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CASEIN AND WHEY PROTEIN INTERACTION AND ITS TECHNOLOGICAL APPLICABILITY

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Key words: calcium chloride, molecular weight of proteins, electrophoretic mobility, rennet, biological value.

The authors have studied the effect of an addition of calcium chloride to milk prior to its pasteurization on the molecular weight of proteins, their electrophoretic mobility, hydration of casein micelles, titration curves of isolated proteins and the coagulation of milk by rennet. The addition of calcium chloride enabled to increase the yield of hard and cottage cheeses. The products obtained contained protein of a higher biological value.

INTRODUCTION

Milk is a complex physico-chemical system. The most important milk constituents which determine the properties of the complex system are proteins and mineral salts. Casein occuring in the form of micelles accounts for about 80% of the total milk protein content [1]. According to Schimminn and Hill [2] the micelles are composed of numerous protein subunits called submicelles and other milk constituents like calcium, phosphorus, magnesium, citrates and others. The quoted authors have argued that particular casein fractions namely: α_s, β and $\varkappa$, display a fairly uniform distribution in the micelles. These fractions differ from one another not only in terms of their numbers in the casein complex but also by the isoelectric point and affinity to calcium ions.

The α_s and $\varkappa$-casein fractions that probably form the micelle nucleus [3] are most susceptible to polymerization and formation of complex combinations. Waugh [4, 5, 6] has shown that there is some stechiometric dependence between the combinations of α_{s1} and $\varkappa$-casein. Kauzman [7]

believes that interactions between particular casein fractions are conditioned by the presence of apolar amino acid groups in the solvent that are dislodged from water into the interior of the protein molecule where they interact with the other apolar groups. The α_s, β and κ-casein fractions contain a lot of hydrophobic apolar groups that are believed to concentrate round the casein fractions. Those factors which tend to diminish the interaction of water with casein fractions also reduce the stability of micelles and thereby destabilize the system i.e. milk.

Thompson et al. [8] have found that casein micelles have a loose structure and contain about 70% water. Under heat treatment they react with globular proteins, and more precisely, with whey proteins that are mainly composed of β-loctoglobulin representing from 40 to 60% of whey proteins and of α-lactoalbumin. The native whey proteins are soluble in milk in all the pH ranges. The detectable changes in these properties are a signal of their denaturation which can occur mainly at higher temperatures and in the presence of chemical compounds. During the denaturation of whey proteins the complex globular configuration is probably destroyed which leads to its transformation into a random configuration. There is a critical threshold of concentration of the thermally denaturated whey proteins above which they undergo aggregation and in consequence are precipitated in the milk [9].

The physico-chemical phenomen of complexes formed by casein fraction with whey proteins is still controversial; the same is true of the mechanism of formation of these complexes, particularly in a complex system like milk. Probably the best known is the effect of heat on the formation of the complex of β-lactoglobulin with κ-casein [10]. Long et al. [10] have suggested that the condition for the formation of the complex is heating the two milk protein constituents above 68°C at which the activation of β-lactoglobulin is to occur by uncovering the sulfhydryl groups in the presence of calcium ions. The formation of β-lactoglobulin — κ-casein complexes during heating leads to the lowering of milk coagulability under the effect of the rennet, which basically lowers the usefulness of that milk for processing into cheeses and other similar products. Most likely the access of the enzyme to κ-casein is hindered by the formation of the κ-casein β-globulin complex [11, 12]. On the other hand our investigations of 1969 [13] and next those of Wilson et al. in 1972 [12] have shown that an addition of calcium ions to milk before pateurization maintains the coagulability of the pasteurized milk at the level of raw milk. Two factors are likely to be responsible for that, namely the protective action of calcium ions on the kinetics of denaturated whey proteins and simultaneous uncovering of the κ-casein groups blocked by denaturated whey proteins accessible to the rennet enzyme. By its divalent charge calcium is to intensify the association of κ-casein molecules and thereby it increases the stability of the complexes formed.

As it results from the above information, the phase milk structure and its thermal stability are modelled by a series of complex interactions between the proteins and ionized salts of milk.

PHYSICAL PROPERTIES OF MILK PROTEINS AFTER INTERACTION

The authors of this paper are interested in the interaction between casein and whey proteins as effected by adding to raw milk small amounts of calcium salt and short-time high pasteurization afterwards. According to earlier finding of the authors, that allows to increase the yields of hard cheese, cottage cheese and milk protein concentrates [14, 15, 16].

It must be stressed that this interaction in spite of its complexity has an exceptional technological significance, although there are other methods for the recovery of whey proteins such as centri whey, reverse osmosis, ultrafiltration and others.

In the first place we wish to present the titration curves of aqueous solutions of proteins obtained by ultra-centrifuged milk protein precipitates. Titration curves characterize the aggregation rate of particles and accessibility of functional groups.

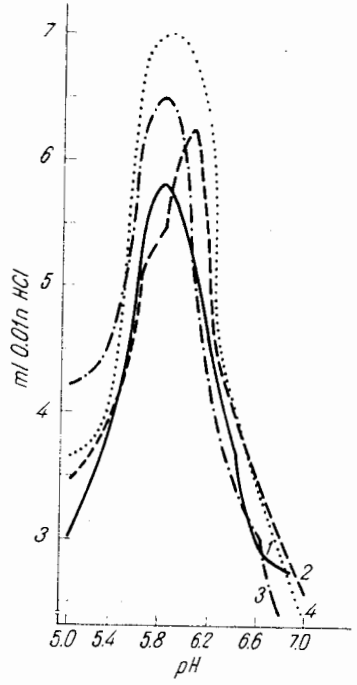

Fig. 1. Acid titration of milk proteins; 1 — raw milk, 2 — milk past. at 92°C/15 s., 3 — milk past. at 92°C/15 min., 4 — milk enriched with 3.6 mM $CaCl_2$ and past. at 92°C/15 s.

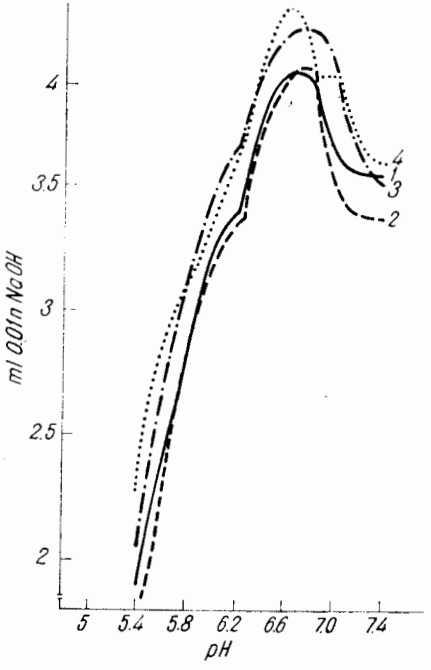

Fig. 2. Base titration of milk proteins; 1 — raw milch, 2 — milk past. at 92°C/15 s., 3 — milk past. at 92°C/15 mM, 4 — milk enriched with 3.6 mM $CaCl_2$ and past. at 92°C/15 min.

The prepared protein solutions were titrated using a titrator radiometer from Denmark with 0.01 hydrochloric acid to a pH of 5.0 and then in the reverse direction with 0.01 N NaOH from a pH of 5 to the initial pH. Figs 1 and 2 seem to indicate that the proteins separated by ultracentrifugation from the pasteurized milk previously treated with calcium salts show different properties than proteins from the other samples. Larger differences were found with acid than with lye titration. Probably, the separated casein-whey-proteins complex shows higher buffer properties than casein itself or the complex obtained from milk pasteurized for 15 minutes at 92°C.

The values of coefficient α, that is the ratio of histeresis area comprised between the titration curves to the area determined by the terminal points on coordinate axes (pH ranges on X-axis and amounts of ml. 0.01 N HCl or 0.01 N NaOH on Y-axis), were also calculated. It appears from Fig. 3 that the coefficients characterizing changes in protein, in this case the rate of denaturation expressed as the coefficient α for three protein samples, differ insignificantly, α = 0.485 for raw milk, α = 0.498 for milk pasteurized at 92°C for 15 seconds, and α = 0.543 for pasteurized calcium-enriched milk. A higher coefficient α seems to point to a liberation of functional groups, probably as a result of the displacement of

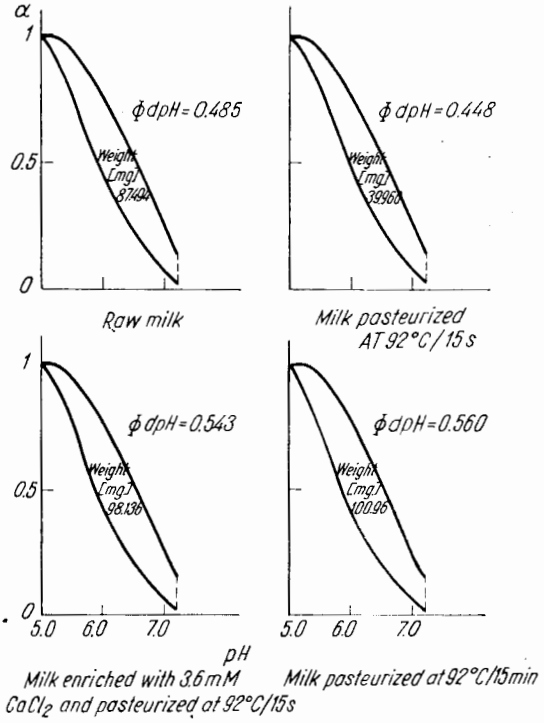

Fig. 3. Acid-base titration of milk proteins

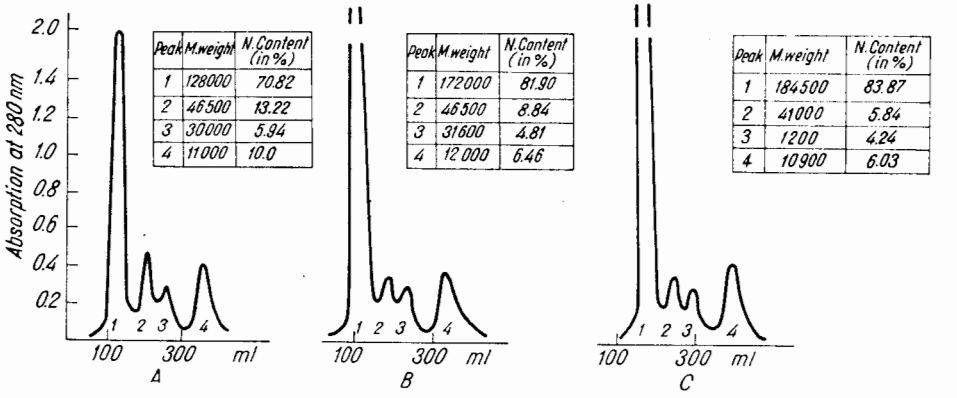

Fig. 4. Partition chromatograms on Sephadex G-100 gel; A — raw milk, B — pasteurized milk, C — milk enriched with 3.6 mM $CaCl_2$ and pasteurized

whey proteins from the globular configuration to a more random configuration.

Using a method of molecular filtration on gel Sephadex G-100, raw milk proteins were separated into four main fractions: casein, β-lactoglobulin, α-lactoalbumin and non-protein compounds (Fig. 4). It was observed that an addition of calcium salt to raw milk at three different ranges followed by pasteurization of these parts of milk resulted in an increase in the content of the first fraction by 84% with a simultaneous decrease in the content of β-lactoglobulin and α-lactoalbumin. There was also an increase in molecular weight of casein and a marked decrease in molecular weight of whey protein fractions that is β-lactoglobulin and α-lactoalbumin and even nonprotein fraction. The increased content of casein protein is confirmed by the experiments of Hartman and Swanson [17] who have shown that high temperature causes an increase in the molecular weight of casein.

The data obtained in this study show that the interaction between whey proteins and casein leads to the formation of combinations in which about 55% of nitrogen has the form of whey proteins, which allows for a better utilization of protein compounds of milk.

Using a method of milk protein separation based on the rate of migration in an electrical field, and more precisely, disc electrophoresis on polyacrilamide gel, changes were also observed in the content of particular whey protein fractions (Fig. 5). In this case the highest differences were found in the level of protein fractions corresponding to α-lactoalbumin and β-lactoglobulin. In this experiment the protein precipitate obtained by centrifuging milk samples in a Beckman LS 65 ultracentrifuge at 69.000 g for 35 minutes at 37°C was analyzed. In the rennet whey samples and in supernatants obtained from the pasteurized calcium-enriched milk there was a decrease in the level of proteins in all the se-

parated whey protein fractions. However, more marked differences were found among whey protein fractions occuring in the supernatant (Table 1).

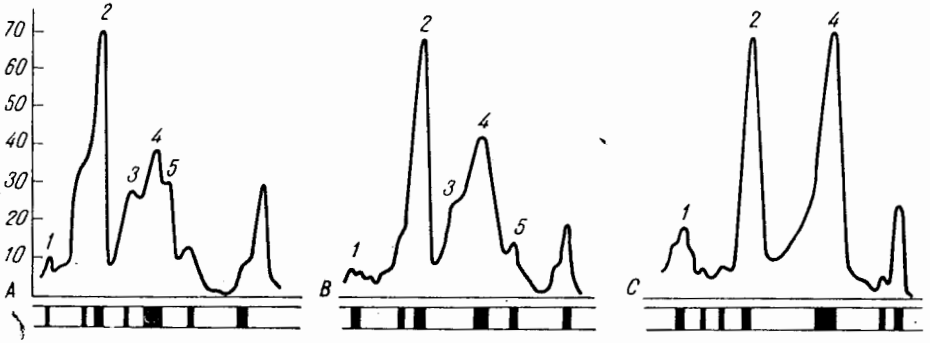

Fig. 5. Electrophoretic separation of milk proteins on PAA gel; A — raw milk, B — milk pasteurized at 92°C/15 s., C — milk enriched with 3.6 mM $CaCl_2$ and pasteurized at 92°C/15 s.

Table 1. Content of whey protein fractions in supernatant*)

Fraction \ Kind of sample	Supernatant from raw milk		Supernatant from milk pasteurized at 92°C for 15 s		Supernatant from milk enriched with 3.6 mM $CaCl_2$ and pasteurized at 92°C for 15 s	
	%	μg	%	μg	%	μg
Immunoglobulin	19.78	13.60	—	—	—	—
Proteose-peptone	9.85	6.70	10.27	6.00	12.27	5.70
Serum albumin	7.66	3.20	8.73	5.15	13.95	4.95
α-lactoalbumin	21.98	15.10	18.20	10.70	19.60	9.20
β-lactoglobulin	41.00	28.29	38.00	22.70	30.15	14.10
Protein insoluble in buffer	—	—	23.60	13.72	23.30	10.95

*) Supernatant from milk after ultracentrifugation at 69 000 g for 35 min content of nitrogen in fractions were calculated from densitometric curves and expressed in % and μg

Determination of the hydration rate of casein micelles by the method of Thompson et al. [18] as modofied by the authors showed that the amount of centrifugated precipitate varied depending on the method of milk treatment. It was also observed that the precipitates from the samples of raw milk and of calcium-enriched milk were more compact, and the precipitates from milk pasteurized with no calcium salt added were less packed. This observation is confirmed by the analysis of hydration of precipitates (Fig. 5a). Water content in the precipitate from raw milk averaged 1.9 g/g protein precipitate, and of that from pasteurized milk it amounted on the average to 1.95 g/g protein. Precipitates of milk treated with 3.6 mM/l calcium chloride and of pasteurized milk were less hy-

drated, 1.67 g/g protein on the average. In relation to variations in hydration of protein precipitate there was a change in thermal milk stability defined in time units since the moment of sample immersion in oil at 140°C to the first flocculation. Treatment of the same milk samples with 0.05% sodium citrate or sodium polyphosphate along with 3.6 mM $CaCl_2$ restores stability to the level of milk pasteurized with no additions. There seems to be a dose correlation between the hydration rate of the precipitate of milk proteins and thermal milk stability.

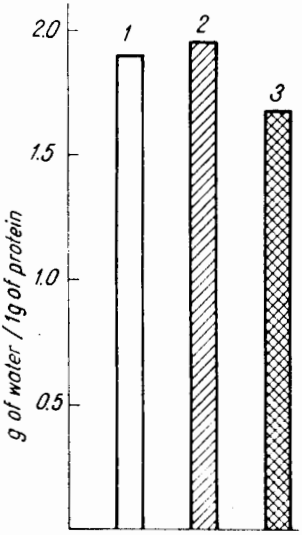

Fig. 5a. Solvation water rate of casein micelles; 1 — raw milk, 2 — milk past. at 92°C for 15 s., 3 — milk enriched with 3.6 mM $CaCl_2$ and past. at 92°C for 15 s.

Evaluation of milk coagulability under the effect of rennet indicated that milk enriched with calcium salts and pasteurized showed a point and time of coagulation very similar to those observed in raw milk, contrary to milk pasteurized only (Fig. 6).

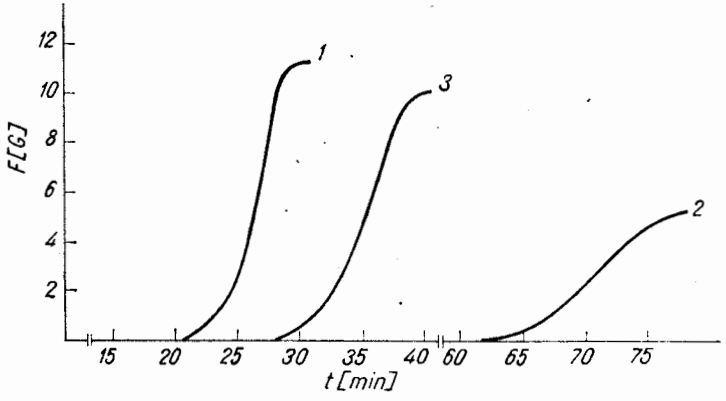

Fig. 6. Curd firmness of 1 — raw milk, 2 — milk past. at 92°C/15 s., 3 — milk enriched with 3.6 mM $CaCl_2$ and past. at 92°C/15 s.

SIZE DIFFERENTATION OF MILK PROTEIN MICELLE

Rheological properties of curd as well as its behaviour during cheese-making seem to indicate that whey proteins are integrally bonded with casein and they compose a homogenous mass of hard cheese or cottage cheese. This was the reason for undertaking a study on the structure of casein micelles using an electric microscope [29]. Our observations agree with the results obtained earlier by Schmidt et al. [19], Knoop et al. [20] and Saito [21] showing that casein micelles have an approximatelly globular shape and a spongy surface. In addition, our experiments showed that a high pasteurization of milk, both with or without calcium ions added does not distort the spongy surface of micelles in spite of enlarging their size. In comparison with raw milk the most significant enlargement of casein micelles was observed in pasteurized calcium-enriched milk [29].

Calculations showed that casein micelles in raw milk occuped 36% of the entire area of the pictures, pasteurized milk 41% and calcium ion-enriched pasteurized milk — 48%. Our microscope observations seem to confirm the experiments of Saborwal and Ganguli [22] showing that casein micelles can stabilize whey proteins before thermal denaturation creating complexes through the intermediary of calcium bridges [23].

Enlargement of casein micelles in raw milk, pasteurized milk and calcium-enriched pasteurized milk is presented graphically (Figs 7a, 7b,

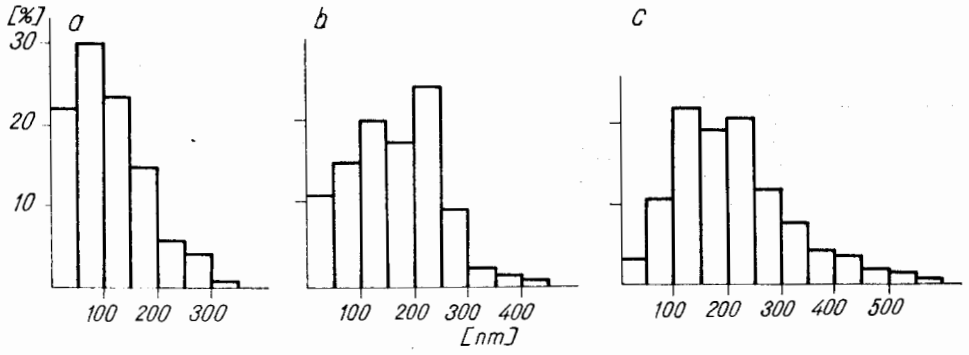

Fig. 7a. Size of casein micelles in raw milk
Fig. 7b. Size of casein micelles in milk pasteurized at 92°C/15 s.
Fig. 7c. Size of casein micelles in milk enriched with 3.6 mM $CaCl_2$ and pasteurized at 92°C/15 s.

7c). Our results, obtained in different conditions, seem to confirm the findings of Schmidt and Buchheim [19] indicating that larger micelles can be formed from combinations of small micelles and even submicelles. Our observations also show that it is highly probable that casein-whey protein complexes are formed through calcium bridges. The possibility

of bonding whey proteins with casein micelles is also evidenced by analysis of nitrogenous compounds in supernatants from milk samples centrifuged at 67.000 g. The supernatant from pasteurized calcium-enriched milk was found to contain merely 8.3% nitrogen compounds precipitable in 12% TCA colution, and that from milk pasteurized with no calcium added 17.5%, and the supernatant from raw milk 22.5% in terms of the total amount of nitrogen compounds in milk. Over 60% of the whey nitrogen compounds from pasteurized calcium-enriched milk were found to be intergrally bonded with casein micelles.

A study by Knoop and Peters [24] of the submicroscopic structure of acid milk curd has shown that in definite conditions at a slow acidifying of milk the action of milk acid phosphatase is manifested. Under the action of phosphatase the curd was desintegrated to the particles corresponding to the size of casein submicelles. This desintegration proceeded from the surface deep into the curd and, on the microtomic sections, it was observed at the edges of curd particles.

Assuming after Knoop and Peters optimum conditions for acid phospatase action in milk (pH 5.2 to 4.8 and temp. 50°C) the effect of phosphatase on acid curd obtained from milk pasteurized with an addition of 3.6 mM $CaCl_2$ was examined [25]. Microscopic examinations of the photographs of acid curd from milk pasteurized at 92°C with no calcium salts added indicate that casein micelles concentrated in the curd exhibit a clearly loose structure at the edges. On this basis it can be presumed that like in the experiments by Knoop and Peters [24] there was a partial desintegration of curd structure caused by the action of acid phosphatase. However, in case of acid curd obtained from pasteurized calcium-enriched milk no clear desintegration of curd to submicelles was observed [25]. In can be supposed that in spite of similar conditions acid phosphate penetrated the curd structure at a far slower rate. This seems to point to the physical difference in the structure of that curd. It can be assumed that the separated whey proteins in association with casein also influence the change of curd destruction caused by phosphatase.

PRACTICAL SIGNIFICANCE OF INTERACTION BETWEEN MILK PROTEINS

The nutritional investigations of Randoin and Lausert [30] have shown that cheese proteins have a much lower biological value than those of raw or dried milk. In our experiments [31] we have found low Net Protein Utilization (NPU) values for cheese protein, amounting to 56-60 vs 75-77 for milk proteins. There is no doubt that from the physiological and economical points of view, simultaneous utilisation of casein and whey proteins in cheese-making should be considered as the most rational utilization of the proteins of milk.

The technological procedure of the 1-stage utilization of milk in cheese manufacture is presented in our patent description on Method of Milk Protein Coagulation [13]. This method aims at casein interaction with whey proteins during pasteurization and rennet coagulation of the complex. The subsequent stages of production are similar to those of traditional cheese manufacture.

Table 2 shows the effects of the new method in comparison with the traditional method of cheese production.

Table 2. Effects of the new method in comparison with the traditional method of cheese production

Type of cheese		Utilization of N compounds of milk (%)		Increase in N compounds utilization (%)		Increase cheese yield from 1000 l milk (Kg)
		total N	proteins	total N	proteins	
	C	70.5	76.6	—	—	—
Grana		88.3	93.6	17.8	16.0	17.5
	C	74.7	77.7	—	—	—
Gruyere	E	86.5	92.2	11.8	15.2	11.4
	C	70.8	74.5	—	—	—
Cheddar	E	83.2	91.1	13.0	16.3	21.0
	C	74.4	76.6	—	—	—
Blue	E	88.2	92.6	14.6	14.9	18.6
	C	72.3	75.6	—	—	—
Stilton	E	87.6	91.0	15.3	15.4	20.0
	C	74.4	79.2	—	—	—
Münster	E	87.8	93.3	13.4	14.5	20.0
	C	77.4	77.3	—	—	—
Limburger	E	86.3	92.9	12.9	15.6	18.0

C — control cheese
E — Experimental cheese

Flavour and consistency of the cheeses manufactured from all milk proteins were similar to those of cheese produced traditionally. Blue cheese and Stilton had the taste of ewe's milk cheese. Protein breakdown during ripening proceeded normally in the experimental cheese and increases in soluble N, non protein N, peptide N, and free fatty acids were similar to those of traditionally made cheese; the chromatographe picture of individual fatty acids did not differ from that of control cheeses.

The 1-stage method of utilization of all milk proteins developed by us allows to increase the yield by 15% and the biological value by 10-25% during manufacture of various types of cottage cheese [15]. At the same time, the higher Ca content adds considerably to the nutritive value of the product. New types of highly nutritive cottage cheese may be produced on the existing manufacturing lines.

The new technological procedure enables utilization of all milk proteins at the level of about 95% giving sodium or calcium milk proteinate better than caseinate [16].

Making use of the interaction between whey proteins and casein in the technology of processing milk into known and new dairy products leads to an increase of the biological protein value. This has been evidenced by the studies of Czarnowska-Misztal et al. [26] and the several times repeated investigations of Cichon et al. [27]. According to Cichon, the NPU and PER values of hard and cottage cheeses obtained from all milk proteins were significantly higher than those of analogical hard and cottage cheeses obtained by a traditional technology. The NPU was higher by 8 to 11 units and PER by 0.36 to 0.62 units (Table 3).

Table 3. NPU and PER of cheeses and quarks*)

	Nutritive value of protein from cheeses and quarks obtained by			
	traditional technology		from all milk proteins	
	NPU	PER	NPU	PER
Italian cheese	61.5	2.52±0.08	71.2	2.90±0.06
Ementaler cheese	62.5	2.58±0.07	70.6	2.94±0.06
Edam cheese	62.9	2.63±0.07	72.2	3.03±0.07
Münster cheese	61.0	2.67±0.06	72.3	3.12±0.07
Blue cheese	56.3	2.23±0.09	65.2	2.58±0.10
Solan cheese	54.4	0.84±0.12	62.4	1.76±0.11
Münster cheese (fresh)	62.6	2.69±0.07	73.2	3.21±0.07
Acid rennet quark	62.7	2.73±0.05	73.1	3.25±0.08
Acid quark	63.0	—	—	—
Co-presipitated	—	—	74.3	3.52±0.06

*) Corrected to NPU of 60 and PER of 2.50

The presented feeding experiments point to a positive effect of using new technology for making hard and cottage cheeses. Utilisation of a considerable part of whey proteins for making hard and cottage cheeses has had a positive effect on the amino acid composition of these products.

To sum up, the technology of recovery of whey proteins developed by us is superior to the recommended ultrafiltration or reverse osmosis method in that it allows to separate milk proteins within a one-stage production cycle and it can be used for milk processing both to all standard (conventional) dairy products and to new products of different physico-chemical characteristic.

The one-stage protein separation method consumes less energy for there is no need of re-pasteurization of whey and ultrafiltrate. Also there are no hygienic and sanitary problems which are encountered when recovering proteins by ultrafiltration or reverse osmosis.

Moreover as it has been found by Bednarski et al. [28] concentrated

whey proteins by the method of reverse osmosis or ultrafiltration and then spray dried show a significantly lower solubility then those obtained by traditional concentration and spray drying.

This phenomenon is particularly observed at higher protein concentrations obtained by reverse osmosis or ultrafiltration. The observations discussed suggest that during reverse osmosis or ultrafiltration a "physical fatigue" of proteins occurs which is most likely to reduce their original physiochemical properties (perhaps the hydrative coating of proteins is distorted).

LITERATURA

1. Thompson M. P., Kiddy C. A.: J. Dairy Sci., 1964, 47, 626.
2. Shimmin P. D., Hill R. D.: J. Dairy Res., 1964, 31, 121.
3. Garnier J.: Reunion Intern. — sur les caseins. Jouy-Josas, France 1965.
4. Waugh D. E., von Hippel: J. Am. Chem., 1956, 78 (4), 576.
5. Waugh D. E.: Soc. Disc., 1958, 25, 186.
6. Waugh D. E.: J. Am. Chem. Soc., 1965, 110.
7. Kauzman W.: Adv. Protein Chem., 1959, 14, 1.
8. Thompson M. P., Farrel H. M.: 28-th Annu Proc., 1970.
9. Morr C. V., Josephson R. V., Jennes R., Manning P. B.: J. Dairy Sci., 1971, 54, 1555.
10. Long J. E., von Q. Winkle, Gould J. A.: J. Dairy Sci., 1963, 46, 1329.
11. Kannan A., Jennes K.: J. Dairy Sci., 1961, 44, 808.
12. Wilson G., Wheelock J.: J. Dairy Res., 1972, 39, 413.
13. Poznański S., Śmietana Z., Surażyński A., Królik T.: Sposób koagulacji białek mleka. Polish patent nr 82692, 1969.
14. Poznański S., Śmietana Z., Jakubowski J. et al. XIX Int. Dairy Congr. 1E, 722, 1974.
15. Poznański S., Rymaszewski J., Śmietana Z. et al. XIX Int. Dairy Congr. 1E., 720, 1974.
16. Chojnowski W., Śmietana Z., Poznański S., Jakubowski J., Rymaszewski J.: Milchwiss., 1975, 30, 7, 407.
17. Hartmann G. H., Swansom A. M.: J. Dairy Sci., 1965, 48.
18. Thompson M. P., Gordon R. T. et al.: J. Dairy Sci., 1969, 52, 1166.
19. Schimdt D. G., Buchheim W.: Milchwiss., 1972, 25, 596.
20. Knoop A. M., Knoop E., Wiechen A.: Neth. Milk Dairy J., 1973, 27, 121.
21. Saito Z.: Neth. Milk Dairy J.: 1973, 27, 143.
22. Sabarwal P. K., Ganguli N. C.: J. Dairy Sci., 1972, 55, 765.
23. Morr C. V., Josephson R. V.: J. Dairy Sci., 1968, 51, 1349.
24. Knoop A. M., Peters K. H.: Milchwiss., 1975, 30, 674.
25. Śmietana Z., Jakubowski J., Poznański S., Kocoń J.: Milchwiss., 1978, 33, 478.
26. Czarnowska-Misztal et al.: Przem. Spoż., 1978, 32, 31.
27. Cichoń R. et al.: (in press).
28. Bednarski W., Sloth-Hansen P. et al.: Le Lait 1978, 57, 571.
29. Śmietana Z., Jakubowski J., Pnznański S., Żuraw J., Hosaja M.: Milchwiss., 1977, 32, 464.
30. Randoin L., Causert J.: Ann. Techn. Agric. 1956, 4, 521, 527.
31. Poznański S., Siudak F.: Ind. J. Nutr. Dietet., 1971, 8, 189.

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INTERAKCJA KAZEINY I BIAŁEK SERWATKOWYCH I JEJ TECHNOLOGICZNE ZNACZENIE

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Streszczenie

Prześledzono wpływ jonów wapniowych dodawanych do mleka surowego przed jego pasteryzacją na interakcję białek serwatkowych z kazeiną. Krzywe miareczkowania wodnych roztworów białek otrzymanych przez odwirowanie w ultrawirówce prób mleka wydają się wskazywać, że białka mleka pasteryzowanego uprzednio wzbogaconego w sole wapnia wykazują inne właściwości niż białka mleka surowego lub pasteryzowanego w tych samych warunkach, lecz bez dodatku jonów wapnia. Obliczone wartości współczynnika α wydają się wskazywać na uwalnianie się grup funkcyjnych, prawdopodobnie w wyniku przejścia białek serwatkowych od konfiguracji globularnej do bardziej przypadkowej. Osady białek mleka z dodatkiem jonów wapnia były mniej uwodnione — 1,67 g wody/g białka wobec 1,9 dla osadu białek mleka surowego.

Stosując metodę sączenia molekularnego stwierdzono w białkach po interakcji wzrost zawartości frakcji pierwszej i wzrost ciężaru cząsteczkowego tych białek przy jednoczesnym obniżeniu się tych wartości dla frakcji II i III.

Stosując technikę mikroskopii elektronowej zaobserwowano zwiększenie się rozmiarów miceli kazeiny w próbkach mleka z dodatkiem jonów wapnia przed pasteryzacją. W porównaniu z mlekiem surowym nie zaobserwowano zmian w gąbczastej powierzchni micel.

Śledząc działanie fosfatazy kwaśnej w próbkach mleka z dodatkiem jonów wapnia nie zaobserwowano wyraźnego rozpadu skrzepu do submiceli.

Wykorzystanie interakcji białek serwatkowych z kazeiną w technologii przetwarzania mleka na znane i nowe produkty mleczne doprowadza do podniesienia wydajności produktu i podniesienia wartości odżywczej białka.

Proponowana przez autorów jednostopniowa metoda wydzielania białek mleka jest mniej energochłonna niż metoda ultrafiltracji, ponieważ nie zachodzi potrzeba ponownej pasteryzacji serwatki i ultrafiltratu. Nie występują też problemy higieniczno-sanitarne, na jakie napotyka się przy odzysku białek serwatkowych metodą ultrafiltracji lub odwróconej osmozy.