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EFFECT OF HEATING THE SOLUTIONS OF MILK PROTEIN CONCENTRATE ON THE COMPOSITION OF PROTEINS, CA AND P IN A GEL NETWORK AFTER RENNET AND ACID-RENNET COAGULATION

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Key words: milk protein concentrate, rennet and acid-rennet curd

The aim of the research was to study the effect of heating the water solutions (pH 7.1) of the milk protein concentrate obtained with ultrafiltration on the content of nitrogen (N), calcium (Ca) and phosphorus (P) and the composition of proteins in the pellets after the ultracentrifugation of rennet curds (pH 6.6) and acid-rennet curds (pH 6.0). It was found that heating the concentrate solutions did not have any influence on the content of Ca and P in the insoluble fractions of rennet curd. However, it contributed, irrespective of the temperature of heating, to an increase in the share of total N by 0.3% (significant differences at $p=0.05$). Electrophoretic analysis of the proteins in pellets after ultracentrifugation of the curds indicates that heating to 92°C caused an increase in the share of whey proteins by 1% on average. At the same time, a decrease in the share of β-lactoglobulin (β-lg) by 4.7% and BSA+Ig by 2.8% was noted in the proteins of the soluble fraction after ultracentrifugation of the curds. α_s- and β-casein were not found in the soluble fractions. On the other hand, para-κ-casein was found (from 2.3% in the unheated substrates to 2.7% in the substrates heated to 92°C). With acid-rennet coagulation heating the substrates at 92°C contributed to a decrease in the share of Ca and P in the pellets after ultracentrifugation of the curds by 1.8% and 1.7%, respectively, with the unchanged share of total N. The content of β-lg, α-lactalbumin (α-la) and BSA+Ig in the soluble fractions of the curds in the substrates heated at 92°C decreased by 13.7%, 1.6% and by 3.9%, respectively.

Heating the substrates at 92°C contributed to a decrease in the share of para-κ-casein in the soluble fractions of acid-rennet curds from 1.7% to 0%. At the same time, it limited the incorporation of dissociated β-casein into a gel network. Its content in the soluble fraction after ultracentrifugation of the curds increased from 0% in the case of the unheated substrates to 2.7% in those heated to 92°C.

INTRODUCTION

In the products obtained with the coagulation of milk proteins the texture, moisture and other variables, which depend on water activity, are determined by syneresis [Walstra *et al.*, 1985; Leliévre, 1997]. A crucial factor determining the course of this process is the content of the denaturated whey proteins incorporated in a gel network. An increase in their content limits syneresis which allows to determine better functional attributes of low fat cheese and yoghurt [Savello & Dargan, 1995; McMahon *et al.*, 1996]. The share of Ca in a gel network also determines its functional properties and affects the firmness of curd. One of the basic functions of Ca is to bind molecules and protein particles during the formation of a gel network [Creamer, 1985; Lefebvre-Cases *et al.*, 1998]. A decrease in its content in curd influences the texture by lowering the number of cross-linkages between protein polypeptide chains. It is unfavourable in the case of rennet hard cheese but favourable for the production of acid-rennet cheese of mozzarella type [Remeuf *et al.*, 1989; Solrza & Bell, 1995; Metzger *et al.*, 2000; Raynal & Remeuf, 2000].

The aim of the research was to study the effect of heating the solutions of milk protein concentrate, which are characterised by a lower, in comparison to milk, ratio of Ca and P to protein, on the composition of proteins forming a gel network and the share of Ca and P in it.

MATERIALS AND METHODS

Materials. The commercial concentrate of milk proteins obtained with spray drying of retentate after ultrafiltration (69.2% of protein, 16.2% of lactose, 4.2% of water, 7.2% of ash, 3.2% of fat) was used in the research. Water solutions of the concentrate of 34 g protein/dm^3 at pH 7.1 were prepared. The solutions were heated at 72°C/15 s or 92°C/60 s, cooled in water with ice to a temperature of 20°C. Then sodium azide (0.02%, w/v) and streptomycin (0.02%, w/v) were added. In some of the solutions pH was reduced to 6.6 and 6.0 (BECKMAN Φ720 pH-meter) with 4.4 mol/L of lactic acid. The differences in volume were settled with redistilled water to obtain the protein concentration of 33 g/dm^3 in all the analysed substrates.

Analytical methods. The content of total nitrogen (N) was determined in the solutions with the Kjeldahl's method [Budsławski & Drabent, 1972], calcium (Ca) was determined with atomic absorption spectrophotometry (UNICAM 939 Solar apparatus, UK) after wet sample mineralisation [Laskowski, 1974; Rutkowska, 1981], and phosphorus (P)

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was determined with colorimetric method (SPEKTROMOM 195D spectrophotometer, Hungary) after wet sample mineralisation [Mattsson & Swartling, 1954]. Chymosin (EC 3.4.23.4) (Sigma Chemical Company, catalogue No. 7751, activity of 23.5 U/mg protein) was used to obtain the curds. The incubation was performed at a temperature of 32°C. The enzyme concentration of 1.07×10^{-6} g/cm^3 was used in the substrates of pH 6.6 whereas the concentration of 0.40×10^{-6} g/cm^3 was used in the substrates of pH 6.0. The curds obtained by the incubation with chymosin were ultracentrifuged at 110 000 g/1 h (OTD COMBI centrifuge made by Sorvall Instrument, DuPont, rotor T-865, volume of centrifugation tube 11.5 cm^3). The time between the addition of chymosin and ultracentrifugation of the curds was twice as long as the previously determined RCT (rennet clotting time). In the case of the substrates heated at 72°C and 92°C, it amounted to 70.2 min and 80.4 min, respectively, at pH 6.6. However, it amounted to 62.0 min and 64.0 min at pH 6.0. The content of N, Ca and P was determined in the insoluble fractions which were treated as a curd matrix. The percentage of the electrophoretically separated proteins in the insoluble and soluble fractions was also determined. The proteins were separated with SDS-PAGE method (MINIPOL 2 apparatus, Poland) in the reducing conditions [Laemmli, 1970]. The standards of casein fractions separated and purified at the Chair of Food Biochemistry [Dziuba & Mioduszewska, 1997; Dziuba *et al.*, 1998] and the standards of whey proteins: α-lactalbumin (α-la) and β-lactoglobulin (β-lg) (BDH Chemicals Ltd Poople, UK, product No. 44171 and 44064), and bovine serum albumin (BSA, International Enzymes Ltd, UK, product No. 565E) were used for the identification. On the basis of densytometric analysis at 578 nm with VITATRON MPS (Modular Photometer System) apparatus, type 940.800 (Germany), their relative content was determined in relation to all the separated fractions.

Handling the results. All the analyses were performed three times. The results were presented as means. Standard error of mean (SEM) and significant differences (p=0.05) between the composition of unheated and heated (72°C/15 s; 92°C/60 s) solutions of milk protein concentrate and the composition of rennet (pH 6.6) and acid-rennet (pH 6.0) curds obtained from the analysed substrates were calculated. The statistical analysis was performed with STATISTICA PL program.

RESULTS AND DISCUSSION

Characteristics of the substrates

The content of total N, Ca and P in the concentrate solutions, from which the enzymatic curds (pH 6.6 and 6.0) were obtained, amounted to 5.24 (± 0.01) mg/cm^3, 0.94 (± 0.01) mg/cm^3, and 0.63 (± 0.01) mg/cm^3, respectively. Heating the solutions did not cause any significant changes (p=0.05) in the content of these components.

The curds were separated with ultracentrifugation assuming that the insoluble fraction contains proteins, Ca and P which take part in the formation of a gel network. Such analysis can be used to predict the properties of a gel but it cannot be compared to the distribution of milk components into curd and whey after syneresis [Creamer, 1985; Horne & Davidson, 1993; Lefebvre-Cases *et al.*, 1998].

Effect of heating on the composition of rennet curds

Heating the concentrate solutions did not affect the content of Ca and P in the insoluble fractions of rennet curd (pH 6.6) (Table 1). However, it contributed to a significant (p=0.05) increase in the share of N. An increase in the temperature of heating from 72°C to 92°C did not have any considerable influence on the content of N in the pellets. On the basis of the electrophoretic analysis of the insoluble proteins after the ultracentrifugation of the curds, it was found that their composition in the heated substrates changed (Figure 1). The increase in the temperature of heating contributed mainly to an increase in the share of β-lg and unidentified fractions which take the position between β-casein and β-lg in a gel. It limited the percentage of casein but did not influence the proportions between its forms significantly (p=0.05). It is confirmed by the analysis of the proteins in the soluble fractions (Figure 2). Heating the concentrate solutions at 92°C contributed to a decrease in the share of β-lg and BSA+Ig by 4.7% and 2.8%. The soluble fractions were predominated by the unidentified fractions which take the position between the standards of β-casein and β-lg after electrophoretic separation. These were probably proteose peptones whose presence was also found in the concentrate solutions during the analysis of proteins soluble at pH 4.6. α_s- and β-Caseins were not found among the proteins of the soluble fraction, although para-κ--casein was found. An increase in its share from 2.3% to 2.7% in the soluble fraction of the curds after heating the concentrate at 92°C, where larger dissociation of κ-casein/ /β-lg complexes [Singh, 1995] could happen, suggests that β-lg which forms complexes with para-κ-casein being a part

TABLE 1. The effect of heating on the content of N, Ca, and P in the pellet after ultracentrifugation (110 000 g/1 h) of the enzymatic curds obtained at pH 6.6 and 6.0.

Component	Mean ± SEM					
	pH 6.6			pH 6.0		
	unheated	72°C/15 s	92°C/60 s	unheated	72°C/15 s	92°C/60 s
Content of N, % total N	92.4^{b}± 0.0	92.7^{a}± 0.1	92.7^{a}± 0.1	92.4^{b}± 0.0	92.4^{b}± 0.0	92.4^{b}± 0.0
Content of Ca, % total Ca	88.7^{a}± 0.4	88.7^{a}± 0.4	88.8^{a}± 0.7	69.5^{b}± 0.4	69.8^{b}± 0.4	67.7^{c}± 0.3
Content of P, % total P	79.9^{a}± 0.5	79.9^{a}± 0.5	80.6^{a}± 0.0	66.2^{b}± 0.5	65.1^{bc}± 0.0	64.5^{c}± 0.0
Ca/P, w/w	1.67^{a}± 0.01	1.67^{a}± 0.01	1.69^{a}± 0.01	1.56^{c}± 0.01	1.60^{b}± 0.01	1.61^{b}± 0.01

Letters a, b, c (p=0.05) refer to the significance of differences between the content of the determined components in the unheated and heated (72°C/15 s; 92°C/60 s) substrates. The alphabetical order of the letters in columns was arranged with the decreasing values of the determined component.

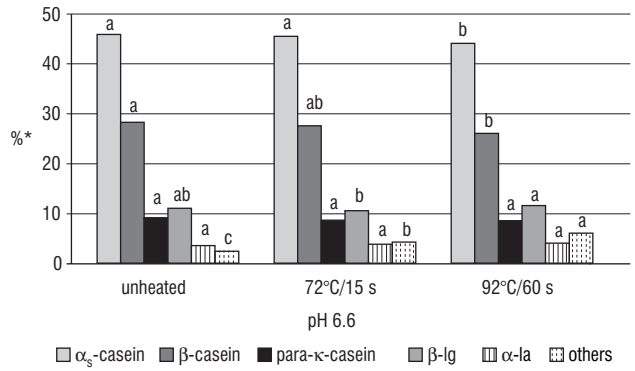

FIGURE 1. Effect of heating on the relative percentage of proteins in the pellet after ultracentrifugation (110 000 g) of rennet curds (pH 6.6). (* – calculated on the basis of densytometric analysis of the proteins separated electrophoretically and expressed in percentage of the sum of the separated fractions). Letters a, b, c (p=0.05) refer to the significance of differences between the content of the determined fractions in the unheated and heated (72°C/15 s; 92°C/60 s) substrates. The alphabetical order of the letters was arranged with the decreasing values of the determined fraction.

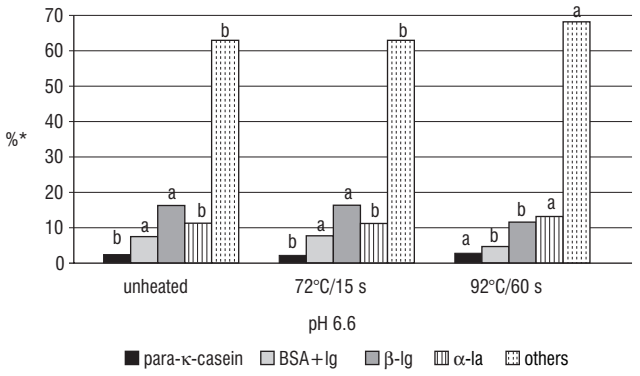

FIGURE 2. Effect of heating on the relative percentage of proteins in the fraction of rennet curds (pH 6.6) soluble at 110 000 g. (* – calculated on the basis of densytometric analysis of the proteins separated electrophoretically and expressed in percentage of the sum of the separated fractions). Letters a and b (p=0.05) refer to the significance of differences between the content of the determined fractions in the unheated and heated (72°C/15 s; 92°C/60 s) substrates. The alphabetical order of the letters was arranged with the decreasing values of the determined fraction.

of κ-casein [Oldfield *et al.*, 1998] can limit its incorporation into a gel network at pH 6.6.

Effect of heating on the composition of acid-rennet curds

Heating the substrates did not have any influence on the distribution of N between the insoluble and soluble fractions of the curds obtained at pH 6.0. On the other hand, it had a considerable (p=0.05) influence on the share of Ca and P in the pellets after ultracentrifugation of the substrates heated to 92°C/60 s. It decreased by 1.8% and 1.7%, respectively, compared to the unheated substrates (Table 1). After heating at 72°C, the differences were insignificant. Larger dissociation to the soluble phase of Ca and P after pH of the substrates heated at 92°C was lowered can confirm the effect of heating at pH 7.1 on the disintegration and larger diversity of the sizes of the protein particles which take part in the formation of a gel network [Singh, 1995; Singh & Latham, 1993; Noh & Richardson, 1989]. Heating the substrates contributed to a significant increase in the above mentioned Ca/P ratio in the insoluble fractions of the curds obtained after proteolysis (Table 1). The increase in the Ca/P ratio can result not only from Ca function in joining disintegrated particles [Walstra *et al.*, 1985; Zoon *et al.*, 1988] but also from the change in the relative percentage of proteins forming a gel network. Electrophoretic analysis of the proteins in the insoluble fractions of acid-rennet curds (Figure 3) indicates a higher, irrespective of heating the substrates, relative percentage of whey proteins, mainly β-lg, than in the corresponding fractions of rennet curds (Figure 1). It is confirmed by the analysis of the soluble fractions of the curds (Figure 4). The content of β-lg, α-la and BSA+Ig in the soluble fractions of the curds obtained from the substrates heated

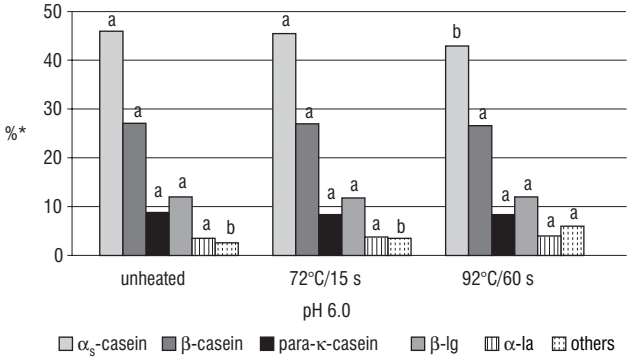

FIGURE 3. Effect of heating on the relative percentage of proteins in the pellet after ultracentrifugation (110 000 g) of acid-rennet curds (pH 6.0). (* – calculated on the basis of densytometric analysis of the proteins separated electrophoretically and expressed in percentage of the sum of the separated fractions). Letters a and b (p=0.05) refer to the significance of differences between the content of the determined fractions in the unheated and heated (72°C/15 s; 92°C/60 s) substrates. The alphabetical order of the letters was arranged with the decreasing values of the determined fraction.

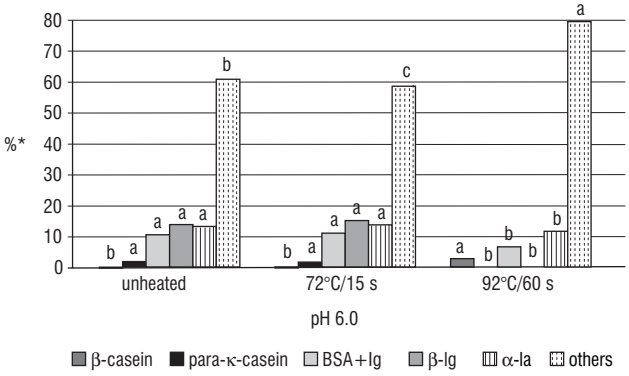

FIGURE 4. Effect of heating on the relative percentage of proteins in the fraction of acid-rennet curds (pH 6.0) soluble at 110 000 g. (* – calculated on the basis of densytometric analysis of the proteins separated electrophoretically and expressed in percentage of the sum of the separated fractions). Letters a, b, c (p=0.05) refer to the significance of differences between the content of the determined fractions in the unheated and heated (72°C/15 s; 92°C/60 s) substrates. The alphabetical order of the letters was arranged with the decreasing values of the determined fraction.

at 92°C decreased by 13.7%, 1.6% and by 3.9%, respectively. The share of β-casein in the proteins of the soluble fraction after ultracentrifugation of the curds from the substrates heated at 92°C was found at the level of 2.7%. Its trace amounts were also found in the proteins of the soluble fraction of the curds obtained from the unheated substrates and the substrates heated at 72°C. The incorporation of β-casein, which dissociates at pH 6.0, into a gel network after proteolysis could contribute to a lower, compared to milk, concentration of Ca in the concentrate. At the same time, a contribution of heating the substrates to a decrease in the share of para-κ-casein in the soluble fractions of acid-rennet curds was observed. In the case of the substrates heated at 92°C only trace amounts of para-κ-casein and β-lg were found in the soluble fractions. It suggests that higher denaturation of β-lg can influence the formation, also with κ-casein of protein aggregates, which at pH 6.0 take part in the formation of acid-rennet gel. The decrease in the share of total Ca in acid-rennet curds from the substrates heated at 92°C indicates a limitation of Ca function in their formation.

CONCLUSIONS

1. Heating the solutions of the UF milk protein concentrate did not affect the share of Ca and P in the rennet gel network. This, however, determined the increase in the share of N represented by whey proteins.

2. Heating the solutions of the UF milk protein concentrate contributed to a limitation of the share of Ca, P and β-casein in the acid-rennet gel network and to the increase in the share of whey proteins and para-κ-casein.

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WPŁYW OGRZEWANIA ROZTWORÓW KONCENTRATU BIAŁEK MLEKA NA SKŁAD BIAŁEK, CA I P W SIECI ŻELU PO KOAGULACJI PODPUSZCZKOWEJ I KWASOWO-PODPUSZCZKOWEJ

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Celem pracy było zbadanie wpływu ogrzewania wodnych (pH 7,1) roztworów koncentratu białek mleka, otrzymanego metodą suszenia rozpyłowego retentatu po ultrafiltracji, na skład białek tworzących pod wpływem chymozyny sieć żelu podpuszczkowego i kwasowo-podpuszczkowego oraz udział w niej Ca i P. Skrzepy uzyskano przy pH 6,6 i 6,0 z wodnych roztworów odpowiednio przygotowanych (nieogrzewane, ogrzewane w 72°C/15 s, ogrzewane w 92°C/60 s) po doprowadzeniu pH kwasem mlekowym roztworów koncentratu o stężeniu białka 33 g/dm^3. Oznaczono zawartość N ogółem, Ca i P w substratach oraz w nierozpuszczalnych po ultrawirowaniu (110 000 g/1 godz.) frakcjach skrzepów. Na podstawie rozdziału elektroforetycznego białek, w oparciu o analizę densytometryczną, ustalono ich względny udział (%) w nierozpuszczalnych i rozpuszczalnych frakcjach otrzymanych po ultrawirowaniu skrzepów. Frakcje nierozpuszczalne potraktowano jako matrycę żelu. Stwierdzono, że ogrzewanie roztworów koncentratu nie miało wpływu na udział Ca i P w sieci żelu podpuszczkowego, wpływało natomiast na ograniczenie ich udziału w sieci żelu kwasowo-podpuszczkowego.

Wpływ ogrzewania substratów na skład białek tworzących sieć żelu był uzależniony od pH koagulacji. W przypadku koagulacji podpuszczkowej dotyczył wzrostu udziału białek serwatkowych i był związany ze wzrostem udziału N ogółem w matrycy skrzepów. Natomiast w przypadku koagulacji kwasowo-podpuszczkowej dotyczył wzrostu udziału białek serwatkowych i kazeiny-para-κ przy jednoczesnym ograniczeniu zawartości kazeiny-β. Zmiana składu białek tworzących sieć żelu w substratach ogrzewanych nie miała wpływu na zawartość N ogółem w tej frakcji.