

## CHARACTERISTICS OF NITROGEN COMPOUNDS AND NUTRITIVE VALUE OF WHEY AND PERMEATE OBTAINED IN THE PRODUCTION OF COTTAGE CHEESES

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The study was aimed at determining the effect of calcium chloride addition, temperature of milk pasteurization and a separation technique of milk components (centrifugation and ultrafiltration) on the content of nitrogen compounds and nutritive value of protein of whey and permeate obtained in the production process of cottage cheeses. Whey obtained after acid coagulation of milk supplemented with calcium chloride at a dose of 0.04% and pasteurised at a temperature of 90°C/15 s, was characterised by statistically significantly lower (at  $\alpha=0.05$ ) contents of total nitrogen compounds, cysteine-cystine, leucine and lysine, compared to the whey obtained from milk pasteurized at 75°C/15 s (with the addition of calcium chloride). Electrophoresis on polyacrylamide gel (Urea-PAGE) demonstrated reduced concentrations of  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin in nitrogen compounds of whey as a result of their interaction with casein upon high pasteurisation of milk enriched with calcium ions. Due to the lower content of protein nitrogen compounds and essential amino acids compared to whey, including the limiting amino acid – leucine, the permeate was characterised by a lower nutritive value of protein expressed by values of chemical score (CS) and essential amino acid index (EAAI).

### INTRODUCTION

Whey and permeate, obtained in the technological process of cottage cheese making, are by-products whose usability for successive processing is determined, to a great extent, by the content of nitrogen compounds of milk, mainly of proteins – highly valuable from the nutritional point of view [McIntosh *et al.*, 1998; Renner & Abd-El-Salam, 1991]. The basic technological processes in cottage cheese making are milk protein coagulation and separation of cottage cheese bulk with the separation of whey or permeate. In traditional technology, protein separation from milk proceeds through acid coagulation of milk pasteurised at a temperature of 75°C/15 s, at the isoelectric point of casein (4.5 pH), as a result of souring run by lactic acid bacteria (LAB). After protein coagulation, the cheese bulk may be separated from whey with the method of centrifugation, which enables 75% utilisation of milk proteins in cottage cheese, or with the technique of ultrafiltration which enables 95% retention of protein in a product [Nielsen, 1998; Szpendowski *et al.*, 2004]. A higher degree of protein utilisation in cottage cheeses, compared to the traditional method, is achieved through milk supplementation with calcium ions (an addition of 0.04% CaCl<sub>2</sub>) and its high pasteurisation (90°–95°C/15 s) [Szpendowski *et al.*, 2005]. While heating milk to a temperature over 80°C, whey proteins, mainly  $\beta$ -

lactoglobulin and  $\alpha$ -lactalbumin, interact with casein [Carbonaro *et al.*, 1998; Singh & Waungana, 2001]. The thermal aggregates of casein and whey proteins induced may be then coagulated with the acidic method, which enables reaching a considerably higher protein retention in cottage cheeses and reduced protein losses in whey [Szpendowski *et al.*, 2005]. Both the preparation method of milk before its coagulation (milk supplementation with calcium ions and pasteurisation temperature) as well as the separation method of cheese bulk are likely to affect the content of nitrogen compounds in milk that are not utilised in the product, but migrate from whey to permeate.

The study was aimed at determining the effect of calcium chloride addition, the temperature of milk pasteurisation and the technique of cheese bulk separation on the content of nitrogen compounds and the nutritive value of whey and permeate proteins.

### MATERIAL AND METHODS

The raw material for the production of cottage cheeses was milk with 1.6% fat content pasteurised at a temperature of 75°C for 15 s or supplemented with 0.04% of CaCl<sub>2</sub> and pasteurised at a temperature of 90°C for 15 s. After cooling the milk to a temperature of 28°C, it was injected with lyophilised inoculum of pure cultures of lactic acid bacteria

at a dose that enabled obtaining a curd with the acidity of pH 4.6 within 16 h. The curd was then centrifuged to separate whey or transferred to an ultrafiltration apparatus to separate permeate. The separation process of cottage cheese bulk with the centrifugation method was carried out at a temperature of 40°C at centripetal acceleration of 6000 g. In contrast, separation with the UF technique was carried out at a temperature of 40°C and a pressure of 0.5 mPa, with the use of an apparatus equipped with polysulfone capillary membranes characterised by the cut-off point of 15.000 daltons. The separated whey and permeate were cooled to a temperature of 4–5°C and subjected to analyses. Six series of large-scale production were carried out for each technological variant. Analyses were conducted on: (1) whey after coagulation of milk pasteurised at a temperature of 75°C/15 s and separation of cottage cheese bulk with the centrifugation method (variant I); (2) whey after coagulation of milk supplemented with CaCl<sub>2</sub> at a dose of 0.04% and pasteurised at a temperature of 90°C/15 s and separation of cottage cheese bulk with the ultrafiltration technique (variant II); and (3) permeate after coagulation of milk pasteurised at a temperature of 75°C/15 s and separation of cottage cheese bulk with the ultrafiltration technique (variant III).

Samples were assayed for protein content – with the standard method according to AOAC [1984] and the content of nitrogen compounds soluble in 12% trichloroacetic acid (TCA) [Alais, 1963]. The content of protein nitrogen compounds was computed from a difference between the contents of total nitrogen compounds and those soluble in 12% TCA.

Protein fractions were characterised with the use of electrophoresis on a polyacrylamide gel with urea (Urea-PAGE) [Mayer, 2001]. Briefly, 10 mg of a defatted and dried sample were dissolved in 1 mL of 0.01 mol/L triglycine buffer, pH 8.3. Electrophoretic separations were carried out with polyacrylamide gel, using an electrophoretic kit by Pharmacia FBE-3000. Protein-peptide fractions were stained with a 0.15% dye solution of Coomassie brilliant blue R-250 in a mixture of water, ethanol and acetic acid (70:30:7 v/v/v). Separations were run at the voltage of *ca.* 200 V for 42 min, with voltage acceleration to *ca.* 400 V. The separation lasted for *ca.* 5.5 h. The amount of an injected sample was 10 µL, at a temperature of 15°C. Protein capacity for dye binding was determined with the densitometric method. Analyses of electrophoretic patterns were carried out based on standards of casein and whey proteins, *i.e.* α<sub>s</sub>-casein, β-casein, κ-casein as well as β-lactoglobulin and α-lactalbumin, respectively. Dyed electrophorograms were

photographed and photocolourimetrically in a VITATRON TYPE 940, 800 MPS apparatus.

Concentrations of amino acids were determined with an automatic amino acid analyser High Performance Analyzer System 6300 made by BECKMAN using a 120-mm column [Slocum *et al.*, 1989]. At the assay of the total amino acids, protein hydrolysis was carried out in 4 mol/L sulfuric acid. Tryptophane was determined after base hydrolysis of proteins in 5 mol/L sodium hydroxide [Opieńska-Blauth *et al.*, 1963], whereas sulfuric amino acids – according to Schram *et al.* [1954]. Based on amino acid composition, the Chemical Score (SC) [Block & Mitchell, 1946] and Essential Amino Acid Index (EAAI) [Oser, 1951] were calculated, compared to the amino acid composition of the standard according to WHO/FAO of 1991 [Gawęcki, 1998].

## RESULTS AND DISCUSSION

The chemical analysis demonstrated the highest concentration of total nitrogen compounds (0.087%) in whey obtained after coagulation of milk pasteurised at a temperature of 75°C/15 s and cheese bulk separation with the centrifugation method (Table 1). The addition of calcium chloride (0.04%) to milk and its high pasteurisation (90°C/15 s) in the production process of cottage cheese resulted in a statistically significantly lower content of total nitrogen compounds (0.052%) in whey (at  $\alpha=0.05$ ). On the contrary, the application of the ultrafiltration process of cottage cheese slurry enabled the obtaining of permeate with 0.029% of total nitrogen compounds. No statistically significant correlation (at  $\alpha=0.05$ ) was observed between the processing method of milk and the separation methods of cheese bulk and the content of non-protein nitrogen compounds (soluble in 12% TCA). The levels of those compounds in wheys and permeate analysed appeared to be similar (0.024–0.025%). In contrast, a significantly higher (at  $\alpha=0.05$ ) concentration of non-protein nitrogen compounds, expressed in percentage of total nitrogen compounds, was demonstrated in permeate (86.2%), compared to their level in wheys (27.6–44.2%). This indicates that permeate contained as little as 0.004% of protein nitrogen compounds, which constituted 13.8% of all nitrogen compounds in that raw material. It may be assumed, therefore, that negligible amounts of whey proteins, mainly α-lactalbumin with a molecular mass of 14 200 daltons, may have penetrated to the permeate through a membrane with a cut-off point of 15 000 daltons [Kinghorn *et al.*, 1995]. The whey obtained from milk pasteurised at a temperature of 75°C/15 s contained nearly twice as much protein nitrogen compounds

TABLE 1. Concentration of nitrogen compounds in wheys and permeate (n=6).

Preparation of milk and whey separation method			Nitrogen compounds (%)				
CaCl <sub>2</sub> addition to milk (%)	Milk pasteurisation (temp./time)	Separation (centrifugation/ultrafiltration)	total	protein	protein in % of total nitrogen compounds	soluble in 12% TCA	soluble in 12% TCA in % of total nitrogen compounds
-	75°C/15 s	centrifugation	0.087 <sup>A</sup> ±0.05	0.063 <sup>A</sup> ±0.02	72.4 <sup>A</sup> ±2	0.024 <sup>A</sup> ±0.02	27.6 <sup>A</sup> ±2
0.04	90°C/15 s	centrifugation	0.052 <sup>B</sup> ±0.03	0.029 <sup>B</sup> ±0.02	55.8 <sup>B</sup> ±2	0.023 <sup>A</sup> ±0.02	44.2 <sup>B</sup> ±3
-	75°C/15 s	ultrafiltration	0.029 <sup>C</sup> ±0.02	0.004 <sup>C</sup> ±0.01	13.8 <sup>C</sup> ±1	0.025 <sup>A</sup> ±0.01	86.2 <sup>C</sup> ±3

A, B, C – values in the same column are statistically significantly different ( $\alpha=0.05$ ). A, A or B, B – values in the same column are not statistically different ( $\alpha=0.05$ ).

(0.063%), as the whey separated from coagulated milk enriched with calcium chloride and subjected to high pasteurisation (0.029%).

In the traditional technology of cottage cheese making, involving pasteurisation of milk at a temperature of 75°C/15 s and separation of cheese bulk with the centrifugation method, *ca.* 25% of nitrogen compounds of milk are observed to penetrate to whey, 65% of which are whey proteins and negligible amount of sprayed casein [McIntosh *et al.*, 1998]. The application of high pasteurisation of milk is likely to affect the formation of complexes between  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin and casein through disulfide-, hydrogen- and ionic bonds with the presence of amorphous calcium phosphate [Visser *et al.*, 1986]. As a result of heating the milk, whey proteins may form stable complexes, mainly with fractions of  $\alpha_{s1}$ -casein,  $\beta$ -casein and  $\kappa$ -casein [Oldfield *et al.*, 2000]. The addition of calcium ions to milk prior to high pasteurisation increased the size area of casein micelles, intensified the polymerisation and aggregation of whey proteins, and consequently increased the effect of the interaction between casein and whey protein aggregates [Britten & Giroux, 2001]. In the heating process of milk, soluble calcium transforms into a colloidal form that participates in the formation of a casein complex with whey proteins [Singh & Waungana, 2001]. Milk supplementation with calcium ions and its high pasteurisation enable reaching a higher degree of milk protein utilisation in the production technology of cottage cheeses, ripening cheeses and protein preparations as well as reducing the protein content of whey [Szpendowski *et al.*, 2005].

The content of nitrogen compounds in permeate is mainly determined by parameters of the ultrafiltration method: the type of membranes (pore diameter), temperature and pressure [Nielsen, 1998; Renner & Abd-El-Salam, 1991]. The entire pool of nitrogen compounds with molecular masses higher than the cut-off point, which characterises the pore sizes of membranes and molecular mass of compounds retained, is accumulated in retentate [Renner & Abd el Salam, 1991]. In a properly-run ultrafiltration process, solely non-protein nitrogen compounds, including peptides, free amino acids, urea, purine bases, creatine and non-nitrogen compounds: lactose, mineral salts and acids, are observed to transfer to permeate [De Witt, 2001]. The mean content of nitrogen compounds in permeate fluctuates from 0.048% to 0.083% [Rattary *et al.*, 1997]. The nitrogen compounds of permeate are constituted in 40.96% by non-protein compounds, in 28.9% by whey proteins and in 0.72% by casein [Punidades & Rizvi, 1998].

The electrophoretic analysis of proteins in the analysed samples of wheys and permeate demonstrated highly differentiated patterns of separated fractions (Figure 1). Separately dyed bands of casein and whey proteins in standards facilitated the identification of particular fractions on an electrophoretic diagram. The electrophoretic image of permeate displayed practically no protein fractions, both in the case of casein and whey proteins. This points to a more effective utilisation of milk proteins in the production of cottage cheeses with the ultrafiltration technique, compared to the traditional technology involving centrifugation. The low concentration of nitrogen compounds in permeate

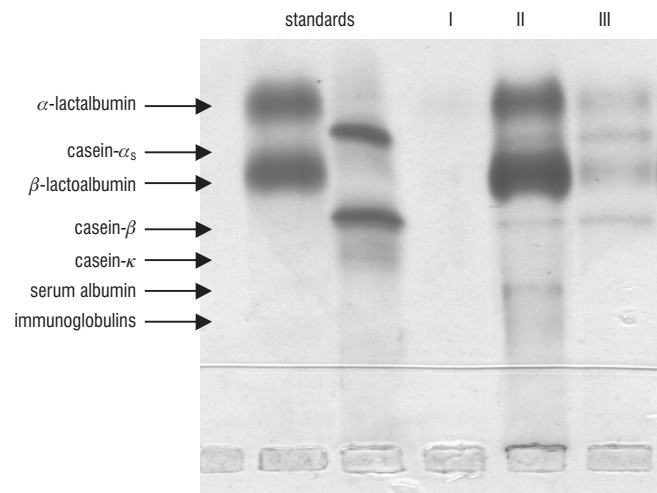


FIGURE 1. Electrophoretic separation of milk proteins in polyacrylamide gel (Urea-PAGE).

I – permeate after coagulation of milk pasteurised at 75°C/15 s (ultrafiltration), II – whey after coagulation of milk pasteurised at 75°C/15 s (centrifugation), III – whey after coagulation of milk supplemented with 0.04% CaCl<sub>2</sub> and pasteurised at 90°C/15 s (centrifugation).

hindered obtaining a clear image of separated protein fractions. It is probable that the ultrafiltration process could have involved a denaturation process of whey proteins, involving immunoglobulins and serum albumin especially and, to a lesser extent,  $\alpha$ -lactalbumin which are susceptible to structural changes as effected by technological factors [Law *et al.*, 1994]. In the electrophoretic image of proteins of whey separated with the centrifugation technique in the production process of cottage cheese, there were bands detected corresponding mainly to  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin as well as less-dyed bands corresponding to fractions of  $\alpha_s$ -casein,  $\beta$ -casein,  $\kappa$ -casein, serum albumin and immunoglobulins, which is consistent with the image of electric separation of whey presented by Elgara *et al.* [2000]. The main nitrogen compounds of whey obtained after cottage cheese making include:  $\beta$ -lactoglobulin – 50%,  $\alpha$ -lactalbumin – 12%, serum albumin – 5%, immunoglobulin – 10%, lactoferrin – 1%, and proteose-peptone – 23% [Kinghorn *et al.*, 1995]. Results of the electrophoretic analysis of that whey indicate that during centrifugation of cheese bulk, a part of pulverised casein curd transfers to whey. The electrophoretic image of whey obtained after coagulation of milk supplemented with 0.04% of calcium chloride and subjected to high pasteurisation (90°C/15 s) displays a considerably lower number bands corresponding to  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin, compared to the whey obtained from coagulated milk pasteurised at a temperature of 75°C/15 s. The results of the electrophoretic separation of whey proteins demonstrate that in milk heated to high temperatures and supplemented with calcium ions, interactions occurred between whey proteins and casein. The whey obtained in this process was remarkably depleted in whey proteins, compared to the whey separated as a result of the traditional technological process of cottage cheese making.

The results obtained indicated that the method of milk preparation before protein coagulation (pasteurisation temperature, the addition of calcium chloride) as well as the method of cheese bulk separation affected the contents of

amino acids in the wheys and permeate examined (Table 2). In a group of essential amino acids, no statistically significant differences (at  $\alpha=0.05$ ) were observed between the contents of phenylalanine (4.91–4.98 g/16 g N), threonine (6.15–6.57 g/16 g N) nor tyrosine (4.71–5.15 g/16 g N) in the wheys and permeate analysed. In contrast, permeate was demonstrated to contain statistically significantly less (at  $\alpha=0.05$ ) cystine-cysteine, leucine, isoleucine, methionine, tryptophan, valine and lysine, compared to whey obtained from milk pasteurised at a temperature of 75°C/15 s. On the other hand, the whey obtained from milk supplemented with calcium chloride and pasteurised at a temp. of 90°C/15 s, compared to that separated from milk pasteurised at a temp. of 75°C/15 s, was characterised by lower contents of cystine-cysteine (1.82 g/16 g N *vs.* 2.75 g/16 g N), leucine (8.45 g/16 g N *vs.* 9.73 g/16 g N) and lysine (7.11 g/16 g N *vs.* 8.71 g/16 g N). The total content of essential amino acids in the permeate appeared to be considerably lower (38.83 g/16 g N) than in the wheys examined (49.62–53.61 g/16 g N).

According to Nielsen [1998], compared to casein, the whey proteins contain higher amounts of essential amino acids, including mainly cysteine, leucine, isoleucine and lysine. Hence, the higher the degree of whey protein utilisation in cottage cheese, the lower the content of essential amino acids was observed in whey or permeate obtained after coagulation and separation of protein. The heating processes of whey proteins applied in the dairy industry, namely, pasteurisation and UHT sterilisation, exert the

greatest impact on the reduction in the contents of sulfuric amino acids – methionine and cysteine-cystine as well as alanine, leucine and tyrosine [Carbonaro *et al.*, 1998].

Differences in amino acid composition of wheys and permeate resulted in a diversified nutritive value of protein. The analysis of the amino acid composition of wheys (Table 2) demonstrated that they contain more essential amino acids than the WHO/FAO standard of 1991 [Gawęcki, 1998], hence the chemical score (CS) and the essential amino acid index reached 100 units. The amino acids that limited the nutritive value of permeate appeared to be leucine, whereas the chemical score (CS) accounted for 75.2 and the essential amino acid index (EAAI) for 94.0.

According to Chojnowski [1985], the amino acid that limited the nutritive value of whey protein concentrates was valine. The value of the chemical score (CS) of whey proteins, calculated with reference to hen egg white as a standard, ranged from 72 to 73, whereas that of the essential amino acid index (EAAI) ranged from 88 to 93, depending on the methods applied for coagulation and isolation of whey proteins [Chojnowski, 1985].

## CONCLUSIONS

1. The method of milk preparation before protein coagulation (CaCl<sub>2</sub> addition and pasteurisation temperature) as well as the method of cheese bulk separation (centrifugation or ultrafiltration), applied in the production process of cottage cheeses exerted a significant effect on the content

TABLE 2. Contents of amino acids in wheys and permeate (n=6).

Amino acid (g/16 g N)	Whey after coagulation of milk pasteurised at 75°C/15 s (centrifugation)	Whey after coagulation of milk supplemented with 0.04% CaCl <sub>2</sub> and pasteurised at 90°C/15 s (centrifugation)	Permeate after coagulation of milk pasteurised at 75°C/15 s (ultrafiltration)
Alanine	4.57 <sup>A</sup>	3.45 <sup>B</sup>	3.19 <sup>C</sup>
Arginine	3.67 <sup>A</sup>	3.52 <sup>A</sup>	2.93 <sup>B</sup>
Cystine-cysteine	2.75 <sup>A</sup>	1.82 <sup>B</sup>	0.94 <sup>C</sup>
Phenylalanine	4.98 <sup>A</sup>	4.97 <sup>A</sup>	4.91 <sup>A</sup>
Glycine	2.06 <sup>A</sup>	2.11 <sup>A</sup>	1.99 <sup>A</sup>
Histidine	1.91 <sup>A</sup>	1.95 <sup>A</sup>	1.97 <sup>A</sup>
Aspartic acid	9.87 <sup>A</sup>	9.97 <sup>A</sup>	9.13 <sup>A</sup>
Glutamic acid	19.82 <sup>A</sup>	19.94 <sup>A</sup>	20.77 <sup>A</sup>
Leucine	9.73 <sup>A</sup>	8.45 <sup>B</sup>	4.96 <sup>C</sup>
Isoleucine	5.82 <sup>A</sup>	5.64 <sup>A</sup>	4.57 <sup>B</sup>
Lysine	8.71 <sup>A</sup>	7.11 <sup>B</sup>	4.94 <sup>C</sup>
Methionine	2.67 <sup>A</sup>	2.78 <sup>A</sup>	1.89 <sup>B</sup>
Proline	8.55 <sup>A</sup>	8.72 <sup>A</sup>	9.00 <sup>A</sup>
Serine	6.91 <sup>A</sup>	6.74 <sup>A</sup>	6.46 <sup>A</sup>
Threonine	6.57 <sup>A</sup>	6.42 <sup>A</sup>	6.15 <sup>A</sup>
Tryptophan	2.36 <sup>A</sup>	2.41 <sup>A</sup>	1.1 <sup>B</sup>
Tyrosine	4.71 <sup>A</sup>	4.74 <sup>A</sup>	5.15 <sup>A</sup>
Valine	5.31 <sup>A</sup>	5.28 <sup>A</sup>	4.22 <sup>B</sup>
Sum of essential amino acids	53.61	49.62	38.83
Chemical score	100	100	75.2 (leucine)
Essential amino acid index	100	100	94.0

A, B, C – values in the same column are statistically significantly different ( $\alpha=0.05$ ). A, A or B, B – values in the same column are not statistically different ( $\alpha=0.05$ ).



and quality of nitrogen compounds penetrating from milk to whey or permeate.

2. Whey obtained after coagulation of milk supplemented with 0.04% CaCl<sub>2</sub> and pasteurised at a temperature of 90°C/15 s and separated with the centrifugation method, compared to the whey obtained from milk pasteurised at 75°C/15 s (without calcium chloride addition), was characterised by lower contents of  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin as well as statistically significantly higher (at  $\alpha=0.05$ ) contents of protein nitrogen compounds, cysteine-cystine, leucine and lysine.

3. Permeate obtained upon ultrafiltration of cottage cheese slurry, due to a lower content of protein nitrogen compounds and essential amino acids (including the limiting amino acid – leucine), compared to the whey, was characterised by a lower nutritive value of protein expressed by the values of chemical score (CS) and essential amino acid index (EAAI).

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## CHARAKTERYSTYKA ZWIĄZKÓW AZOTOWYCH I WARTOŚCI ODŻYWCZEJ SERWATKI ORAZ PERMEATU OTRZYMYWANYCH W CZASIE PRODUKCJI SERÓW TWAROGOWYCH

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Badano wpływ dodatku chlorku wapnia i wysokości temperatury pasteryzacji mleka oraz techniki separacji składników mleka (wirówkowa i ultrafiltracja) na związki azotowe oraz wartość odżywczą białka serwatki i permeatu, otrzymywanych w czasie produkcji serów twarogowych. W serwatce otrzymanej po koagulacji kwasowej mleka wzbogaconego chlorkiem wapnia w ilości 0.04% i pasteryzowanego w temperaturze 90°C/15 s, stwierdzono statystycznie istotnie niższą (dla  $\alpha=0.05$ ) zawartość związków azotowych ogółem (0.052%, w tym 55.8% związków białkowych) oraz zawartością cysteiny-cystyny, leucyny i lizyny, w porównaniu z serwatką uzyskaną z mleka pasteryzowanego w temperaturze 75°C/15 s (bez dodatku chlorku wapnia), która zawierała odpowiednio 0.063% związków azotowych ogółem, w tym 72.43% związków białkowych. Permeat, otrzymywany w procesie ultrafiltracji gęstwy twarogowej, zawierał 0.029% związków azotowych ogółem, z czego 13.8% stanowiły białka (tab. 1). Elektroforeza w żelu poliakrylamidowym (Urea-PAGE) wykazała obniżenie udziału  $\beta$ -laktoglobuliny i  $\alpha$ -laktoalbuminy w związkach azotowych serwatki, jako efektu interakcji tych białek z kazeiną, pod wpływem wysokiej pasteryzacji (90°C/15 s) mleka wzbogaconego w jony wapniowe (rys 1). Permeat, ze względu na niższą, w porównaniu z serwatką, zawartość związków azotowych białkowych oraz aminokwasów egzogennych, w tym aminokwasu ograniczającego – leucyny, charakteryzował się niższą wartością odżywczą białka, określoną wskaźnikiem aminokwasu ograniczającego (CS) – 75.2 i zintegrowanym wskaźnikiem aminokwasów egzogennych (EAAI) – 94.0 (tab. 2).