Original scientific paper - Izvorni znanstveni rad UDK: 637.12'639/637.35.045

Primary proteolysis of white brined goat cheese monitored by high molarity Tris buffer SDS- PAGE system

Miroljub B. Barać[1], Milenko Smiljanić[2], Mirjana B. Pešić[1], Sladjana P. Stanojević[1], Snežana T. Jovanović[1], Ognjen D. Maćej[1]*

[1]Institute of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11081 Belgrade, Serbia
[2]Faculty of Technology, Karakaj bb, 75400 Zvornik, Bosnia and Herzegovina

Received - Prispjelo: 10.05.2013.
Accepted - Prihvaćeno: 08.07.2013.

Summary

The aim of this work was to investigate primary proteolysis of white brined goat cheese prepared from raw milk and to correlate with the results obtained with high-molarity Tris buffer electrophoretic system. Proteolytic changes of white brined cheese were monitored by three parameters, the total protein, the water soluble proteins and the degree of proteolysis, whereas the change of major casein fractions was followed by electrophoresis in reducing conditions. Ripening caused a decrease of the total protein content, whereas in the first forty days the water soluble protein and the degree of proteolysis increased. Both casein fractions (α_s- and β-casein) of goat cheese were susceptible to primary proteolysis but to the different extent. α_{s1}- casein disappeared during processing, whereas during 60-days of ripening the content of α_{s2} and β-casein was reduced by 38.90 %, and 30.72 %, respectively. Such trends of major casein fractions were strongly correlated with the degree of proteolysis and the moisture content, whereas no correlation was found between them and the water soluble protein content. The results of our investigation clearly suggested that SDS-PAGE method based on high-molarity Tris-buffer system could be a very useful for purposes of monitoring the white goat cheese proteolysis.

Key words: proteolysis, SDS-PAGE, goat cheese

Introduction

Proteolysis is the most complex and perhaps the most important primary biochemical process during ripening of most cheese varieties (McSweeney, 2004; Kalit et al., 2005), which substantially influences cheese texture, aroma and flavour (Mc Sweeney and Sousa, 2000; McSweeney, 2004). Proteolysis in cheese can be divided into the primary and the secondary phase. Major process involved in the primary proteolysis is a degradation of caseins into large, well defined polypeptides. Further proteolytic processes (secondary proteolysis) cause formation of the small polypeptides and free amino acids responsible for cheese aroma and taste.

The degree of casein degradation is determined by various factors such as the type and the processing history of milk and curd (Faccia et al., 2007); the type, the residual concentration and the activity of rennet in the curd (Van Hekken et al., 2007; Pino et al., 2009); the presence and activity of indigenous milk proteases, especially plasmin (Cortellino et al., 2006) and the presence of starter, adjunct or non-starter microorganisms (McSweeney, 2004). Rennet and indigenous milk enzymes are responsible for the primary proteolysis, whereas microorganisms developed through the ripening cause secondary proteolytic changes. Proteolytic changes during the ripening of different cheese varieties have been studied extensively. Most of these studies were per-

*Corresponding author/Dopisni autor: Phone/Tel: +381 112 199 711; E-mail: baracm@agrif.bg.ac.rs

formed on different varieties of cheese prepared from cow milk (Kirmachi et al., 2011; Yasar and Guzeler, 2011; Sarić et al., 2002; Picon et al., 2010; Radulović et al., 2011). Over the last decade, numerous authors (Ferrandini et al., 2011; Abellán et al., 2011; Mallatou et al., 2004; Sarić et al., 2002; Tejada et al., 2008; Franco et al., 2003) investigated the proteolytic changes during ripening of traditional, mostly Mediterranean varieties of goat cheese.

White cheeses in brine belong to widely consumed varieties in the Southeast European countries. Consumption of these varieties comprises about 60 % of total cheese consumption in Serbia (Radulović et al., 2011). Specificity of this type of cheese is that the maturation of cheese occurs in the salt brine, usually for two or three months. It is commonly made as artisanal cheese from raw or thermally treated cow or sheep milk and rarely from goat milk. In the industrial scale, white brined cheese was prepared only from cow milk. In the last fifteen years, due to nutritive and medical benefits, there has been an increased interest for goat milk production and its conversion into high-valuable products such as cheeses. But, there was still no industrial production of white brined goat cheese.

Proteolysis in cheese is monitored by a wide range of techniques including electrophoresis, HPSE-chromatography (high pressure size exclusion) and RP-HPLC (reverse phase high pressure liquid chromatography), or through different parameters (water soluble proteins, TCA-soluble and PTA-soluble proteins). Electrophoresis, particularly urea-PAGE, is usually used for monitoring the primary proteolysis. Less attention has been paid to the usage of SDS-PAGE under reducing conditions for this purpose, although several investigations (Van Hekken et al., 2004; 2007; Trujillo et al., 1995; 1997; 2002; Jin and Park, 1996) showed that this method could be effective for cheese protein analysis. Pesic et al. (2012) showed that electrophoretic technique proposed by Fling and Gregerson (1986), which is based on a high-molarity Tris buffer system without urea, could be very useful for milk protein separation. According to our knowledge, current literature lacks on data about the possible use of this method for analysis of cheese proteolysis, especially proteolysis of white brined goat cheese. Thus, the aim of this study was to investigate primary proteolytic changes during proteolysis of white goat cheese in brine and to correlate them with the results obtained with high molarity Tris-buffer electrophoretic system.

Material and methods

Cheese making

White brined cheese was made from raw goat (total protein content 3.32 %, fat content 4.7 %, lactose content 4.98 %, pH 6.71; dry matter 13.57 %) milk. The following methods were used to determine the basic composition of the 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).

Fresh 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 a sample was taken, the brine was partly replaced. For these investigations, process of cheese making was performed twice.

Compositional analysis

The 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 the 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 cheese samples 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 the following three parameters: the total protein, the water soluble proteins and the degree of proteolysis (DP). The amount of the 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, the suspension was centrifuged (at 6.000 x g, Janetzki, Czech Republic) for 60 minutes, to obtain a completely clear supernatant. The supernatant was then carefully separated and the nitrogen content was determined by the Kjeldahl method (AOAC, 1999). Also, the same amount of cheese samples was used for the total nitrogen content determination. The total and water soluble protein content was determined as total nitrogen x 6.38.

The degree of proteolysis (DP) was determined according to Kim et al. (1990). DP was estimated by measuring the amount of soluble nitrogen in 10 % trichloracetic acid and 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 12.000 x g for 15 min. Nitrogen content was determined in the supernatant. All analyses were determined in triplicate.

SDS-PAGE

SDS-PAGE was conducted according to the procedure proposed by Fling and Gregerson (1986) using 5 % (wt/vol) stacking and 12.5 % (wt/vol) resolving gel. Electrophoresis was performed using a vertical slab unit (Gel electrophoresis apparatus, LKB-2001-100, LKB, Sweden) with 180x140x1.5 mm slabs, equipped with a cooling bath type Multitemp II and an Electrophoresis Power Supply (EPS 500/400, LKB, Sweden).

For SDS-electrophoresis, the cheese proteins were extracted according to the method described by Kalit et al. (2005) with some modifications. A quantity of 1.2 g of previously grounded cheese was extracted with continuous shaking in 15 mL of buffer (0.055 M Tris-HCl, pH 6.8, 2 % (wt/vol) SDS, 7 % (vol/vol) glycerol, 5 % β-mercaptoethanol (vol/vol)) for 30 minutes at 40 °C. The obtained suspension was centrifuged for 15 minutes at 2.600 g (Janetzki, Czech Republic). Then, the clear solution beneath the upper layer was carefully 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). Extract to sample buffer ratio was 1:3. Diluted samples were frozen. Prior to electrophoresis, samples were heated at 90 °C for 5 min and cooled down to the room temperature. A quantity of 25 μL of each sample intended for the SDS-PAGE were loaded per well. The gels were run at 30 mA per gel for 4 hours to completion. Gels were fixed, stained with 0.23 % (wt/vol) 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 and destained with 8 % acetic acid and 18 % (vol/vol) ethanol. Molecular weights of the polypeptides were estimated by using the low molecular weight calibration kit (Pharmacia, Upsalla, Sweden). Molecular weight markers included: phosphorylase B (94.0 kDa), bovine serum albumin (67.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (30.0 kDa), soybean trypsin inhibitor (20.1kDa), and α-lactalbumin (14.4 kDa). Besides the molecular weight, the results of Pesic et al. (2012) were used for casein fraction determinations. SDS-PAGE was performed in duplicate.

Densytometric analysis

SDS-gels were digitised by PC scanner (HP, USA) and analyzed by SigmaGel software version 1.1 (Jandel Scientific, San Rafalel, CA). Caseins and polypeptides were quantitatively determined by the integration of peak volumes. Each pattern was analyzed at least in triplicate. The residual content of identified caseins was expressed as a percent of their initial content of fresh cheese.

Statistical analysis

The data were analyzed using Statistica software version 7.0 (StatSoft Co., Tulsa, OK). Significant difference between mean values was determined by *t*-test procedure at $p<0.05$. Regression analyses were also carried out at same level and correlation coefficients were calculated with the same software.

Results and discussion

Chemical analysis

The average protein contents as well as the moisture, the fat in dry matter and the pH of fresh and ripened white brined goat cheese are presented in Table 1. The total protein content of fresh goat cheese was 45.20 g/100 g, whereas the moisture and the fat in the dry matter were 54.21 and 50.67 g/100 g. These values were in agreement with previously reported range for tradition goat cheese varieties after manufacture (Ferrandini et al., 2011). The average pH of fresh cheese was 6.63.

In general, ripening in brine affected the moisture content, the fat in dry mater (F/DM), the pH as well as the total protein content (TP). The moisture content of goat cheese increased during ripening whereas pH decreased. According to our results, moisture content increased significantly ($p<0.05$) up to 40 days of ripening. Longer ripening time had no significant ($p<0.05$) effect on the moisture content. In opposite to our results, Radulović et al. (2011) reported that there were no significant changes of the moisture content during ripening of white brined cow cheese. These deviations between goat and cow white brined cheeses could be attributed to different hydrophilic characteristics of their gel networks. The change of pH of cheese samples was completely different. During 40 days of ripening pH of cheese continually decreased up to 4.26. Longer periods of ripening had no significant effect on pH. Consequently, very strong positive correlation (0.93, $p<0.05$) between the moisture content and the time of ripening and very strong negative correlation (-0.90, $p<0.05$) between time of ripening and pH of cheese was observed (Table 2). During 30 days of ripening F/DM decreased up to 48.92 g/100 g and then increased to 54.87 g/100 g.

Total proteins, water soluble proteins and degree of proteolysis

As it could be expected, TP of fresh goat cheese decreased during ripening. Nevertheless, no significant correlation ($p<0.05$) between the total protein content and the ripening time was observed (Table 2). During 60 days of ripening TP significantly ($p < 0.05$) decreased from 45.20 g/100 g to 36.90 g/100 g. The most intensive decrease was observed after the initial 10 days, but minimum values were obtained after 40 days. During this period (40 days) protein content was reduced for 23.78 %. As previously described, the ripening of white brined cheese occurred in salt brine. Consequently, the reduction of TP could be the result of diffusion of weakly bounded proteins and/or partially hydrolysed proteins into the brine. Comparing the results from the literature concerning proteolysis of white brine cheese, it could be concluded that the change of TP

Table 1. 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 goat milk[a]*

Time of ripening (days)	Moisture (g/100 g)	T.P. (g/100 g)	F/DM (g/100 g)	pH*	WSP (g/100 g)	DP (%)
0	$54.21\pm1.10^{b,c}$	45.20^{a}	50.67^{d}	6.63^{a}	9.77 ± 0.17^{e}	3.47 ± 0.15^{f}
10	$52.16\pm0.70^{b,d}$	36.58^{c}	48.08^{f}	5.67^{b}	15.88 ± 0.41^{d}	3.68 ± 0.09^{f}
20	$55.37\pm0.49^{b,c}$	38.51^{b}	49.29^{e}	4.83^{c}	23.72 ± 0.86^{c}	4.49 ± 0.09^{e}
30	58.08 ± 1.13^{a}	36.43^{c}	$48,92^{e,f}$	4.56^{d}	33.73 ± 0.11^{b}	8.33 ± 0.18^{d}
40	60.14 ± 0.67^{a}	34.45^{d}	52.68^{c}	4.26^{e}	37.93 ± 1.42^{a}	9.52 ± 0.36^{c}
50	60.89 ± 0.85^{a}	$35.54^{d,c}$	53.35^{b}	4.24^{e}	23.88 ± 0.18^{c}	12.82 ± 0.1^{a}
60	60.79 ± 0.92^{a}	36.90^{c}	54.87^{a}	4.20^{e}	17.88 ± 0.77^{d}	11.61 ± 0.5^{b}

[a]Abbreviations: TP - total protein in DM, WSP - water soluble protein, DP - degree of proteolysis, F/DM - fat in dry matter
*Means within the same column with the same small letters were not significantly different at $p<0.05$

Table 2. Correlation coefficients between protein composition and some of parameters of ripened cheese[a,*]

	Time of ripening	Moisture	TP	F/DM	pH	residual β-CN	residual αs-CN	residual αs/β-CN	WSP	DP	LMWP
Time of ripening	1,00	0,93*	-0,67	0,80*	-0,90*	-0,95*	-0,98*	-0,84*	0,42	0,95*	0,89*
Moisture		1,00	-0,53	0,83*	-0,84*	-0,85*	-0,86*	-0,69	0,54	0,96*	0,94*
TP			1,00	-0,21	0,85*	0,58	0,72	0,94*	-0,72	-0,62	-0,33
F/DM				1,00	-0,52	-0,84*	-0,77*	-0,43	0,06	0,80*	0,87*
pH					1,00	0,78*	0,88*	0,93*	-0,71	-0,83*	-0,74
residual β-CN						1,00	0,98*	0,71	-0,19	-0,93*	-0,87*
residual αs-CN							1,00	0,83*	-0,34	-0,93*	-0,83*
residual αs/β-CN								1,00	-0,66	-0,72	-0,54
WSP									1,00	0,44	0,28
DP										1,00	0,90*
LMWP											1,00

[a]Abbreviations: TP - total protein content in DM, WSP - water soluble protein, DP - degree of proteolysis, αs - CN, β-CN - residual content of corresponding caseins, F/DM - fat in dry matter, LMWP - low molecular weight products. *These correlation coefficients correspond to correlations that are significant at $p<0.05$

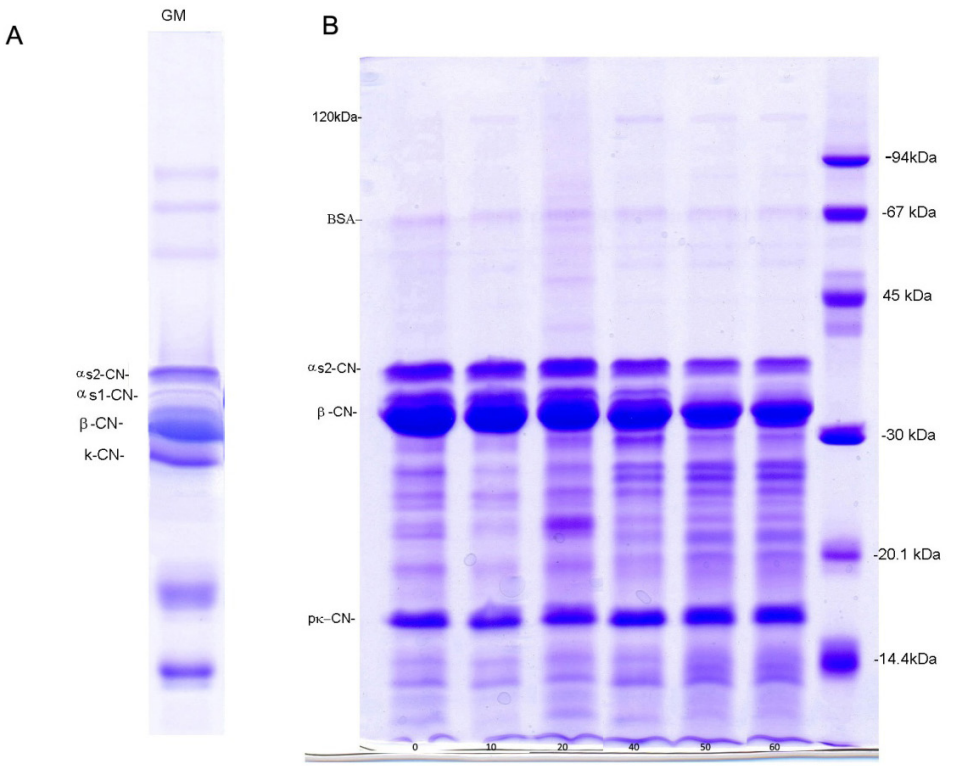
in goats and cow cheeses are significantly different. Investigation of Radulović et al. (2011) as well as our unpublished results related to proteolysis of white brined cow cheese confirmed these observations. The change of TP during proteolysis of goat cheese was in higher extent compared to that in cheese made of cow milk. Such differences between changes in protein contents reported for cow cheese and our results for goat cheese indicated to different stability of their gel networks. It seemed that gel network of goat cheese was less stable and more susceptible to proteolysis which provoked that proteolysis products more easily diffused into the brine, especially throughout the 40 days of ripening. During this period, in the case of goat cheese, maximal diffusion occurred. This conclusion is supported by the fact that the dry matter of goat cheese intensively decreased by 12.95 %.

The proportion of water soluble nitrogen (and consequently water soluble protein) has traditionally been regarded as a "ripening index" for cheese because it reflects the extent of proteolysis and it is an indicator of casein hydrolysis by the action of coagulant enzymes and milk proteases present at the beginning of ripening (Tejada et al., 2008). On the other hand, data for TCA soluble nitrogen (usually soluble in 12 % TCA) is traditionally considered as an index of "ripening depth" (Tejada et al., 2008) since it indicates progress of secondary proteolysis and the degree of formation of low molecular peptides. It is known that proteolytic processes take place in milk during curd processing (Fox et al., 1993). Consequently, fresh goat cheese had small quantities of water soluble and TCA-soluble nitrogen. The average WSP value of fresh goat cheese was 9.77 g/100 g, whereas degree of proteolysis was 3.47 %. During ripening the level of both parameters increased and depending on time of ripening WSP index was in the range from 15.88 to 37.93 g/100 g, whereas DP values were in the range from 4.49 to 12.82 % (Table 1). Trend of their changes was partly different and there was no significant correlation ($p>0.05$) between them. Namely, WSP continually and intensively increased in initial phase of ripening and reached maximum (37.93 g/100 g) after 40 days. Further ripening (50 and 60 days) significantly ($p<0.05$) reduced WSP to 17.88 g/100 g (Table 1). On the other hand, the DP index increased more slowly but continuously during 50 days of ripening

(up to 12.81 %) and then slightly decreased. Due to such trends, in the case of goat cheese ripening, there was no correlation between WSP index and time of ripening. Also, no significant correlation was found between WSP and other investigated parameters (Table 2). In opposite to this, DP was strongly and positively correlated (0.95, $p<0.05$) with the time of ripening and moisture content whereas moderate negative correlation between DP and F/DM and pH was observed (Table 2).

Protein profiles

The primary proteolysis of white brined cheese of goat milk was investigated by SDS-PAGE method of Fling and Gregerson (1986). Pesic et al. (2012) showed that this method based on a high-molarity Tris buffer system without urea could be useful for investigation of differences between milk proteins from different species and milk protein interactions. The SDS-PAGE profiles of goat milk proteins, fresh and cheeses ripened for 10, 20, 30, 40, 50 and 60 days are presented in Fig. 1. Under the applied experimental conditions milk proteins separated into three major casein components (α_s, β- and κ-CN), and five whey proteins including α-lactalbumin (α-La), β-lactglobulin (β-Lg), lactoferrin, blood serum albumin (BSA) and Ig`s (Fig. 1A) with molecular weight and electrophoretic mobility that were consistent with those reported in literature (Van Hekken et al., 2004; Pesic et al., 2012). α_s-casein of goat milk was registered as band of αs_2- and less intensive band of α_{s1}-CN. β-CN of goat milk and cheese (Fig. 1) was detected as two overlapped bands with slightly different electrophoretic mobility. This was consistent with previous work of Trujillo et al. (2000). These authors detected two genetic forms of β-CN which were different according to degree of phosphorylation.



[a]Abbreviations: GM - goat milk; BSA - blood serum albumin; α_s-CN, α_s-casein; β-CN, β-casein, κ-CN, κ-casein; pκ-CN; paraκ-casein; MWS - molecular weight standard. 0 - fresh cheese; 10-60 - days of cheese ripening

Figure 1. Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) of raw goat milk proteins (A) and goat cheese proteins (B)

Protein profiles of fresh and ripened goat cheese showed a major β-CN band and less intensive α_{s2}-CN band. SDS-electrophoretic profiles (Fig. 1B) clearly confirmed that relatively intensive proteolysis occurred throughout curd processing. Namely, SDS-PAGE pattern of fresh cheese (Fig. 1B) contained a large number of low molecular weight peptides (26-10 kDa), which accounted for almost 50 % (data was not shown) of all detected polypeptides. Most of these fractions, including para-κ-casein were not detected in the initial milk and were products of proteolysis. Several factors including the residual rennet, the indigenous milk proteases, the starter and non-starter microbiological cultures are responsible for proteolysis obtained in cheese. Since no starter culture was added and the pH of fresh cheese was high (6.63), it could be assumed that at that phase of production most of these fractions represented chymosin and plasmin generated products. According to investigations of Trujilo et al. (1998) based on model systems, and work of Van Hekken et al. (2007), most of these products with molecular weights in the range of 10-20 kDa were rennet generated fragment from β-CN, κ-CN, and plasmin generated fragments of β-CN and α_s-CN. The most susceptible to proteolysis was α_{s1}-CN. During the goat cheese processing a less intensive band of this casein completely disappeared (Fig. 1, 0 days). Due to the low content and high sensitivity to chymosin (Trujillo et al., 1998), the loss of this casein fractions could be expected. Greater sensitivity of α_{s1}-CN of raw goat milk compared to α_{s2}- and β-CN was observed by Sanchez-Macias et al. (2011), but these authors found residual α_{s1}-CN content after processing. This discrepancy is probably a consequence of different content of α_{s1}-CN fraction in the initial milk. It is known that the content of α_{s1}-CN in goat milk could vary from null to high content (Park, 2006).

The ripening of goat cheese influenced the distribution of proteins and peptides. With ageing and hydrolysis of the protein matrix, the amount of the intact proteins, α_s-CN and β-CN, decreased ($p<0.05$) with concomitant increase in peptide levels (Fig. 2). The only exception was 10-days' ripened cheese in which was registered reduced level of low molecular weight peptides (LWMP) compared to initial level by 14.11 %. (Fig. 1 and Fig. 2). Decrease of LMWP in the initial phase of ripening could be due to higher availability of these smaller peptides to enzymes and indigenous bacteria than caseins.

Our results showed that caseins, α_s-CN and β-CN were prone to hydrolysis, but in different extent (Fig. 2). In general, α_s-CN was more susceptible than β-CN. After 60 days of ripening, residual

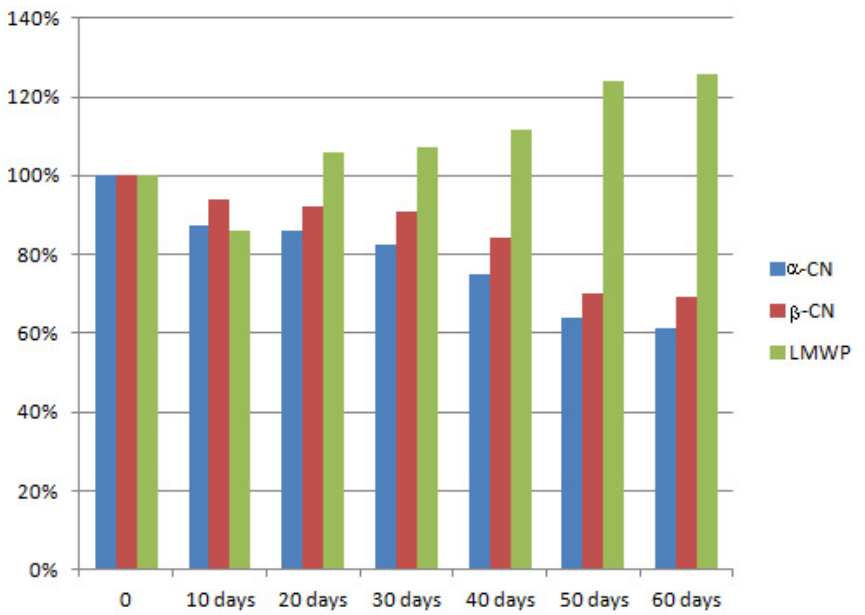


Figure 2. The change (%) of residual content of α_s-casein, β-casein and low molecular weight fraction (LMWP) during ripening of white brined goat cheese

content of these caseins were 61.10 % and 69.28 %, respectively. β-CN was more stable to proteolysis than α_s-CN especially during 30 days. During that period the initial content of β-CN was reduced by 9 %, whereas α_s-CN was reduced by almost 18 %. Longer ripening caused more intensive proteolysis of β-CN which is basically a consequence of intensive degradation of β-CN form with lower electrophoretic mobility. This form of β-CN was completely hydrolysed after 50 days (Fig. 1). This degradation of β-CN could be due to microbiological activity concerning that pH of cheese decreased and reached constant value after 40 days of hydrolysis.

Such trends of major caseins were in a good agreement with results obtained for goat cheddar cheeses reported by Jin and Park (1996) and the results of Sarić et al. (2002) obtained on artisanal cheese named Travnik cheese and were supported by the DP values. Strong negative correlations (-0.93, $p<0.05$) between the residual content of both caseins and the DP was registered.

As previously described, proteolysis of caseins was followed by an increase of low molecular weight products (<26 kDa, except in the case of 10 day's ripened cheese). But in percentage terms, there was an disagreement between the reduction of major caseins content and the increase of LMWP content. Namely, after 60 days caseins were reduced approximately by 30-40 %, but LMWP weigh content increased by 25.81 % (Fig. 2). Such disagreement could be a result of the weakening of gel matrix and facilitated diffusion into brine and/or more intensive secondary proteolysis which is supported by the absence of correlation between LMWP content and WSP as well as by a very strong correlation (0.94, $p<0.05$) between LMWP and moisture content.

Conclusion

The results of this investigation suggested that the SDS-PAGE method based high-molarity Tris-buffer system could be a very useful for purposes of monitoring the white goat cheese proteolysis. The results obtained with this method were in a good agreement with the change of other investigated parameters. This method clearly reflected different susceptibility of major caseins to proteolysis. α_{s1}- casein disappeared during processing, whereas during 60-days of ripening the initial content of α_{s2} and β-casein was reduced by 38.90 %, and 30.72 %, respectively.

Acknowledgement

We are grateful to the Serbian Ministry of Education and Science for providing Grant III 46009 for this study.

Primarna proteoliza kozjeg bijelog sira u salamuri praćena SDS-PAGE sistemom visokog molariteta

Sažetak

Cilj ovog rada bio je ispitati proces primarne proteolize bijelog sira u salamuri pripremljenog od sirovog kozjeg mlijeka i staviti u korelaciju s rezultatima dobivenim SDS-elektroforetskom metodom koja se bazira na primjeni Tris pufernog sistema visokog molariteta. Proteolitičke promjene bijelog sira u salamuri praćene su pomoću tri parametra: ukupnih proteina, u vodi topljivih proteina i stupnja proteolize, dok je promjena glavnih kazeinskih frakcija praćena elektroforezom u reducirajućim uvjetima. Zrenje sira uzrokuje pad sadržaja ukupnih proteina, dok sadržaj u vodi topljivih proteina i stupanj proteolize raste tijekom 40 dana zrenja. Obje kazeinske frakcije (α_s- i β-kazein) podliježu procesu proteolize, ali u različitom stupnju. α_{s1}-kazein se razgrađuje tijekom pripreme sira, dok se tijekom zrenja sira sadržaji α_{s2}- i β-kazeina reduciraju za 38.90 % i 30.72 %. Ovakva promjena glavnih kazeinskih frakcija u jakoj je korelaciji sa stupnjem proteolize, sadržajem ukupnih proteina i sadržajem vlage, dok nije uočena korelacija između ovih parametara i u vodi topljivih proteina. Rezultati naših istraživanja jasno pokazuju da SDS-elektroforetski sistem zasnovan na visoko molarnim Tris- puferima može biti vrlo korisna metoda za praćenje proteolitičkih promjena tijekom zrenja kozjeg bijelog sira u salamuri.

Ključne riječi: proteoliza, SDS-PAGE, kozji sir

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