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CHANGES OF TOTAL PROTEINS DURING MATURATION PERIOD OF SJENICA CHEESE

PROMENE UKUPNIH PROTEINA TOKOM ZRENJA SJENIČKOG SIRA

Željko Savić[a], Aleksandar Čukić[a*], Ljiljana Andjušić[a] and Božidar Milošević[a]

[a]Faculty of Agriculture, University of Priština in Kosovska Mitrovica, Kopaonička bb, 38219 Lešak, Serbia
*Corresponding author: aleksandar.cukic@pr.ac.rs

Ž.S. https://orcid.org/0009-0006-5932-3551, A.Č. https://orcid.org/0000-0001-9063-3431, Lj.A. https://orcid.org/0000-0002-0054-1128, B.M. https://orcid.org/0000-0003-4381-0276

ABSTRACT

Proteins are one of the most important milk quality parameters and the course of coagulation, composition and technological properties of cheese curd depend on them. Proteins form the basis of the structure of curds and cheese in which milk fat is incorporated, and represent the substrate for enzymes during the ripening of Sjenica cheese. Sjenica cheese is originally produced on the Sjenica-Pester plateau, from whole, fresh, sheep and cow milk, and belongs to the group of white cheeses in brine. Considering the importance of proteins, this research aimed to determine the protein content after cheese making, and to monitor changes after 30, 60, 120, 180 days of ripening, and the extent of degradation using electrophoresis. The results of the research showed that the total protein content was the highest in Sjenica cheese after 30 days of ripening (15.54%), and in Sjenica type cheese after 60 days of ripening (16.55%). After 30, i.e. 60, 120, 180 days of ripening, there is a slight decrease in total protein in both cheeses, as a result of the transfer of soluble nitrogen from the cheese to the ripening brine. After 180 days, the total protein content was 12.78% for Sjenicki and 13.92% for Sjenicki type cheese. Electrophoretic studies showed that protein degradation occurred mostly during the first 60 days of ripening, after that the protein fractions appear in traces as diffuse zones.

Keywords: Sjenica cheese, total proteins, proteolysis, ripening, electrophoresis.

SAŽETAK

Proteini su jedan od najvažnijih parametara kvaliteta mleka, od kojih zavisi tok koagulacije, sastav i tehnološke osobine gruša. Proteini čine osnovu strukture gruša i sira u koje je inkorporirana mlečna mast, a predstavljaju supstrat za enzime tokom zrenja Sjeničkog sira. Sjenički sir izvorno se proizvodi na Sjeničko-pešterskoj visoravni, od punomasnog, svežeg, ovčijeg i kravljeg mleka, a pripada grupi belih sireva u salamuri. Obzirom na značaj proteina, istraživanja su imla za cilj da se utvrdi sadržaj proteina nakon izrade sireva, a zatim pratiti promene tokom zrenja nakon 30, 60, 120, 180 dana, a elektroforezom i obim razgradnje. Rezultati istraživanja su pokazali da je sadržaj ukupnih proteina imao maksimum kod Sjeničkog sira nakon 30 dana zrenja(15,54%), a kod sira u tipu Sjeničkog posle 60 dana zrenja(16,55%). Nakon 30 odnosno 60, 120, 180 dana zrenja beleži se blago smanjenje ukupnih proteina kod oba sira, kao rezultat prelaska rastvorljivog azota iz sira u salamuru za zrenje. Nakon 180 dana sadržaj ukupnih proteina iznosio je 12,78% za Sjenički i 13,92% za sir u tipu Sjeničkog. Elektroforetska ispitivanja su pokazala da je do razgradnje proteina u najvećoj meri došlo tokom prvih 60 dana zrenja, nakon čega se proteinske frakcije javljaju u tragovima kao difuzne zone.

Ključne reči: Sjenički sir, ukupni proteini, proteoliza, zrenje, elektroforeza.

1. INTRODUCTION

For majority types of cheeses, the greatest importance of all milk components in the production belongs to casein. Technological process of cheese production is based on the specific properties of casein. The ability to coagulate and to form milk curd stands out, as well as syneretic property of

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formed curd, and the tendency to proteolysis, under the influence of proteolytic enzymes found in cheese. The specific properties of casein, that are needed for cheese production, come from its structure and micellar form (1).

Proteins build a protein matrix that forms the basis of the cheese structure, in which a large part of the mineral complex of the cheese is incorporated, so they are the bearers of the firmness and elasticity of the cheese and the greatest potential for changes in the cheese dough during ripening under the influence of enzymes. All enzymes involved in the ripening process are in the watery phase of the cheese. As cheese ripening processes take place predominantly on cheese proteins, this means that the relationship between the watery phase and the protein matrix phase is important in regulating enzymatic processes during ripening period (2, 3).

Cheese ripening caused by enzymatic coagulation of milk implies changes in proteins. This is the longest phase that can last up to 2 years, and during this period the texture and taste characteristic of the corresponding type of cheese develop (4).

Biochemical reactions that take place in cheese during ripening include glycolysis, lipolysis and proteolysis. In many varieties of cheeses, proteolysis is the most important and the most complex of all biochemical transformations that occur during ripening (4).

For the most types of cheeses and their variants, the initial hydrolysis of casein occurs by the action of the residual coagulant enzyme, and by the action of plasminin fewer extent. The products of these reactions are peptides of high molecular weight. Further degradation of these peptides takes place through coagulant enzymes, native enzymes, as well as enzymes of starter and non-starter cheese microflora. All this indicates that cheese ripening is essentially an enzymatic process (5, 6).

Traditional way of cheese production still occupies a very important place in the overall cheese production in the world and in our country. For the production of these types of cheeses, only animal origin rennet is used. It is mainly made from calves stomach, in which the most common enzymes are chymosin and pepsin, and their weight ratio depends on the animal age (5). Residual coagulant, which is, in a certain percentage retained in curd (0-15%), makes the main and essential contribution to cheese proteolysis during ripening, and thus to the development of taste and texture (7).

2. MATERIALS AND METHODS

The experiments in this paper were performed on Sjenica-Pester plateau, where Sjenica cheese is traditionally produced by autochthonous technology. The cheese is produced from fresh full-fat sheep's milk like the original Sjenicki and cow's milk in the Sjenicki type.

According to the plan of conducting the experiment in the wide area of Sjenica-Pester plateau, we selected 6 households that produce cheese with indigenous technology from sheep and cow milk in the following way:

The raw material for production is fresh, whole milk that is curdled with rennet immediately after milking at a temperature of 28-32 ℃, without adding any additives or starter cultures, for 50-60 minutes. Curd processing is simple. The curd is not cut, but transferred to cheesecloths where the whey is separated by squeezing for 4-8 hours by self-pressing or pressing under the pressure. Curd shaping is done in cheesecloths, after whey separation. The resulting cheese bundle is cut into slices, which are stacked and successively salted with dry coarse sea salt. Cheese ripening lasts for 30-40 days in salted whey, which is released by the cheese, or is added, thus providing anaerobic ripening conditions.

After the production of fresh cheese, a certain content of total proteins was determined, and we followed the changes in total proteins after 30, 60, 120, 180 days, which was the determined ripening period.

The analyzes were performed in the chemical laboratory of the Faculty of Agriculture in Belgrade, by the following method:

Percentage of total proteins by Kjeldahl method, IDF standard (8).

Procedure: About 0.5 g of cheese sample is put into a Clydal cuvette, then 10 ml of concentrated H_2SO_4 is added, and after that it is burnt on a hot plate (> 420 ℃) until the contents become clear. Then, the content is cooled to room temperature for about 25 min, and 75 ml of distilled water is added into it. Next, 25 ml of 4% boric acid is poured into the Erlenmeyer flask, and distillation is performed up to 150 ml of distillate into a Klejtek apparatus. The distillate is titrated with 0.1N H_2SO_4 until the green color disappears, ie. decolorization-a moment before the formation of pink color.

The content of total nitrogen substances is calculated using the following formula:

$$UN = \frac{1{,}4007 \cdot (V1 - V2) \cdot c \cdot F}{m}$$

V1 - ml of H_2SO_4 spent for sample titration;

V2 - ml of H_2SO_4 spent for blank test;

C - normality of H_2SO_4 (0,1);

F - morality factor H_2SO_4;

m - mass of the measured cheese sample (g).

The total protein (TP) content is calculated when the total nitrogen (TN) is multiplied by a milk protein factor of 6.38%.

TP (%) = TN • 6.38

The extent of protein degradation by SDS-PAGE electrophoresis.

Statistical evaluation: The basic characteristics of a series of obtained data are shown through the mean values. The deviations of the individual data in each serie from the arithmetic mean of the corresponding serie, as well as the strength of their grouping around the mean, are shown through measures of variation (standard deviation and coefficient of variation). Student's t-test was applied to test the differences of the mean values (9). The SPSS statistics software (IBM, USA) was used.

3. RESULTS AND DISCUSSION

The most important and most complex changes during the ripening of cheeses occur to proteins. Therefore, ripening of cheese actually implies changes of proteins. The extent and dynamics of protein degradation are closely related to the technology of cheese production, ripening conditions, activity of coagulant enzymes, starters, non-starter microflora, etc.

If we talk about the dynamics of nitrogenous substances, and changes to proteins, it should be considered several factors in indigenous cheese production: milk is curdled fresh, without heat treatment, and it has a corresponding effect on the protein characteristics, as a changing substrate subject during ripening. Actually, to the cheeses, which are produced in traditional way, the basis of the protein matrix is casein, which easily subject to changes during ripening. Also, starter cultures are not added to these types of cheeses during the production, but the basic factors of ripening are enzymes of coagulants and non-starter-autochthonous microflora that naturally exist in milk.

The original raw material for Sjenica cheese is sheep milk. That raw material should be preserved as much as possible in the production of his type of cheese. Mirecki et al. (10) states that in the traditional production of Njegusi cheese, originality increasingly disappears, because sheep milk is increasingly replaced by cow, goat or a mixture of these types of milk.

Protein breakdown gradually takes place during ripening. In the first stage of protein breakdown, insoluble products are formed, while in the second stage, soluble but non-volatile products of separation are formed. They affect on consistency formation of white cheese. In the last, third phase of ripening, further decomposition of protein breakdown products results in the introduction of soluble and volatile products that affect the formation of the taste and smell of white cheese (11).

Table 1 and chart 1 shows the dynamics of total proteins during ripening of Sjenica cheese, and Table 2 and chart 2 shows the dynamics of total proteins in dry matter.

Based on the results from *Table 1*, it can be seen that the content of total proteins after production in fresh cheese was 13.43% for Sjenica, and 13.77% for the type of Sjenica cheese. The largest increase of total proteins in Sjenica cheese is recorded in the first 30 days of ripening (2.11%), when it reaches a maximum value of 15.54%, which is in the line with increase of cheese dry matter content. For the type of Sjenica cheese, an increase in total proteins of 2.78% is recorded during 60 days of ripening, when it reaches a maximum of 16.55%. After 30 days, for Sjenica cheese, or 60 days for the type of Sjenica cheese, a slight continuous decrease of total proteins was observed in both types of cheeses. After 180 days of ripening the content of total proteins was 12.78% for Sjenica cheese and for the type of Sjenica it was 13, 92%. The decrease happened due to transfer of a part of soluble nitrogenous substances from cheese to ripening brine.

The obtained results are in accordance to the results from this cheese group, stated by the following authors: white cheese 15.12%, Živić et al. (12);

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Homolje cheese, 17.37%, Maćej et al. (13); Sjenica, 17.34%, Savić (14); Polimlje-Sjenica cheese, 14.04%, Dozet et al. (15); Homolje cheese 17.32%, Zlatar cheese 16.91%, Jovanović et al. (11); Svrljig cheese 16.32%, Maćej et al. (13); Teleme, 16.42%, Abdelsalam et al. (16);

How important is a regular ripening process on the proteolysis course, Bontinis et al. (17) state that the course of proteolysis determines the development of texture and cheese sensory properties.

Activity of chimosin during ripening and the amount of residual chymosin are influenced by several factors: the amount of added rennet, the water content in the cheese and the salt content in the cheese. The pH value during curd processing has a great importance in the activity and amount of retained rennet. The lower the pH value of curd, the higher the percentage of coagulant retained in cheese, and therefore its activity during cheese ripening is higher (18). This statement is consistent with the pH conditions characterized by Sjenica cheese.

Sensory properties are also affected by free lipid acids. Barac et al. (19) analyzing the lipid acid content of three types of white cheeses in brine from the Republic of Serbia (Homolje cheese, Svrljig cheese, Zlatar cheese) has found that the content of saturated lipid acids was the lowest in Sjenica cow cheese, and the content of desired lipid acids (46-34%) was the best. Therefore, this type of cheese had the most favorable health aspects.

Protein degradation products contain significant antioxidants, ACE inhibitors, and antimicrobial properties and potential. The main ingredients responsible for the antioxidant properties of mature white cheeses in brine, are of low molecular weight peptides and amino acids. Considering the fact that they have other properties, traditional white cheeses play a significant role in maintaining the human antioxidant and antihypertensive immune system (20).

Considering that the autochthonous technology of Sjenica cheese is characterized by variability in the method of production and quality, significant variations of the results in the data series are observed, which is reflected in the values of minimum and maximum. When such variable results in series are reduced to an average value, the differences between them are minimal. Therefore, differences were not statistically significant among the cheeses for all ripening periods.

Electrophoretyc testing of protein change during cheese ripening

The proteolytic processes that take place during the ripening of indigenous white cheeses in brine are very complex, which is a consequence of some specificities in the production of these cheeses and in a large extent are dependent on the indigenous present microflora. Indigenous white cheeses in brine are made from raw whole fat milk without heat treatment and additives. This means that native protein ases of milk have a significant role in proteolytic processes during ripening. In addition, starter cultures are not used in the production of these cheeses, and besides the coagulants, enzymes of native microflora play a significant role. Among other factors, these factors are essentially the basis of differences in the chemical composition of indigenous white cheeses in brine. The large differences in composition are the consequence of the action of different indigenous microflora (21).

The SDS-polyacrylamide gel electrophoresis was performed as the aim of the experiment, in order to obtain a more complete picture of ripening of Sjenica cheese and the type of Sjenica cheese. The picture of the electrophoregram shows that the proteolytic changes mostly occurred during the first 60 days of ripening.

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Table 1. Dynamics of total proteins during cheese ripening (%)

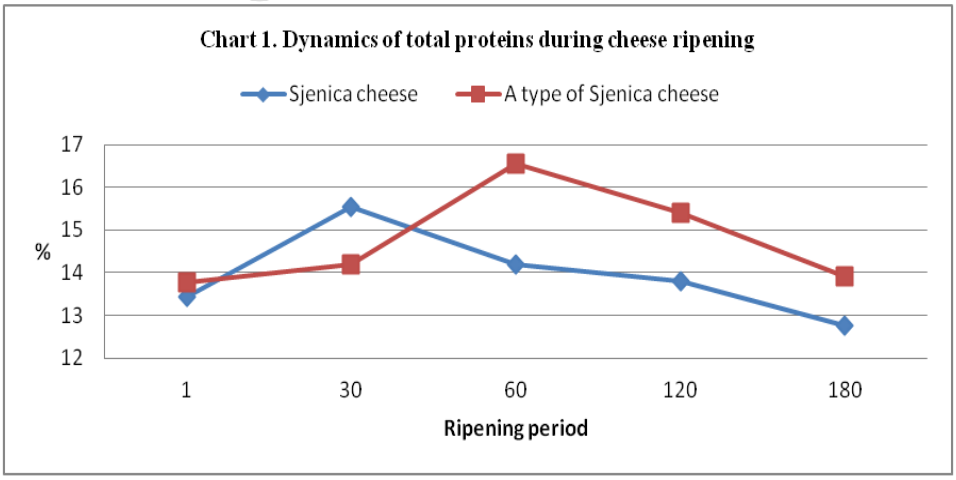
Type of cheese	Parameters	Ripening period (days)				
		1	30	60	120	180
Sjenica cheese	*min*	11.33	14.08	11.46	10.94	10.88
	max	15.10	17.28	17.28	15.55	15.81
	$\bar{x}$ *(n=6)*	13.43	15.54	14.21	13.79	12.78
	Sd	1.57	1.30	2.29	1.93	1.84
	CV(%)	11.66	8.38	16.09	13.96	14.40
The type of Sjenica cheese	*min*	13.18	14.21	15.23	13.95	12.35
	max	14.53	14.21	17.79	19.01	17.47
	$\bar{x}$ *(n=6)*	13.77	14.21	16.55	15.39	13.92
	Sd	0.51	0.00	0.85	1.88	2.03
	CV(%)	3.69	0.00	5.12	12.18	14.61

Theoretical values of the t-arrangement refer to the number of degrees of freedom (df) = 10 and are p < 0.05 = 2.28; p < 0.01 = 3. 16; t-calculated by days: 1 = 0. 58; 30 = 2. 08; 60 = 3. 15; 120 = 2. 02: 180 = 1. 44.

Table 2. Dynamics of total proteins in dry matter.

Type of cheese	Parameters	Ripening period (days)				
		1	30	60	120	180
Sjenički cheese	*min*	30.13	29.44	25.63	24.18	25.30
	max	38.46	35.03	34.00	29.66	30.67
	$\bar{x}$ *(n=6)*	34.18	32.18	28.72	27.12	28.09
	Sd	3.20	1.94	2.88	2.38	2.35
	CV(%)	9.36	6.01	10.04	8.77	8.35
The type of Sjenički cheese	*min*	29.02	28.35	31.11	26.43	26.46
	max	35.46	31.97	36.48	34.48	37.59
	$\bar{x}$ *(n=6)*	32.05	30.19	34.22	30.61	29.47
	Sd	2.21	1.62	1.92	2.67	4.13
	CV(%)	6.89	5.35	5.62	8.72	14.02

Theoretical values of the t-arrangement refer to the number of degrees of freedom (df) = 10 and are p < 0.05 = 2.22; p < 0.01 = 3. 16< t-calculated by days: 1 = 2.26*; 30 = 2.61*; 60 = 6.17**; 120 = 3.83**: 180 = 1.33.



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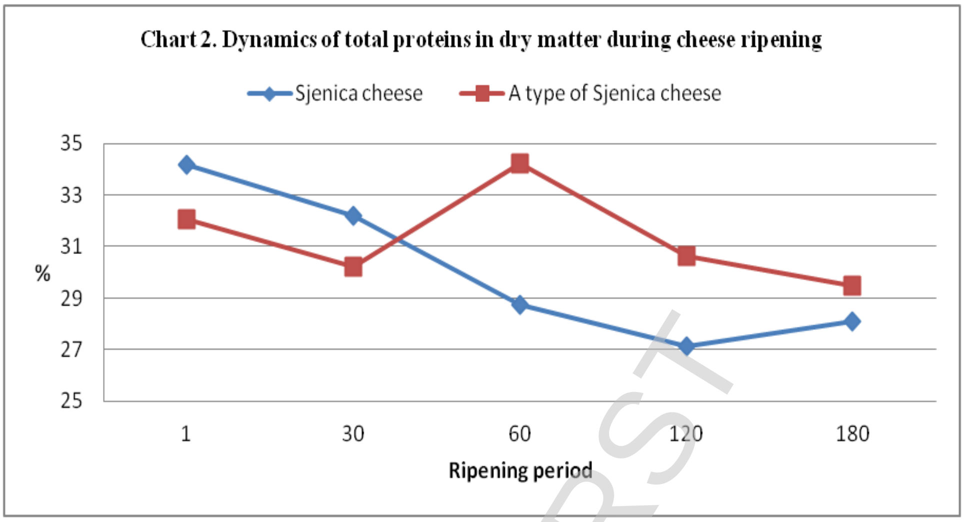

Chart 2. Dynamics of total proteins in dry matter during cheese ripening

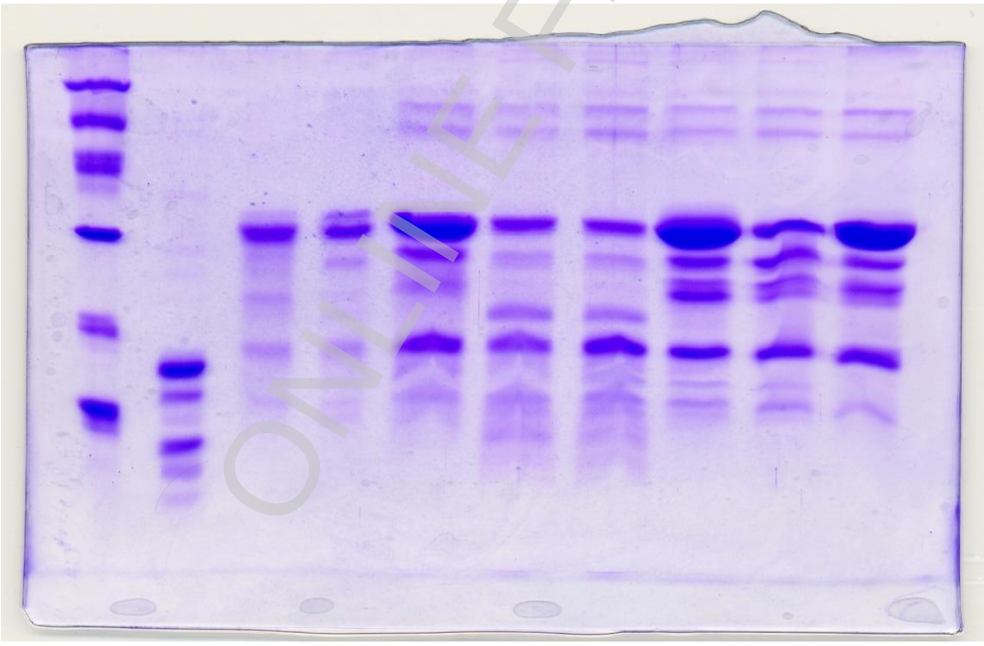

Figure 1. Electrophoregram 1 sheep cheese sample and 2 cow cheese samples (k1 and k2) after 1 day, with casein and proteins of known molecular weight (from left to right: O_1, O_2, O_3, O_4, O_5, O_6 – sheep's cheese samples 1 day old, K_1, K_2, - cow's cheese samples 60 days old, 9-β- kazein, 10- low molecular weight standard)

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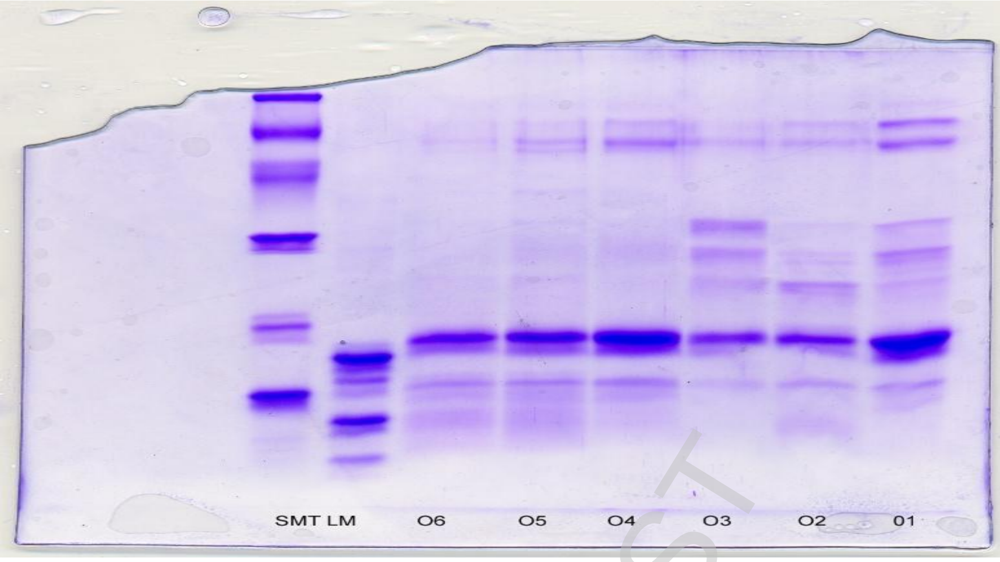

Figure 2. Electrophoregram 2 sheep cheese samples after 60 days of ripening, with casein and proteins of known molecular weight (O_1, O_2, O_3, O_4, O_5, O_6– samples of sheep's cheese 60 days old, LM-β- kazein, SMT-low molecular weight standards)

4. CONCLUSIONS

Proteins are the most important parameter of milk, which affects the course of coagulation. The composition and technological properties of curd form structure basis and rheological characteristics of cheese. Sjenica cheese, which is produced in the traditional way by enzymatic coagulation using rennet, without addition of pure cultures, indicates that the key role during ripening is played by rennet enzymes and non-starter microflora that is naturally present in milk. Cheese ripening is a typical enzymatic process, and the most important and complex changes occur on proteins. Changes to proteins during ripening determine the texture and sensory properties of mature cheese. The biggest changes in total proteins occurred in Sjenica cheese during the first 30 days of ripening, and in the type of Sjenica cheese in the first 60 days of ripening. After this period, there is a slight continuous decrease of total proteins in both types of cheeses, as a result of nitrogenous substances transition from cheese to brine. Electrophoretic tests showed that the proteins were mostly degraded during the first 60 days of ripening, after which the fractions appear as diffuse zones in traces.

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