

## PROPERTIES OF CO-PRECIPIATES OBTAINED BY DIFFERENT ACIDS AND DISTRIBUTION OF MILK NITROGEN MATTER

**O. Maćej, Snežana Jovanović and Jelena Denin\***

**Abstract:** For the production of co-precipitates, skim milk (0.05 % milk fat) was used. The chemical complex between casein and whey protein was formed by heating of milk at 87 °C during 10 min, and at 95 °C during 20 min, respectively. The pasteurised milk (80-85 °C during 20 sec) was used as a probe.

Co-precipitates were obtained from heat-treated milk by coagulation with 2.8 M HCl, 1.4 M H<sub>2</sub>SO<sub>4</sub>, 2.8 M lactic and 2.8 M acetic acids, respectively. Co-precipitates obtained by acetic acid were rather dry, lax and white, while co-precipitates obtained by H<sub>2</sub>SO<sub>4</sub> and HCl, respectively, were a little sticky, compact and had pronounced yellow undertone. Co-precipitates obtained by lactic acid were compact and softer, probably due to higher moisture content (67.32%).

As a measure of recoveries of milk nitrogen matter into co-precipitates, sera nitrogen content was used. Milk sera obtained by separation of co-precipitates, had a little nitrogen matter content, regardless of the acid used (0.0440% for HCl; 0.0465% for H<sub>2</sub>SO<sub>4</sub>; 0.0500% for acetic acid and 0.0464% for lactic acid). Nitrogen matter that remained in sera represents non-protein nitrogen, which represents 4-6% of total nitrogen matter of milk. Utilisation of nitrogen matter was greater when milk was treated at 87 °C during 10 min than by pasteurisation of milk. Co-precipitates obtained from heat treated milk (87 °C; 10 min) by HCl had 11.61%. Co-precipitates obtained from pasteurised milk had 11.38% nitrogen matter. Nitrogen matter utilisation was greater when lactic acid was used (11.62%).

**Key words:** co-aggregates, co-precipitates, skim milk, nitrogen matter utilisation.

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## Introduction

When milk is subjected to high heat treatment, the complex between casein and whey proteins is formed. This complex is known as co-aggregates of milk proteins (Long et al., 1963, Shalabi and Wheelock, 1976, Elfagm and Wheelock, 1977, Elfagm and Wheelock, 1978, Mottar et al., 1989, Maćej, 1989, Doi et al., 1983, Paulson and Dejmek, 1990, Corredig and Dalgleish, 1996). According to the results of Long et al., 1963, the fastest rate of interaction between casein and whey proteins was when milk was subjected to heat treatment at 85 °C and 99 °C, respectively. The formed co-aggregates could be precipitated by the action of different organic and inorganic acids and  $\text{CaCl}_2$ , respectively, or by their combination. The formed coagulum is known as co-precipitates of milk proteins.

It has been suggested (Jovanović et al., 1997, Vukićević et al., 1998) that heat treatments used for the production of co-precipitates, influence the lactose content decreasing. According to the results of Jovanović et al. (1997), the greatest decrease of lactose content is noted when milk is subjected to heat treatment at 95 °C, while heat treatment at 87 °C induces the smallest decrease of lactose content.

The properties and composition of co-precipitates depend on method used for their production (Kožev et al., 1970, Müller, 1971, Maćej, 1983, Đorđević et al., 1987, Carić, 1990). The technological methods for production of co-precipitates with defined composition have been established.

The properties and behaviours of co-precipitates greatly differ from casein coagulum, which is the consequence of physico-chemical transition of milk proteins and formation of co-aggregates. Maćej et al. (1998) reported low solubility of co-precipitates in water and the agents which resolve acid-casein very easily. Kožev et al. (1970) reported a great influence of  $\text{Ca}^{2+}$  content on solubility of co-precipitates, and concluded that the acid co-precipitates showed the greatest solubility, especially those obtain by HCl (Maćej, 1983, Maćej et al., 1998, Maćej et al. 2000).

According to the experiments of Maćej, 1983, Đorđević et al., 1987, and Maćej et al. (1998), different organic (lactic and acetic) and inorganic (HCl and  $\text{H}_2\text{SO}_4$ ) acids could be used for the production of co-precipitates. The technological process for the production of co-precipitates doesn't differ greatly from the technological process for acid casein production. More than 95% nitrogen matter of milk (Maćej, 1983, Southward, 1978) could be recovered by the production of co-precipitates at the optimal processing condition, which is significantly greater compared with nitrogen matter recovering from pasteurised milk (83.7%).

It could be concluded that production of co-precipitates is a relatively cheap and simple manner for high utilisation of total milk proteins. The co-precipitates are widely used in food industry, because of their good technological characteristics.

The aim of these experiments was to investigate the influence of different acids on co-precipitates' properties, dry matter content, moisture content of gained

coagulum, and distribution of nitrogen matter in sera obtained by filtration of co-precipitates.

## Materials and Method

### Milk samples

For the production of co-precipitates skim milk was used (0.05% milk fat) obtained from the "Beograd" PKB - Imlek.

### Heat treatment of milk

Two different temperature-time heat treatments, 87 °C during 10 min and 95 °C during 20 min were used. Pasteurised milk was used as a probe.

### Production of co-precipitates

1) Co-precipitates were produced by acid coagulation of milk treated at 95 °C during 20 min. Two inorganic (2.8M HCl and 1.4M H<sub>2</sub>SO<sub>4</sub>) and two organic (2.8M lactic and 2.8M acetic) acids were used for coagulation. Co-precipitates were separated from sera by filtration and self-pressing during 15 min.

2) Co-precipitates were produced from milk treated at 87 °C during 10 min and pasteurised milk (80-85 °C during 20 s) by acid coagulation with 2.8M HCl and 2.8M lactic acid. The obtained co-precipitates were separated from sera by filtration and self-pressing during 15 min.

### Dry matter and nitrogen analyses

The standard drying method at 102 °C was used for dry matter content determination, IDF Standard 21B:1987.

Total nitrogen content of co-precipitates and sera were determined by Kjeldahl method, IDF Standard 92:1979.

## Results and Discussion

The investigation of dry matter content in co-precipitates, which was obtained by sedimentation of milk protein co-aggregates formed by heat treatment at 95 °C during 20 min, was the first part of the experiments. HCl, H<sub>2</sub>SO<sub>4</sub>, CH<sub>3</sub>CHOHCOOH and CH<sub>3</sub>COOH were used for precipitation. The results are shown in table 1.

Tab. 1. - Dry matter content of co-precipitates obtained by precipitation of coagulum obtained with different acids

Parameters	HCl		H <sub>2</sub> SO <sub>4</sub>		CH <sub>3</sub> COOH		CH <sub>3</sub> CHOHCOOH	
	DM(%)	M(%)	DM(%)	M(%)	DM(%)	M(%)	DM(%)	M(%)
X	34.39	65.61	35.48	64.52	34.65	65.35	32.68	67.32
Sd	1.4720	1.4720	2.0740	2.0740	1.6591	1.6591	1.7507	1.7507
Cv (%)	4.28	2.24	5.84	3.21	4.79	2.54	5.36	2.60

Legend:

DM - dry matter; M - moisture; X - average means; Sd - standard deviation; Cv - coefficient of variation

Results shown in Table 1. indicate that acids mentioned above didn't have a great influence on dry matter content of co-precipitates. However, there were differences among co-precipitates in their appearance, colour and compactness of coagulum. Co-precipitates obtained by acetic acid were rather dry, lax and white, while co-precipitates obtained by  $H_2SO_4$  and  $HCl$ , respectively, were a little sticky, compact and had pronounced yellow undertone. Co-precipitates obtained by lactic acid were softer, probably due to higher moisture content, with less pronounced yellow undertone. The obtained results were a consequence of different action of these acids on milk proteins.

According to Inihov (1956),  $HCl$  doesn't only influence casein clotting, but also partially hydrolyses casein, especially if it has been added too fast or in greater quantity. Co-precipitates obtained by  $HCl$  have smaller mineral matter content, because the salt formed with mineral components of milk (Ca, Mg, Na, K) could be readily washed from casein coagulum. Greater dry matter content of co-precipitates obtained by  $H_2SO_4$  could be explained by small solubility of salt formed between  $H_2SO_4$  and Ca, which remained in co-precipitates. The acetic acid forms soft coagulum, which has longer draining time.

Our results differ a little from those of Inihov (1956), because we found that co-precipitates obtained by acetic acid formed firm and drier coagulum. This is probably the result of milk proteins deeper transition, due to high heat treatment, as well as properties of co-precipitates which differ from acid casein properties.

As a measure of milk nitrogen matter recoveries into co-precipitates, sera nitrogen content has been used. The differences among sera nitrogen contents clearly indicate how different acids influence the distribution of nitrogen matter in co-precipitates and sera, as well as a degree of this influence, because the same milk samples were used for the production of co-precipitates. Table 2. shows the results of nitrogen contents in sera obtained by separation of co-precipitates, which have been produced by acidification of milk treated at  $95\text{ }^\circ\text{C}$  during 20 min.

Tab. 2. - Average means of nitrogen in milk sera obtained by separation of co-precipitates

Acid	N in sera (%)	% *
$HCl$	0.0440	100
$H_2SO_4$	0.0465	105.7
$CH_3COOH$	0.0500	113.6
$CH_3CHOHCOOH$	0.0464	105.5

N - nitrogen matter content

\* - N in sera from co-precipitates produced by  $HCl$  as 100%

It could be seen that milk sera of co-precipitates has a little nitrogen content, regardless of the acid used for coagulation. This could be associated with the fact that in the production of co-precipitates both casein and whey proteins are utilised, due to formation of co-aggregates. Nitrogen matter that remained in sera represents non-protein nitrogen, which doesn't coagulate and most likely doesn't

react with casein. Non-protein nitrogen matter represents 4-6% of total nitrogen matter of milk, Đorđević, 1987. A part of non-protein nitrogen is utilised by the production of co-precipitates but only when it was mechanically rabbed in coagulum.

HCl and lactic acids achieve the greatest utilisation of nitrogen, as indicated in table 2.

Further experiments were directed to the investigation of effects of these acids on the production of co-precipitates from milk treated at 87 °C during 10 min as mentioned above.

The results of Jovanović et al. (1997) and Vukićević et al. (1998) suggest that when milk has been subjected to this heat treatment, there is the smallest decrease of lactose content and the smallest part of  $\epsilon$ -amino acids react with lactose. This means that nutritive value of milk protein has been totally saved.

The results of dry matter content of co-precipitates and distribution of milk nitrogen matter into co-precipitates and milk sera are presented in table 3.

Tab. 3. - Utilisation of skim milk nitrogen matter as influenced by heat treatment and acid used for precipitation

Used acid	Heat treatment	N in milk (%)	N in sera (%)	N in coagulum (%)	DM in sera (%)	DM in coagulum (%)	N in DM of coagulum (%)	Nitrogen utilisation (%)
HCl	87 °C during 10 min	0.4962	0.0421	2.5817	6.10	22.23	11.61	93.07
	80-85 °C during 20 sec	0.4962	0.0942	2.7867	6.20	24.51	11.38	84.00
Lactic acid	87 °C during 10 min	0.4962	0.0439	2.5769	6.19	22.17	11.62	92.72
	80-85 °C during 20 sec	0.4962	0.0966	2.7934	6.30	24.42	11.43	83.49

Figure 1. shows dry matter content of co-precipitates obtained by precipitation with different acids.

The dry matter contents of co-precipitates obtained by HCl and lactic acid from milk treated at 87 °C during 10 min were smaller than those obtained from pasteurised milk, as indicated in table 3. and figure 1. Our results agree with those from Đorđević et al. (1987) and Southward (1978) which indicate that whey proteins are not fully utilised by the production of co-precipitates from pasteurised milk. Whey proteins have a great nutritive value due to a high content of essential amino acids, Đorđević, 1987, so the production of co-precipitates is a possible way of their utilisation.

Dry matter content was the smallest in co-precipitates obtained from milk treated at 87 °C during 10 min by lactic acid, which indicates greater hydrophilic properties of co-precipitates when this heat treatment was used. These results agree with those from Mottar et al. (1989) who suggested that hydrophilic properties of milk protein co-aggregates were greater when milk was treated at 90 °C

during 10 min than by UHT system. Greater hydrophilic properties of formed co-aggregates at pH = 4.6 may be linked with greater amount of  $\alpha$ -lactalbumin in complex.

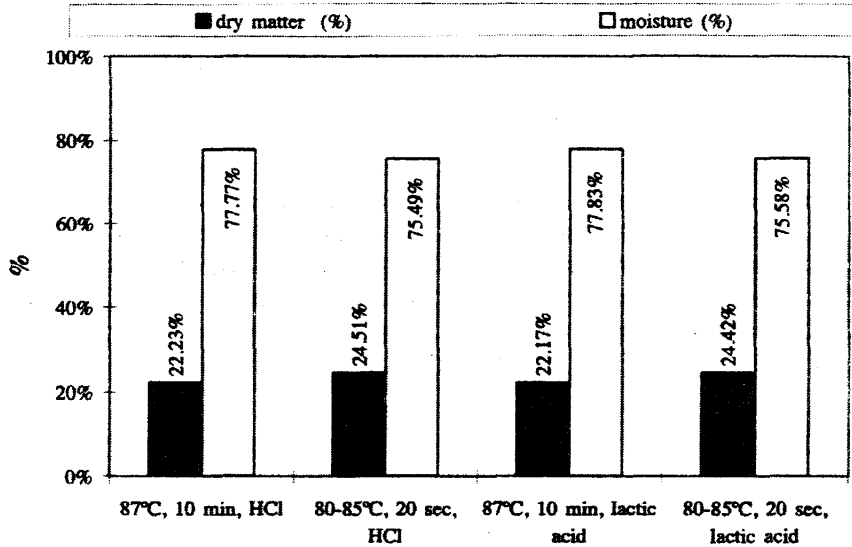


Fig. 1. - Dry matter and moisture content of co-precipitates from milk treated at 87°C during 10 min and from pasteurised milk obtained by precipitation by HCl and lactic acid

The distribution of milk nitrogen matter into co-precipitates and sera is shown in figure 2.

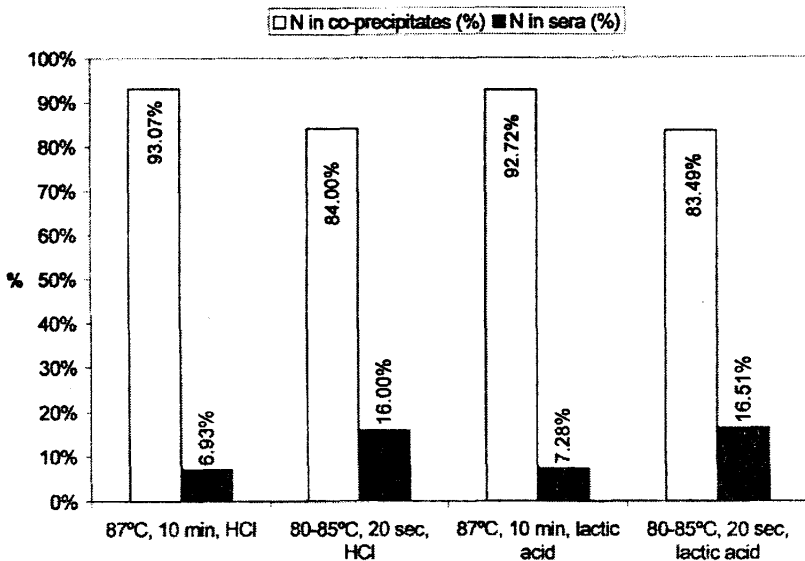


Fig. 2. - Distribution of nitrogen matter in co-precipitates and sera

It could be concluded that greater utilisation of nitrogen matter is achieved by precipitation of co-aggregates from milk treated at 87 °C during 10 min than from pasteurised milk, as indicated in table 3. and figure 2. As could be seen in table 3. greater nitrogen matter utilisation is achieved when co-precipitates were precipitated by HCl than by lactic acid. However, there is the greatest nitrogen content (11.62%) in dry matter of co-precipitates obtained by lactic acid from milk treated at 87 °C during 10 min, and the smallest nitrogen content (11.38%) in dry matter of co-precipitates obtained by HCl from pasteurised milk.

Bearing in mind all aforementioned, it could be concluded that the great part of whey proteins is utilised when milk is treated at 87 °C during 10 min.

### Conclusion

According to all aforementioned, we could conclude:

Gained co-precipitates differ in their appearance, colour and dry matter content. Co-precipitates obtained by H<sub>2</sub>SO<sub>4</sub> have the greatest dry matter content, probably due to the presence of insoluble sulphate, which remains in co-precipitates after washing.

According to dry matter content co-precipitates gained by different acids could be ranked:

- Co-precipitates obtained by H<sub>2</sub>SO<sub>4</sub>,
- Co-precipitates obtained by acetic acid,
- Co-precipitates obtained by HCl, and
- Co-precipitates obtained by lactic acid

There was the smallest nitrogen matter content in sera of co-precipitates obtained by HCl and lactic acid from milk treated at 95 °C during 20 min, which indicates the greatest utilisation of whey proteins.

Co-precipitates had pronounced hydrophilic properties, i.e. they had a bigger water holding capacity, when heat treatment at 87 °C during 10 min was used. At the same time, nutritive value of whey proteins was fully saved.

The greatest milk nitrogen matter utilisation was achieved by the production of co-precipitates by HCl, while co-precipitates gained by lactic acid had the greatest nitrogen matter in dry matter.

## REFERENCES

1. Carić, M. (1990): Tehnologija koncentrovanih i sušenih mlečnih proizvoda. Naučna knjiga. Beograd.
2. Corredig, M., Dalgleish, D.G. (1996): The binding of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin to casein micelles in milk treated by different heating systems. *Milchwissenschaft*, 51 (3), 123-127.
3. Doi, H., Tokuyama, T., Kuo, F.-H., Ibuli, F., Kanamori, M. (1983): Heat induced complex formation between  $\kappa$ -casein and  $\alpha$ -lactalbumin. *Agri. Biol. Chem.* 47 (12), 2817-2824, abstract.
4. Đorđević, J., Maćej, O., Milčić, M. (1987): Uticaj termičke obrade na stepen iskorišćenja azotnih materija mleka. *Mljekarstvo* 37 (10), 305-309.
5. Đorđević, J. (1987): Mleko. Naučna knjiga. Beograd.
6. Elfagm, A.A., Wheelock, J.V. (1977): Effects of heat treatment on structure and solubility of whey proteins. *J. Dairy Res.* 44, 367-371.
7. Elfagm, A.A., Wheelock, J.V. (1978): Heat interaction between  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and casein in bovine milk. *J. Dairy Sci.* 61, 28-32.
8. Inihov, G.S. (1956): Biohemija mleka. Moskva.
9. International Dairy Federation (IDF) (1987): IDF Standard Documents 21B.
10. International Dairy Federation (IDF) (1979): IDF Standard Documents 92.
11. Jovanović, S.T., Maćej, O.D., Jokić, A.M., Mikuljanac, A.M. (1997): Promena sadržaja laktoze u mleku u zavisnosti od primenjenih režima termičke obrade. *Preh. Ind. Mleko i mlečni proizvodi.* 8 (3-4), 25-29.
12. Kožev, K. S. I. A., Račev, R., Pavova, V. (1970): Fiziko-himični i tehnološki proučavanja vlrhru polučavaneto na vodnoraztvorimi i neraztvorimi Ko-precipitati na mlečnite beltilični. Naučnoizsledovatel'ski Institut po mlečna promišlenost - Vidin, tom IV, 145-153.
13. Long, J.E., Van Winkle, Q., Gould, I.A. (1963): Heat induced interaction between crude  $\kappa$ -casein and  $\beta$ -lactoglobulin. *J. Dairy Sci.* 46, 1329-1334.
14. Maćej, O. (1983): Prilog proučavanju koprecipitata radi potpunijeg iskorišćavanja belančevina mleka. Magistarski rad. Poljoprivredni fakultet. Beograd.
15. Maćej, O. (1989): Proučavanje mogućnosti izrade mekih sireva na bazi koagregata belančevina mleka. Doktorska disertacija. Poljoprivredni fakultet. Beograd.
16. Maćej, O., Đorđević, J., Jovanović, S. (1998): Uticaj pH i polifosfata na rastvorljivost koprecipitata. *Preh. ind. Mleko i mlečni proizvodi* 9 (1-2), 24-28.
17. Maćej, O., Jovanović, S., Vukićević, D. (2000): Uticaj tehnoloških činilaca na osobine mleka. *Arh. Poljopr. Nauke.* 61, 212 (1-2), 203-218.
18. Mottar, J., Bassier, A., Joniau, M., Beart, J. (1989): Effect of heat induced association of whey proteins and casein micelles in yogurt texture. *J. Dairy Sci.* 72, 2247-2256.
19. Müller, L.L. (1971): Manufacture and uses of casein and co-precipitate. *D. Sci. Abstr.* 33 (9), 659-674.
20. Paulson, M., Dejmeck, P. (1990): Thermal denaturation of whey proteins in mixtures with caseins studied by differential scanning calorimetry. *J. Dairy Sci.* 73, 590-600.
21. Shalabi, S.T., Wheelock, J.V. (1976): The role of  $\alpha$ -lactalbumin in the primary phase of chymosin action on heated casein micelles. *J. Dairy Res.* 43, 331-335.
22. Southward, C.R. (1978): La fabrication du co-précipité, 20. congrés international de laiterie, Paris, France, 26-30 juin 1978, Brèves communications, 914-915.
23. Vukićević, D., Jovanović, S.T., Maćej, O.D., Jokić, A.M. (1998): Uticaj termičkog tretmana na promenu sadržaja laktoze i pH vrednost mleka. Zbornik radova III jugoslovenskog simpozijuma prehrambene tehnologije. Beograd. 127-131.

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## OSOBI NE KOPRECIPITATA DOBIJENIH POMOĆU RAZLIČITIH KISELINA I DISTRIBUCIJA AZOTNIH MATERIJA MLEKA

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### Rezime

Za dobijanje koprecipitata korišćeno je obrano mleko sa 0.05 % mlečne masti. Hemijski kompleks između kazeina i serum proteina obrazovan je zagrevanjem mleka na 87 °C u toku 10 min, i na 95 °C u toku 20 min. Kao referentni uzorak korišćeno je pasterizovano mleko (80-85 °C, 20 sec).

Koprecipitati su dobijeni koagulacijom termički tretiranog mleka pomoću 2.8 M HCl, 1.4 M H<sub>2</sub>SO<sub>4</sub>, 2.8 M mlečne i 2.8 M sirćetne kiseline. Koprecipitati dobijeni pomoću sirćetne kiseline bili su suvi i rastresiti, bele boje, dok su koprecipitati dobijeni pomoću HCl i H<sub>2</sub>SO<sub>4</sub> bili malo lepljivi, kompaktni, sa izraženom žučkastom nijansom. Koprecipitati dobijeni pomoću mlečne kiseline bili su kompaktni, mekši i imali su najveći sadržaj vode (67.32%).

Kao merilo prelaska azotnih materija iz mleka u koprecipitate korišćen je sadržaj azota u mlečnom serumu. Mlečni serum posle izdvajanja koprecipitata imao je mali sadržaj azota, bez obzira na korišćenu kiselinu (0.0440% za HCl; 0.0465% za H<sub>2</sub>SO<sub>4</sub>; 0.0500% za sirćetnu i 0.0464% za mlečnu kiselinu). To znači da azotne materije koje su zaostale u serumu predstavljaju praktično neproteinski azot, koga prema literaturnim podacima ima 4-6% u odnosu na ukupne azotne materije mleka.

Istraživanja su pokazala da se zagrevanjem mleka na 87 °C u toku 10 min postiže znatno veće iskorišćenje azotnih materija mleka u obliku koprecipitata dobijenih pomoću HCl (11.61%), nego kod pasterizovanog mleka (11.38%) i da je ono nešto veće kada se koprecipitati dobijaju koagulacijom pomoću mlečne kiseline (11.62%).

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