

Primary proteolysis of white brined cheese prepared from raw cow milk monitored by high-molarity Tris buffer SDS-PAGE system

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Abstract

The aim of this work was to investigate primary proteolysis of white brined cow cheese prepared from raw milk by SDS-electrophoretic method based on high-molarity Tris buffer system and to correlate with the results of other commonly used parameters. Proteolytic changes of white brined cow cheese were monitored by three parameters: total protein, water soluble proteins and degree of proteolysis. Changes of major casein fractions were followed by SDS-electrophoretic system in reducing conditions. The total protein content and moisture content of white brined cow cheese were significantly affected by ripening. Ripening in brine increased water soluble proteins and degree of proteolysis. Major caseins were differently resistant to proteolysis; α -CN was more susceptible than β -CN. The α -CN content was highly and negatively correlated with time of ripening and water soluble proteins whereas no significant correlation ($p < 0.05$) between β -CN content and these parameters was found. Also, a strong significant correlation ($p < 0.05$) between the amount of low molecular weight products and time of ripening, water soluble proteins and α -CN content was observed. SDS-PAGE method used in this study could be useful for monitoring the white cow cheese proteolysis.

Key words: proteolysis, SDS-PAGE, cow cheese

Introduction

White cheese in brine is the most widely manufactured and consumed cheese variety in Serbia. Usually it is made as artisanal cheese from raw or thermally treated cow or sheep milk, but rarely from goat milk. In general, this type of cheese is characterised by high acidity, sharp and salty flavour.

Cheese and cheese ripening are dynamic systems that are chemically, microbiologically and enzymatically complex (Mallatou et al., 2004). One of the major biochemical processes during cheese ripening is proteolysis. Due to the action of rennet enzymes, proteinases and peptidases from starter bacteria, secondary microflora and indigenous milk enzymes, peptides and amino acids are released, which influences the flavour and texture of final products. Each

type of cheese is characterised by specific way of proteolysis. Specificity of white brined cheese is that the ripening occurs in salt brine, usually for two or three months. In addition, cheese preservation was also achieved.

Proteolysis of white brined cheese has been studied extensively. Numerous authors (Sarić et al., 2002; Picon et al., 2010; Kirmaci et al., 2011; Yasar and Guzler, 2011; Radulović et al., 2011) characterized proteolysis of white brined cow cheeses prepared from thermally-treated milk. Less attention has been paid to investigation of cow cheeses prepared from raw milk. The objectives of previous investigations conducted by Sarić et al. (2002) and Hayaloglu et al. (2008) were proteolysis of traditional Mediterranean varieties of cheese prepared

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from raw cow milk. Yet, in these investigations, proteolysis of white brined cheese was monitored by standard parameters such as water soluble protein or total protein content, trichloroacetic acid-soluble (TCA) and phosphotungstic acid-soluble nitrogen (PTA) etc. usually combined with urea-polyacrylamide gel electrophoresis (urea-PAGE) or other chromatographic techniques. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions was rarely used although it was suggested as a useful technique for goat milk protein separation (Van Hekken et al., 2004; Pesic et al., 2012; Barać et al., 2013).

In our previous work (Barać et al., 2013), proteolysis of white brined goat cheese prepared from raw milk was characterized by SDS-PAGE method based on high-molarity Tris buffer system. Since, cow and goat milk have different protein composition and rennet clotting properties (Park, 2007), proteolysis of cheese might be also different. According to our knowledge, no studies were made to compare proteolytic changes during ripening of cow white brined cheeses made from raw milk under the same conditions. Thus, the objective of this investigation was to characterize primary proteolytic changes of white brined cow cheese, prepared under the same conditions as previously described for goat cheese, and to compare them with the results obtained by high-molarity Tris buffer SDS-PAGE system.

Material and methods

Milk analysis and cheese making

White brined cheese was produced from raw cow milk according to completely identical procedure as was used for goat cheese (Barać et al., 2013). The following methods were used to determine the composition of milk: total protein (TN x 6.38), (AOAC, 1999); dry matter, (IDF, 1982); fat, (Ardö and Polychroniadou, 1999); lactose, (FIL-IDF, 1974). The pH was measured with pH meter (Consort, Belgium).

Raw milk was tempered at 34 °C for 30 minutes and rennet (Maxiren, DSM, Denmark) was added in concentration of 0.014 g x L⁻¹. No starter cultures were added. Coagulation took place within 40 min at 32-33 °C. Once curdling was completed, the

cheese mass was carefully transferred from cheese vats into the mould. After about 2 h of draining without pressing, the cheese curd was cut into pieces of 10x10x3 cm and dry salted with 3.0 % NaCl. The next day, cheese was placed into plastic cans and covered with brine (8 % solution of NaCl). Ripening was conducted at 13 °C during 60 days. During this period, cheese was sampled every 10 days and frozen. Each time when sample was taken, brine was partly replaced. For these investigations, process of cheese making was performed twice.

Analysis of cheese

Total nitrogen content of cheese samples was determined according to Kjeldahl method (AOAC, 1999). Total protein (TP) was determined as total nitrogen x 6.38 and was expressed as total protein in dry matter. Dry matter content was measured by the oven drying method at 105 °C (IDF, 1982). Fat content was measured by the Van Gulik-Gerber method (Ardö and Polychroniadou, 1999). The pH of cheeses was measured using a pH meter (Consort, Belgium) in slurry prepared by dispersing 5 g of grated cheese in 10 ml of deionised water.

Assessment of proteolysis

Proteolytic changes of white brined cheese were monitored by three parameters: total protein, water soluble proteins and degree of proteolysis (DP). The amount of water soluble protein was determined in extracts prepared as following: the amount of 5 g of previously homogenized cheese was extracted in 50 mL of miliQ water for 60 minutes. Then, suspension was centrifuged (at 6000 x g, Janetzki, Czech Republic) for 60 minutes, to obtain a completely clear supernatant. Supernatant was carefully separated and nitrogen content was determined by Kjeldahl method (AOAC, 1999). Also, the same amount of cheese was used for total nitrogen content determination. Total and water soluble protein content was determined as total nitrogen x 6.38 per dry matter.

Degree of proteolysis (DP) was estimated by measuring the amount of soluble nitrogen in 10 % trichloroacetic acid according to Kim et al. (1990). DP was calculated using formula:

% DP = (Soluble nitrogen in 10 % TCA/total nitrogen) x 100.

The amount of nitrogen soluble in TCA was determined by extracting of 5 g of homogenized cheese with 20 mL of 10 % TCA for 30 min, centrifuging at 12000 x g for 15 min. Nitrogen content was determined in the supernatant. All analyses were determined in triplicate.

SDS-PAGE

Cheese proteins were separated by SDS-PAGE using method of Fling and Gregerson (1986). Analysis were performed on 5 % (wt/vol) stacking and 12.5 % (wt/vol) resolving gel. A vertical slab unit (Gel electrophoresis apparatus, LKB-2001-100, LKB, Sweden) with 180x140x1.5mm slabs, equipped with a cooling bath type Multitemp II and an Electrophoresis Power Supply (EPS 500/400, LKB, Sweden) were used.

Protein samples of cheese were prepared according to following procedure. Grounded cheese (1.2 g) was extracted with continuous shaking in 15 mL of Tris-HCl, buffer pH 6.8 [0.055 M, 2 % (wt/vol) SDS, 7 % (vol/vol) glycerol, 5 % β -mercaptoethanol (vol/vol)] for 30 minutes at 40 °C. Suspension was centrifuged for 15 minutes at 2600 x g (Janetzki, Czech Republic). Then, the upper layer was carefully removed and clear solution was taken and diluted with sample buffer [0.055 M Tris-HCl, pH 6.8, 2 % (wt/vol) SDS, 7 % (vol/vol) glycerol, 4.3 % (vol/vol) β -mercaptoethanol, 0.0025 % (wt/vol) bromophenol blue]. The ratio of protein extract to sample buffer was 1:3. Diluted samples were frozen. Prior to electrophoresis, samples were heated at 90 °C for 5 min and cooled at room temperature. The SDS-PAGE was performed on two gels with different amount (25 μ L and 5 μ L) of loaded samples. The gels were run at 30 mA per gel for 4 hours to completion. Gels were stained in 0.23 % (wt/vol) solution of Coomassie Blue R-250 [dissolved in 3.9 % (wt/vol) trichloroacetic acid (TCA), 6 % (vol/vol) acetic acid, and 17 % (vol/vol) methanol] for 45 min. This was followed by two destaining steps of 20 h and 2 h, with 1000 mL and 500 mL of destaining solution [18 % (vol/vol) ethanol and 8 % (vol/vol) acetic acid], respectively. Destaining of gels was performed with continuous agitation.

Molecular weights of separated polypeptides were estimated by using low molecular weight markers (Pharmacia, Upsalla, Sweden). Molecular

weight markers contained: phosphorylase B (94.0 kDa), bovine serum albumin (67.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (30.0 kDa), soybean trypsin inhibitor (20.1 kDa), and α -lactalbumin (14.4 kDa). Also, the results of our previous investigation (Pestic et al, 2012) were used for determinations of major casein fractions. SDS-PAGE was performed in duplicate.

Densitometric analysis

SDS-gels were scanned by PC scanner (HP, USA). Scanned gels were analysed by SigmaGel software version 1.1 (Jandel Scientific, San Rafael, CA). Caseins and polypeptides were quantitatively determined by integration of peak volumes. Intensity of casein bands was quantified from the gel on which were applied 5 μ L of the samples. Each pattern was analysed in triplicate. Residual content of identified caseins and low molecular products was expressed as a percent of their initial content of fresh cheese.

Statistical analysis

The obtained data were analysed using Statistica software version 7.0 (StatSoft Co., Tulsa, USA). Results are given as the mean values \pm standard deviation. Significant difference between mean values was determined by *t*-test procedure at $p < 0.05$. Also, regression analyses were carried out at the same level. Correlation coefficients were calculated with the same software.

Results and discussion

Chemical analysis

Raw milk used in this study showed typical cow milk composition with 3.19 % total protein, 3.70 % fat, 4.70 % lactose and 12.63 % dry matter. The pH of milk was 6.74. The change of moisture content, total protein content (TP), water soluble protein (WSP), fat in dry matter (F/DM) and degree of proteolysis (DP) during ripening of white brined cheese prepared from raw cow milk are presented in Table 1. The average total protein content, moisture and F/DM of fresh cow cheese were in agreement with previously reported range for white brined cheese varieties (Hayaloglu et al., 2002; Radulović et al., 2011).

Table 1. The change of moisture content, total protein content, water soluble protein, fat in dry matter and degree of proteolysis during ripening of white brined cheese prepared from raw cow milk

| | Moisture g/100 g | TP/DM | WSP g/100 g | DP | F/DM | pH |
|----|-----------------------------|--------------------|-------------------------|------------------------|--------------------|-------------------|
| 0 | 51.49±0.50 ^d | 33.71 ^a | 5.16±0.12 ^s | 4.21±0.08 ^d | 47.41 ^c | 6.63 ^a |
| 10 | 52.85±1.12 ^{b,c} | 32.24 ^b | 12.15±0.08 ^f | 4.33±0.15 ^d | 48.46 ^c | 5.16 ^b |
| 20 | 53.86±1.04 ^{a,b,c} | 33.39 ^a | 14.29±0.22 ^e | 3.94±0.25 ^d | 50.35 ^b | 4.83 ^c |
| 30 | 54.84±0.60 ^{a,b} | 33.32 ^a | 20.22±0.16 ^d | 5.05±0.21 ^c | 51.08 ^a | 4.74 ^d |
| 40 | 52.10±0.85 ^{b,c,d} | 33.21 ^a | 21.94±0.08 ^c | 8.97±0.15 ^a | 51.36 ^a | 4.74 ^d |
| 50 | 54.10±1.11 ^{a,b,c} | 31.96 ^b | 31.60±0.34 ^a | 8.82±0.07 ^a | 50.58 ^b | 4.67 ^e |
| 60 | 55.70±0.89 ^a | 30.75 ^c | 30.42±0.17 ^b | 7.87±0.11 ^b | 50.43 ^b | 4.60 ^e |

Abbreviations: TP/DM - total protein in dry matter, WSP - water soluble protein, DP - degree of proteolysis, F/DM - fat in dry matter. ^{a,b,c,d,e,f}Means within the same column and not sharing the same superscript letter are significantly different ($p < 0.05$)

Moisture content, fat in dry matter and pH of cow cheese was significantly influenced by ripening in brine. During 60 days of ripening, the moisture content as well as the fat in dry matter of white brined cow cheese increased. During this period of ripening, the moisture content ranged from 51.49 g/100 g to 55.70 g/100 g. Similar variations in moisture content during ripening of white brined cheese prepared from different types of milk were reported by several authors (Özer et al., 2002, 2004; Atasoy and Turkogly, 2008; Lavasani et al., 2012; Achachlouei et al., 2013; Barać et al., 2013). Although, the moisture content increased more intensively during goat and ovine cheese ripening. Özer et al. (2002) reported more intensive increase of moisture content during ripening of a traditional Turkish cheese named Urfa prepared from raw ovine milk than those prepared with bovine milk. They suggested that higher protein content of ovine cheese may have contributed to this phenomenon due to water binding capacity. Greater variation in the moisture content was also observed during goat cheese ripening (Barać et al., 2013). Higher increase in moisture in this type of cheese could be a result of releasing of new ionic groups through extended proteolysis and/or the different texture of bovine and goat cheeses. The cow cheese possibly became more firm than goat cheese and the brine could not easily penetrate into cheese matrix.

The increase in moisture was followed by the increase of fat in dry matter content. During 40 days this parameter continually increased from 47.41

g/100 g to 51.36 g/100 g and then slightly decreased up to 50.48 g/100 g (60 days). No significant differences between 50- and 60-days of cheese ripening were observed. Such results indicated that more intensive diffusion processes of soluble compounds into the brine occurred during 40 days of ripening after which an equilibrium was established. Almost identical increase of F/DM during 60 days of ripening of white brined cow cheese was observed by Atasoy and Turkogly (2008).

In opposite to moisture content and F/DM, during 60 days of ripening, pH continually decreased up to 4.67. The significant positive correlation between time of ripening and F/DM (0.76, $p < 0.05$; Table 2) as well as negative correlation between time of ripening and pH (- 0.76, $p < 0.05$; Table 2) was observed. There was no significant correlation between time of ripening and moisture content. In addition, F/DM and pH values were highly and negatively correlated.

Total proteins, water soluble proteins and degree of proteolysis

Ripening significantly affected content of total protein in dry matter of white brined cheese. Total content of protein (TP) in dry matter decreased from 33.71 g/100 g to 30.75 g/100 g on sixty days of ripening which was supported by the decrease of a dry matter content. The decrease in TP content of traditional cow cheese throughout ripening and cold storage was reported earlier (Özer et al., 2002; 2004; Atasoy et al., 2008; Atasoy and Turkogly,

2008). Also, similar changes of TP were registered during ripening of white goat cheese (Barač et al., 2013) and white ovine cheese (Özer et al., 2002). However, cheeses manufactured from goat and ovine milk had a higher level of total protein loss than those prepared from cow milk. The loss of total protein is a consequence of diffusion of nitrogen compounds into brine (Michaelidou et al., 2005; Monteiro et al., 2007). Monteiro et al. (2007) showed that the migration of both, intact casein and products of hydrolysis of casein matrix occurred during ripening. Same group of authors also suggested that differences observed between two types of cheese in terms of total protein loss could be related to their different microstructure. It is possible that white brined cow cheese had more compact structure of matrix than cheeses of other species and this could be the main reason for these differences.

It is known that proteolysis takes place in milk during curd processing (Fox et al., 1993). Consequently, fresh cow cheese had small quantities of water soluble and TCA-soluble nitrogen. Hence, WSP and DP values of fresh cow cheese were 5.16 g/100 g and 4.21 %, respectively. As expected, 60 days of proteolysis induced significant increase of both parameters, but in a different extent (Table 1). WSP increased more extensively than DP; the WSP value of 60 days old cheese was 30.42 g/100 g whereas DP value was 7.87 %. In other words, after 60 days of ripening WSP and DP index was 5.89 times and 1.87 times higher than in unripened cheese. The

maximum value of WSP was registered in 50 days old cheese (31.60 g/100 g). After that period this parameter slightly, but significantly ($p < 0.05$) decreased. The decrease of WSP observed in the last 10 days of ripening could be the result of more intensive degradation of low molecular weight products and their more intensive passage into brine. The maximum DP value was observed after 40 days (8.97 %). After that period, DP slightly decreased to 7.87 % (Table 1). Both parameters were highly and positively correlated with time of ripening (0.98, WSP; 0.85, DP, $p < 0.05$). Also, a significant correlation (0.83, $p < 0.05$) between WSP and DP values was observed (Table 2).

Protein profile of white brined cow cheese

The SDS-PAGE profiles of fresh cheese and cheese ripened for 10, 20, 30, 40, 50 and 60 days are presented in Fig. 1. The applied electrophoretic method separated cheese proteins into multiple components with molecular weights ranged from 120 kDa-8 kDa.

Protein profiles of fresh and ripened cow cheeses showed two major bands, α -CN and β -CN with molecular weight consistent with those reported in literature (Van Hekken et al., 2004; Pesic et al., 2012). The ratio of α -CN to β -CN of fresh cheese was 1.71. Besides these two fractions, the SDS-PAGE profile of fresh cheese also contained a lot of low molecular weight peptides (LMWP) in the

Table 2. Correlation coefficients between protein composition and some of parameters of ripened cheese

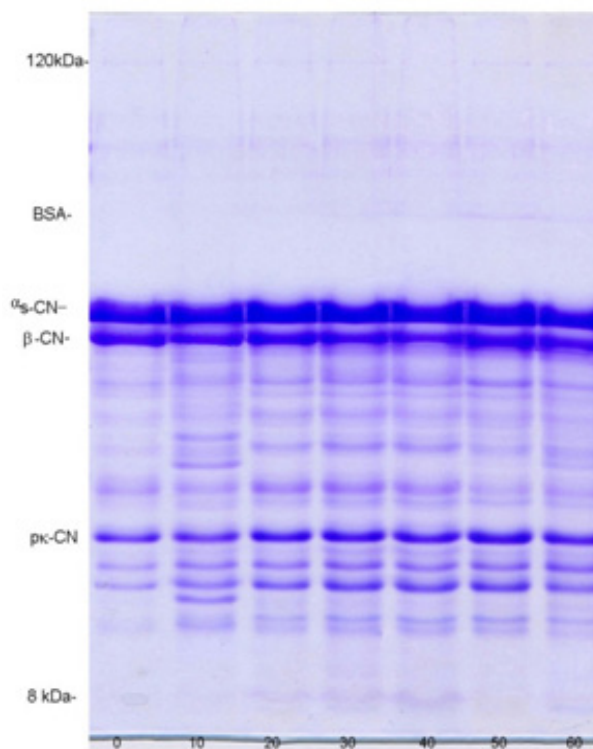
| | Time | Moisture | TP | WSP | DP | F/DM | pH | α -CN | β -CN | LMWP |
|--------------|------|----------|-------|-------|-------|-------|--------|--------------|-------------|--------|
| Time | 1,00 | 0,69 | -0,67 | 0,98* | 0,85* | 0,76* | -0,77* | -0,97* | 0,03 | 0,99* |
| Moisture | | 1,00 | -0,52 | 0,70 | 0,21 | 0,55 | -0,70 | -0,68 | 0,08 | 0,71 |
| TP/DM | | | 1,00 | -0,68 | -0,56 | -0,11 | 0,37 | 0,49 | 0,45 | -0,65 |
| WSP | | | | 1,00 | 0,83* | 0,74 | -0,79* | -0,95* | 0,01 | 0,99* |
| DP | | | | | 1,00 | 0,60 | -0,52 | -0,79* | -0,05 | 0,83* |
| F/DM | | | | | | 1,00 | -0,88* | -0,85* | 0,13 | 0,80* |
| pH | | | | | | | 1,00 | 0,83* | 0,20 | -0,82* |
| α -CN | | | | | | | | 1,00 | -0,15 | -0,97* |
| β -CN | | | | | | | | | 1,00 | 0,01 |
| LMWP | | | | | | | | | | 1,00 |

Abbreviations: TP/DM - total protein in dry matter, WSP - water soluble protein, DP - degree of proteolysis, F/DM - fat in dry matter, LMWP - low molecular weight products

*These correlation coefficients correspond to correlations that are significant at $p < 0.05$.

range of 26 kDa-8 kDa. Most of these peptides, including para- κ -casein were products of proteolysis. They were not registered in raw milk (electrophoretic pattern of milk proteins was not shown). This clearly confirmed that proteolysis started during curd processing which was consistent with the results of WSP and DP, as well as with previous results obtained for goat cheese (Mallatou et al., 2004; Barać et al., 2013) and ovine cheese (Mallatou et al., 2004). It is well known that several factors including residual rennet, indigenous milk proteases, starter and non-starter microorganisms are responsible for proteolysis of cheese. Since pH of fresh cheese was high (6.63) and no starter culture was added, most of these low molecular weight peptides detected by the SDS-PAGE pattern of fresh cheese could be generated by action of chymosin and plasmin.

The distribution of major proteins and peptides of cow cheese was also affected by ripening. Ripening reduced the content of both caseins, α_s -CN and β -CN, but to a different extent. As a result of their degradation, the amount of LMWP significantly ($p < 0.05$) increased. α_s -CN was more susceptible to proteolysis than β -CN. During sixty days of ripening the content of α_s -CN continually reduced to 63.40 % of initial value of fresh cheese (Fig. 2). The similar susceptibility of α_s -CN was observed for white brined goat cheese prepared from raw milk; the residual content of α_s -CN was 61.10 % (Barać et al., 2013) The amount of residual α_s -CN and WSP were highly and negatively correlated ($p < 0.05$) with time of ripening (-0.97 and -0.95, respectively) whereas significant ($p < 0.05$) negative correlation (-0.79), between α_s -CN and DP were registered (Table 2). In opposite to α_s -CN, β -CN degradation progressed much more slowly and the level of residual β -CN at the end of 60 days of ripening was 91.02 %. Consequently, the ratio of α_s -CN/ β -CN decreased from 1.71 (fresh cheese) to 1.21 in 60 days ripened cheese. No significant correlations ($p < 0.05$) between residual β -CN and the other monitored parameters (Table 2) were observed. High resistance of β -CN to proteolysis was highlighted in literature (Viser and de Groot-Moster, 1977; Hayaloglu et al., 2002; Sarantinopoulos et al., 2002). Several authors showed that the type of microbiological cultures involved in proteolysis, as well as type of milk greatly influenced the level of β -CN degra-



Abbreviations: BSA - blood serum albumin; α_s -CN, α_s -casein; β -CN, β -casein; κ -CN, κ -casein; $\rho\kappa$ -CN, para- κ -casein; 0- fresh cheese; 10-60- days of cheese ripening

Figure 1. Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) of proteins of cow cheese ripened for 10-60 days

dation. For example, Hayaloglu et al. (2002) reported that the level of β -CN of white cow cheeses prepared with different starter culture and ripened for sixty days was in the range of 79.7-88.7 %. In addition, Barać et al. (2006) showed that sixty days of ripening of two types of autochthonous Serbian cheeses (prepared with cow and ovine milk) showed different reduction of β -CN content. Different level of β -CN these authors attributed to different proteolytic activity of non-starter cultures. Furthermore, one of the reasons might be different casein composition of these two types of milk which have strong influence on physicochemical and renneting properties of milk (Park, 2007; Pesic et al., 2012).

As previously mentioned, proteolysis of caseins was followed by an increase of LMWP. According to electrophoretic and densitometric analysis most of these products came from degradation of α_s -CN.

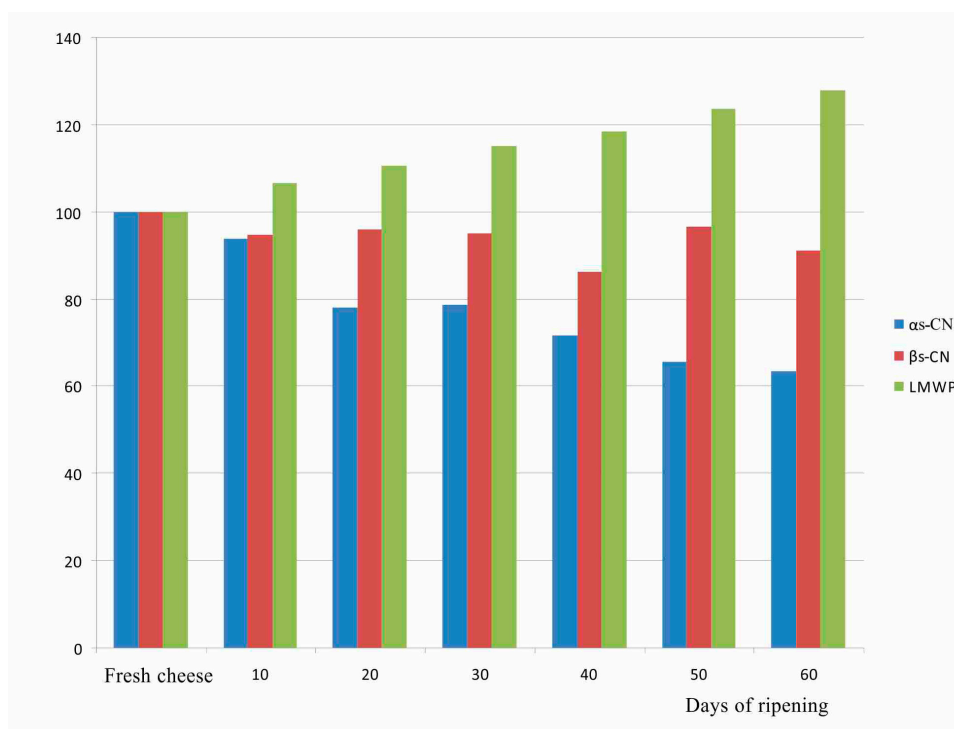


Figure 2. The change (%) of residual content of α_s -casein, β -casein and low molecular weight products (LMWP) during ripening of white brined cow cheese

Consequently, a high negative correlation between LMWP and α_s -CN existed (Table 2). Depending on the time of ripening the amount of LMWP (<26 kDa) continually increased for 6.49 % (10 days) to 27.80 % (60 days) (Figure 2). In percentages, there was a discrepancy between the reduction of major caseins content and the increase of LMWP content. During 60 days, caseins (in total) were reduced approximately by 15 % to 46 % (depending on period of ripening), but LMWP content increased approximately for 6 % to 27 %. Such discrepancy could be a result of two processes, the weakening of the gel matrix and facilitated diffusion into brine and more intensive secondary proteolysis due to activity of non-starter lactic acid bacteria (NSLAB) taking into consideration that the cheese was produced from raw milk. This is supported by the presence of high correlation (0.99, $p < 0.05$) between LMWP and WSP as well as by significant ($p < 0.05$) correlation (0.83), between LMWP and DP (Table 2).

Conclusions

The results of this study clearly suggested that the SDS-PAGE based on high-molarity Tris buffer system could be a useful method for monitoring white cow cheese proteolysis. The results obtained with this method were in a good agreement with the change of water soluble protein content, degree of proteolysis and the other investigated parameters. Most susceptible to proteolysis was α_s -CN fraction. The change of this casein was highly and negatively correlated with time of ripening, water soluble content and the content of low molecular weight products. No significant correlation between β -casein content and analysed parameters was observed. The obtained results also indicated that some differences existed between proteolysis of white brined cow and goat cheeses.

*Primarna proteoliza bijelog sira
u salamuri proizvedenog od sirovog kravljeg
mlijeka praćena visokomolarnim
SDS-PAGE elektroforetskim sistemom*

Sažetak

Cilj ovog rada bio je istražiti proces primarne proteolize bijelog sira u salamuri pripremljenog od sirovog kravljeg mlijeka metodom SDS-poliakrilamidne gel elektroforeze zasnovane na primjeni visoko molarnog sistema Tris pufera i korelirati s rezultatima uobičajeno korištenih parametara. Proteolitičke promjene bijelog sira u salamuri praćene su pomoću tri parametra: ukupnih proteina, proteina topljivih u vodi i stupnja proteolize. Promjena glavnih kazeinskih frakcija praćena je SDS-elektroforetskom metodom u reducirajućim uvjetima. Zrenje je značajno utjecalo na sadržaj ukupnih proteina i sadržaj vlage u kravljem bijelom siru. Ovaj proces povećao je sadržaj proteina topljivih u vodi i stupanj proteolize. Glavne kazeinske frakcije bile su različito otporne na proteolizu. α -CN je bio mnogo osjetljiviji na djelovanje proteolitičkih enzima u odnosu na β -CN. Količina α -CN u jakoj je negativnoj korelaciji sa trajanjem zrenja i proteinima topljivim u vodi, dok nije utvrđena značajna korelacija između sadržaja β -CN i ovih parametara. Također, značajna korelacija utvrđena je između količine produkata male molekulske mase i trajanja zrenja, sadržaja α -CN i proteina topljivih u vodi. SDS-elektroforetska metoda korištena u ovim istraživanjima može biti korisna za praćenje proteolitičkih promjena tijekom zrenja kravljeg bijelog sira u salamuri.

Ključne riječi: proteoliza, SDS-PAGE, kravljji sir

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