin-Djurdjević, 2001, Fetahagić et al., 2001, Jovanović, 2001, Maćej, 1983, 1989, and Maćej and Jovanović, 1998.

According to the results shown in Table 2. and Fig. 3., it can be seen that nitrogen matter content in sera decreased when temperature of applied heat treatment increased. Gained results agree with the results of Corredig and Dalgleish, 1999. According to these authors, the amount of whey protein present in milk influences the amount of formed complex. The degree of interaction, besides time of heat treatment, depends on mass fraction of particular protein. Maximum value of δ ($\delta = g_{\beta\text{-lg}}/g_{\kappa\text{-casein}}$) is 2.2 during heating at 85°C/20 and 1.4 during heating at 99°C/20 min (Long et al., 1963, Maćej, 1983, 1989). However, Corredig and Dalgleish, 1999, concluded that addition of β -lactoglobulin (2 g/l) does not lead to increase of amount of formed complex between β -lactoglobulin and κ -casein during heating at 90°C/60 min. Addition of α -lactalbumin in milk in the amount of 2 g/l leads to balance of α -lactalbumin and β -lactoglobulin concentration in milk, so both proteins show similar kinetics of reaction. Also, similar amount of both proteins was found in formed complex (Corredig and Dalgleish, 1999). When 2 g/l of β -lactoglobulin was added in milk, which is later heat treated at 80°C, the amount of formed complex attained maximum value after few minutes of heating. However, the amount of formed complex did not differ considerably from milk without added β -lactoglobulin. This indicates that casein micelle has only certain number of place for interaction with β -lactoglobulin (Corredig and Dalgleish, 1999). The amount of complex formed between κ -casein and α -lactalbumin linearly increased during the first 20 minutes of heating. The amount of α -lactalbumin that interacts with κ -casein increased at higher temperatures and extended time of heating. Since processing conditions, primarily heat transfer conditions, have the influence on the amount of formed complex, the amount of formed complex was greater during indirect UHT sterilization than during direct sterilization (DSI) (Corredig and Dalgleish, 1996). According to all aforementioned, it could be concluded that the increase of temperature leads to the increase of amount of α -lactalbumin, and therefore amount of total whey proteins that reacts with casein micelle. It also explains smaller amount of nitrogen matter in sera gained from heat-treated milk.

According to the presented results, it could be concluded that heat treatment at 95°C/10 min had the greatest influence on coaggregate formation, which agrees with the results of Corredig and Dalgleish, 1996, 1999, Dalgleish, 1990, Fetahagić et al., 2001, Maćej, 1983, Maćej et al., 2000, Parnell-Clunies et al., 1988. On the other hand, several investigations showed that extended time of heating at 85°C had the same influence on coaggregate formation as heating at 90°C or 95°C/10 min (Corredig and Dalgleish, 1996, 1999, Dalgleish, 1990, Denin-Djurdjević, 2001).

The distribution of nitrogen matter from milk to sera

The distribution of nitrogen matter from milk to sera gained after acidification with GDL and separation of coagula is shown in Fig. 3.

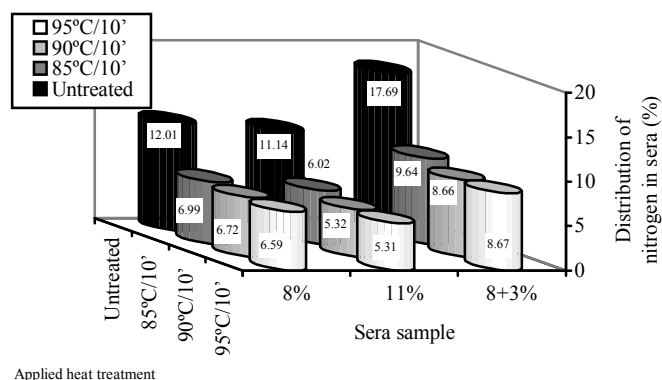


Fig. 3. - The influence of dry matter content and applied heat treatment on distribution of nitrogen matter in sera

The results shown in Fig. 3. indicate that increase of temperature from 85°C to 95°C had significant influence on whey protein denaturation and formation of coaggregates. Also, it could be concluded that increase of dry matter content had the smallest influence on nitrogen matter distribution from milk heat treated at 85°C/10 min. Distribution of nitrogen matter decreased from 6.99% (sera gained from milk with 8.01% DM) to 6.02% (sera gained from milk with 11.15% DM). On the other hand, samples heat treated at 90°C/10 min and 95°C/10 min had more manifested decrease of nitrogen matter distribution from milk to sera. Nitrogen matter distribution from milk heat treated at 90°C/10 min to sera decreased from 6.72% (sera gained from milk with 8.01% DM) to 5.32% (sera gained from milk with 11.15% DM), while nitrogen matter distribution from milk heat treated at 95°C/10 min to sera decreased from 6.59% (sera gained from milk with 8.01% DM) to 5.21% (sera gained from milk with 11.15% DM).

Gained results agree with the results of Maćej, 1983, who concluded that nitrogen matter utilization was 93.26-95.34% during co-precipitate production, and the results of Maćej et al., 2000, who reported that nitrogen matter distribution from milk heat treated at 87°C/10 min to sera gained by precipitation with HCl and lactic acid, respectively, was 6.93% and 7.28%. According to the results of Denin-Djurđević, 2001, nitrogen matter distribution from milk to sera decreased when dry matter increased.

The greatest distribution of nitrogen matter from milk to sera was observed for milk samples standardized with demineralized whey powder. Nitrogen matter

distribution from milk heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min, respectively, was 9.64%, 8.66% and 8.67%. Gained results indicate that although addition of DWP changes casein:whey protein ratio, heat treatment induces formation of the significant level of coaggregates. The greatest distribution of nitrogen matter in sera (9.64%) was from milk heat treated at 85°C/10 min, which indicates that 90.36% of nitrogen matter remained in coagula.

Gained results agree with the results of Denin-Djurdjević, 2001, and Maćej et al., 2001, so it can be concluded that DWP can be used for standardization of milk dry matter. As a result, gained product has high nutritive value due to high content of essential amino acids.

The degree of whey protein denaturation

Applied heat treatment, dry matter content and added DWP had significant influence on sera nitrogen content as well as on nitrogen matter distribution from milk to sera. For obtaining as good as possible rheological properties of acid casein gel, it is necessary to obtain appropriate degree of whey protein denaturation. According to Mottar et al., 1989, the degree of whey protein denaturation directly influences proteins water-binding capacity, their hydrophobic-hydrophilic properties at pH 4.6 and rheological properties of acid casein gel. In this part we calculated degree of whey protein denaturation as influenced by selected factors.

The degree of whey protein denaturation increases with both temperature and time of heat treatments. The degree of whey protein denaturation was calculated with modified method after Savello and Dargan, 1997, as described by Denin-Djurdjević, 2001. The method is based on difference of soluble nitrogen matter in sera gained from untreated and heat treated milk at pH 4.60. The difference was shown as a percentage of soluble nitrogen of sera gained from untreated milk, while loss of solubility at pH 4.6 was expressed as relative index of whey protein denaturation (WPD%) (Denin-Djurdjević, 2001).

The influence of investigated factors on the degree of whey protein denaturation is shown in Fig. 4.

According to gained results it can be concluded that applied heat treatments have significant influence on WPD, especially in combination with dry matter content.

Smaller degree of WPD at lower temperatures can be explained with information reported by Corredig and Dalgleish, 1996, 1999, that a longer time is needed for interaction between β -lactoglobulin and α -casein at lower temperatures (80°C) of heating, while higher temperatures induce faster protein-protein interaction.

WPD were smaller in milk samples standardized with demineralized whey powder than in samples with the same dry matter content standardized with skim-milk powder. WPD was 40.77%, 43.59% and 43.72%, respectively, when heat treatment at 85°C/10 min, 90°C/10 min and 95°C/10 min was used. According to the above results, it can be concluded that applied heat treatment (namely, temperature of heat treatment) had significant influence on WPD of milk samples standardized with demineralized whey powder (with exchanged casein: whey proteins ratio).

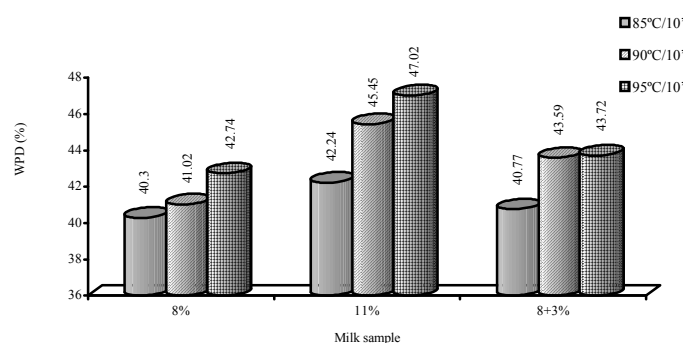


Fig. 4. - The influence of milk dry matter content and applied heat treatment on WPD (%)

Gained results agree with the results of Savello and Dargan, 1997, who concluded that the greatest WPD could be obtained with heating by VAT system. Denin-Djurdjević, 2001, concluded that addition of demineralized whey powder up to 2% did not have influence on WPD, which agrees with the results of our investigation.

Conclusion

According to all aforementioned, it could be concluded:

Nitrogen matter content of milk increases with increase of dry matter. On the other hand, milk nitrogen matter content decreased during heat treatments. Nitrogen matter content in sera decreased when temperature of applied heat treatment increased. Nitrogen matter content of sera gained from untreated milk increased with the increase of milk dry matter content and with the addition of DWP. Sera samples obtained from milk 8% heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min had 69.50 mg%, 66.20 mg% and 66.00 mg% of nitrogen matter, respectively.

Applied heat treatment had significant influence on the distribution of nitrogen matter from milk to milk sera. The distribution of nitrogen matter from milk with 8% DM and 11% DM to sera decreased when temperature of applied

heat treatment increased. On the other hand, distribution of nitrogen matter from milk 8%+3%DWP heat treated at 85°C/10 min, 90°C/10 min and 95°C/10 min to sera samples, were 9.64%, 8.66% and 8.67%, respectively.

Whey protein denaturation increased with increasing of the temperature of the applied heat treatment. The denaturation was the most significant for milk sample 11%.

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UTICAJ PRIMENJENIH TERMIČKIH TRETMANA NA DENATURACIJU SERUM PROTEINA

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R e z i m e

Obrano mleko je rekonstituisano na sadržaj suve materije 8.01%. Ovako dobijeno mleko je standardizovano dodatkom 3% SM obranog mleka u prahu i 3% demineralizovane surutke u prahu, pri čemu su dobijeni uzorci 11% SM i 8+3.0% DSUP. Svi uzorci su termički tretirani na 85°C/10 min, 90°C/10 min i 95°C/10 min. Kao kontrolni uzorci korišćena su termički netretirana mleka. Uzorci mleka su koagulirani pomoću glukono- δ -laktone (GDL) na temperaturi od 45°C do postizanja pH 4.60. Nakon koagulacije, serum je odvajan od koaguluma samopresovanjem u toku 10 minuta.

Tokom termičkih tretmana dolazi do smanjenja sadržaja azotnih materija mleka, ali nije ustanovljena linearna korelacija sa povećanjem temperature termičkog tretmana. Sadržaj azotnih materija seruma se povećavao sa povećanjem sadržaja suve materije mleka i sa dodatkom demineralizovane surutke u prahu, kako kod netretiranih, tako i kod termički tretiranih mleka. Sa povećanjem temperature termičkog tretmana, smanjenje sadržaja azotnih materija seruma je više izraženo. Kod termički netretiranih mleka sadržaj azota u serumu je u proseku iznosio 64.90 mg%, 96.80 mg% i 117.3 mg% za mleka 8%, 11% i 8%+3.0% DSUP, respektivno. Kod uzoraka mlečnog seruma dobijenih od mleka

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8% termički tretiranih na 85°C/10 min, 90°C/10 min i 95°C/10 min, sadržaj azota je u proseku iznosio 38.70 mg%, 38.30 mg% i 37.20 mg%, respektivno. Kod uzoraka mlečnog seruma dobijenih od mleka 11% termički tretiranih na 85°C/10 min, 90°C/10 min i 95°C/10 min, sadržaj azota je u proseku iznosio 55.90 mg%, 52.80 mg% i 51.30 mg%, respektivno. Kod uzoraka mlečnog seruma dobijenih od mleka 8%+3% DSUP termički tretiranih na 85°C/10 min, 90°C/10 min i 95°C/10 min, sadržaj azota je u proseku iznosio 69.50 mg%, 66.20 mg% i 66.00 mg%, respektivno.

Distribucija azota iz mleka u mlečni serum kod uzoraka dobijenih od termički netretiranih mleka prosečno je iznosila 12.01%, 11.14% i 17.69% za mleka 8%, 11% i 8%+3.0% DSUP, respektivno. Kod uzoraka dobijenih od mleka 8% termički tretiranih na 85°C/10 min, 90°C/10 min i 95°C/10 min, iznosila je 6.99%, 6.72% i 6.59%, respektivno. Kod uzoraka dobijenih od mleka 11% termički tretiranih na 85°C/10 min, 90°C/10 min i 95°C/10 min, iznosila je 6.02%, 5.32% i 5.21%, respektivno. Kod uzoraka dobijenih od mleka 8%+3% DSUP termički tretiranih na 85°C/10 min, 90°C/10 min i 95°C/10 min, iznosila je 9.64%, 8.66% i 8.67%, respektivno.

Denaturacija serum proteina se kod svih uzoraka mleka povećavala sa povećanjem temperature termičkog tretmana, a denaturacija je najizraženija kod uzoraka mleka 11%.

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