Proteolytic activity of the Carpathian traditional liquid milk coagulant

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Abstract

Introduction. In the Ukrainian Carpathians, a special Carpathian liquid milk (CLM) coagulant is used for the production of traditional soft cheeses such as brynza and budz. Since CLM coagulant has not been studied before, the aim of the work was to characterize its milk coagulant and proteolytic properties.

Materials and methods. Milk coagulants were obtained from farms in Vorokhta, Carpathians. Milk-clotting activity was examined by the Soxhlet method. The content of proteins was determined using the Kjeldahl method. Homogeneous α_{S1}-, β- and ϰ-casein substrates were separated by preparative electrophoresis. The concentration of the products of casein substrates proteolysis was determined spectrophotometrically. The specificity of casein fractions proteolysis was established by analytical electrophoresis.

Results and discussion. CLM coagulant has high milk coagulant activity, which is slightly lower than of the standard rennet enzyme and is close to the solid milk coagulant preparation Glek. It was found that the activity of CLM coagulant increases during its storage, which is apparently achieved through the proteolytic enzyme extraction from calf's stomach cells. The milk clotting time for fresh CLM coagulant is 159 min, and after 18 months of storage it is reduced to 49 min. Milk-clotting activity of the fresh CLM coagulant is 2515 Soxhlet Units (SU), whereas after storage during 18 months the milk-clotting activity increased to 8100 SU.

In the production of Carpathian brynza and budz, it is recommended to use the amount of CLM coagulant, which provides the beginning of milk coagulation at 33–35 °C after 20–25 min. The analysis of the casein substrates proteolysis products by electrophoretic and spectrophotometric methods shows the high specificity of CLM coagulant to ϰ-casein. Such specificity is characteristic for the purified natural milk coagulant enzyme - chymosin. Along with that, CLM coagulant, unlike pepsin and, to a lesser extent, standard rennet, practically does not cleave α_{S1}- and β-casein substrates.

Conclusions. Milk-clotting activity of CLM coagulant during its storage for 18 months increases 3 times. According to the specificity of α_{S1}-, β- and ϰ-casein substrates proteolysis, CLM coagulant is close to chymosin.

Introduction

In the Carpathian ecologically clean region of Ukraine, traditional products, which are able to provide full-valuable nutrition, have been produced since ancient times. The original waste-free technologies of their production allow the use of all milk components. Such products have good sensory properties (Cais-Sokolińska et al., 2020; Weichselbaum et al., 2009), as well as high nutritional and biological value (Blazic et al., 2017; Rybak 2016). These include guslyanka, pretaned milk, urda, budz, and Carpathian brynza (Braichenko et al., 2020). The interest in these products has significantly grown (Slyvka et al., 2018). They can be of great importance for the development of tourism in the Carpathians. Their production will become highly profitable if they get international recognition and the status of National Product.

It is worth noting that traditional products are characteristic of many countries. Some of them are protected by EU regulations (Commission regulation (EC) No 642, 2007; Commission Regulation (EC) No 676, 2008). In particular, Bryndza Podhalanska (Poland) was granted the Protected Designation of Origin (PDO) of EU as well as Slovenská Bryndza was granted the Protected Geographical Indication (PGI) as they are produced in a defined mountainous regions of Poland and Slovak Republic, respectively (Najgebauer-Lejko et al., 2022; Semjon et al., 2018).

Traditional soft cheeses are considered to be produced locally or regionally, usually seasonally, for many generations, and they have an important place in rural regional food culture (Hernik et al., 2022; Weichselbaum et al., 2009). Furthermore, they reflect the country's history, geography, climate and agriculture (Braichenko et al., 2020; Trichopoulou et al., 2007). In many central European countries varieties of bryndza are made. In different countries this cheese is designated with different names: Bryndza Podhalańska in Poland (Nalepa et al., 2023), Bryndza in Slovakia (Kačániová et al., 2020), Brânză de burduf in Romania (Donnelly et al., 2016), tulum cheese in Turkey and Bulgaria (Dimitrova et al., 2018; Karagozlu et al., 2009).

Another cheese is urda, which has a long tradition of production in mountainous areas of Montenegro (Rasovic et al., 2017) as well as in high mountainous areas of the region of Konitsa, Epirus in Greece (Pappa et al., 2016). However, in Bulgaria it is called the izvara, in Italy ricotta (El-Sheikh et al., 2010; Kamel et al., 2013). Despite this, in different regions the production of urda is similar and based on warming the whey and separating its proteins (Law et al., 2010; Rasovic et al., 2017).

It is known that coagulants play an important role in the clot formation, as well as in the rheological and sensory properties of cheeses (Law et al., 2010; Walstra et al., 2006). In our laboratory, the milk coagulant properties, as well as the proteolytic activity of the solid milk coagulant Glek (Iukalo, 2015) were studied. The regularities of changes of the coagulant proteolytic activity during storage and the specificity of its action in relation to the main casein fractions were established. However, the authors' expedition to the Carpathians showed that today, for the production of budz and Carpathian brynza, more often is used a Carpathians liquid milk (CLM) coagulant, but not Glek. This coagulant differs in manufacturing technology and can be used for cheese production during three years after its manufacture, and according to some reports, up to five years. The properties of CLM coagulant have not been investigated for today and required to be studied.

In this regard, the aim of this work was to study the milk coagulant properties, proteolytic activity and specificity of the Carpathian liquid milk coagulant, which is used for the production of budz and Carpathian brynza.

Materials and methods

Materials

CLM coagulants of different life shelf were used for the study. They were obtained from farms in the urban-type village of Vorokhta, Ivano-Frankivsk region (Figure 1). Also, standard rennet enzyme and pepsin (Kaunas, Lithuania) were used to compare the proteolytic action. Skimmed milk (18 °T) for determination of milk coagulant activity and fresh whole milk for isolation of casein substrates were obtained from the Ternopil Milk Factory. Buffer solutions and reagents for electrophoresis were prepared from reagents of the Reanal Company (Hungary) and domestic reagents of a high degree of purification.

Figure 1. CLM coagulant of different shelf life:
1, freshly isolated; 2, during 6 months; 3, during 18 months

Characteristics of CLM coagulant

Milk-clotting activity of the CLM coagulant was the Soxhlet method as previously described in (Hyslop, 2003; Teplý et al., 1976).

Determination of moisture content in CLM coagulant was carry out by the drying of samples to a constant mass at 100 °C.

The protein content of CLMC was determined according to the Kjeldahl method, using the conversion coefficient 6.25 for the calculation of protein's content (Fox et al., 2015).

The formation and quality of a milk clot's structure during coagulation were visually determined.

Casein fraction proteolysis

Proteolysis of each casein fraction was carried out with enzyme preparations: CLM coagulant, standard rennet enzyme and pepsin. The amounts of enzyme preparations was

taken in such way that they had the same milk coagulant activity. At the same time, the enzyme: substrate ratio for pepsin was set at 1:100.

Proteolysis occurred at a temperature of 35 °C and a pH value of 7.2. The same conditions were used earlier in the study of the action of the solid preparation Glek (Iukalo, 2015). During proteolysis, after 15, 30, 60 and 120 min, samples were taken to determine the cleavage products of the casein fraction, soluble in 12% solotion of trichloroacetic acid (TCA). The concentration of proteolysis products was represented as the optical density determined at a wavelength of 280 nm by a SF-46 spectrophotometer (JSC "Lomo", Russia). For greater objectivity, when comparing the proteolysis of casein fractions, the difference in the values of their absorption coefficients at 280 nm was taken into account. Also, at the 60th min of proteolysis, samples were taken for electrophoretic analysis.

Concentration of total casein and casein fractions and products of their proteolysis

The concentration of total casein and casein fractions was determined spectrophotometrically by absorption in the ultraviolet region of the spectrum at a wavelength of 280 nm. The optical density of casein solutions was measured on a SF-46 spectrophotometer. Absorption coefficients ($D_{1\ cm}^{1\ \%}$) were used for calculation: for total casein, 8.2; for $\varkappa$-casein, 9.6; for β-casein, 4.6; for α_{S1}-casein, 10.0 (Farrell et al., 2004).

During determining the proteolysis products of α_{S1}-, β-, and $\varkappa$-casein substrates, soluble in 12% trichloroacetic acid, their concentration was represented as the value of the optical density at 280 nm. For an objective comparison of the concentrations of different fractions proteolysis products, their optical density values were adjusted to the α_{S1}-casein proteolysis products. At the same time, the value of optical density for β-casein hydrolysates was multiplied by 2.17 and for $\varkappa$-casein by 1.04.

Isolation of casein from cow milk

Fresh whole milk was skimmed by centrifugation at 4000 g (15 min). The procedure was repeated twice: the first time at 30 °C, and the second time at 4 °C. In skim milk, caseins were precipitated at the isoelectric point. The pH value was adjusted to 4.6 by adding a solution of hydrochloric acid with a concentration of 1 mol/l[3]. The precipitate was washed with distilled water and dissolved with 1 mol/l[3] sodium hydroxide at pH values not higher than 7.7–7.9. The precipitation procedure was repeated twice. Inactivation of milk proteases was carried out by casein incubating in acetic acid (pH 4.0) at a temperature of 4 °C for 5 hours.

Electrophoresis in polyacrylamide gel

Analytical electrophoresis of total casein, homogeneous casein substrates and their proteolysis products was performed in polyacrylamide gel (PAG) plates in an anodic electrophoretic system in the presence of 4.5 M urea (Fox et al., 2015; Tremblay et al., 2003). The details of the analytical electrophoresis method of caseins were described by us earlier (Yukalo et al., 2019).

Preparative electrophoresis of total casein was performed in a Stadier-type apparatus with an enlarged chamber for homogeneous PAG. The electrophoretic system for the isolation of electrophoretically homogeneous fractions of α_{S1}-, β-, and $\varkappa$-caseins is described in other works (Iukalo, 2014; Yukalo et al., 2018).

Statistical processing

All analyses were performed on duplicate samples, and the experiment was repeated 3 times. Statistical analysis was performed using the program Statistika 10. Differences were considered significant at $P \leq 0.05$. Standard deviations are indicated in the table or shown as error bars in the figures.

Results and discussion

Milk-clotting activity of the CLM coagulant

Milk-clotting activity and moisture content were determined for CLM coagulant of different shelf life time at 4 °C. Clots formed in milk under the action of the preparations were also evaluated. The results of the research are presented in Table 1.

Table 1

Milk-clotting activity of the CLM coagulant at different storage periods

Enzyme preparation	Milk clotting time, min	The milk's clot quality	Milk-clotting activity by Soxhlet, SU	Moisture content, %
Standard rennet	16±1.0	Strong clot	100 000±1000,0	7.2±0.2
CLM coagulant (fresh)	159±11.0	Weak clot	2515±70.0	85±3.0
CLM coagulant (6 months)	69±4.0	Strong clot	5790±120.0	84±2.5
CLM coagulant (12 months)	56±3.0	Strong clot	6937±137.0	84±3.2
CLM coagulant (18 months)	49±3.0	Strong clot	8100±190.,0	82±2.7
CLM coagulant (24 months)	73±4.0	Strong clot	5100±130.0	80±2.5

The milk clotting time and the formation of milk's clot for the fresh CLM coagulant sample are quite long and is about 2.5 h (159 min). It takes place due to the low milk-coagulant activity of the CLM coagulant because of the small amount of enzymes that are extracted from secretory cells of the calf's stomach into the surrounding water environment. During the storage of CLM coagulant, the amount of chymosin and other enzymes increases, as their extraction time lengthened. Therefore, the clotting time is reduced to 69 min after 6 months of CLM coagulant storage, 59 min after 12 months of storage, and 49 min after 18 months of storage. With these samples of CLM coagulant, a strong clot is formed, which in its characteristics is similar to the clot formed by pepsin.

It was established that the milk-clotting activity of the freshly prepared liquid coagulant, when calculated on a dry basis, is close to that of the standard rennet enzyme, as well as the preparation Glek (Iukalo, 2015). This ratio of activity is predictable, taking into account the high water content in CLM coagulant, which varies within the range of 80 to 85% at different periods of its storage. Moreover, in contrast to the preparation Glek, in CLM coagulant the milk-clotting activity increases during storage.

The milk-clotting activity of fresh CLM coagulant is 2515 SU whereas after 6, 12, 18 months of storage are 5790, 6937, 8100, respectively. CLM coagulant exhibits maximum proteolytic activity at 18th month of its storage that is caused by the maximum number of enzymes in the solution, which were extracted from secretory cells of the calf's stomach. During further storage, extraction processes stop, only denaturation processes occur, causing a decrease in the activity of chymosin and other cell's proteolytic enzymes. In particular, the milk-clotting activity in the CLM coagulant after 24 months of storage decreased to 5100 SU. At the same time, the milk's clot becomes a little weaker, although this does not prevent the use of the preparation in the traditional Carpathian cheese's production. The decrease in the milk-clotting activity of CLM coagulant, established after 24 months of storage, requires its use in a larger amount to ensure the necessary quality characteristics of cheese.

During the expedition to Vorokhta, we discovered and researched CLM coagulant with a maximum shelf life of 18 months. According to the testimony of local farmers, liquid coagulants keep their activity for up to three years. It is recommended to use CLM coagulant in the production of Carpathian brynza and budz no earlier than one month after its production. Before this period, the preparations do not provide the formation of a strong clot. Before a clot obtaining, milk is heated to 32-35 °C. Then we add such an amount of CLM coagulant, which ensures the start of milk coagulation during 20-25 min.

Proteolytic properties of CLM coagulant

An important question in the characteristic of a milk coagulant is the establishment of its proteolytic properties (Hyslop, 2003). This primarily concerns its proteolytic activity towards the main casein fractions. To study the specificity of the CLM coagulant proteolytic action, we obtained α_{S1}-, β-, and ϰ-casein fractions by preparative electrophoresis. As known, the proteolysis of these fractions is of great importance for the process of milk coagulation, as well as the formation of cheeses' organoleptic indicators (Guinee, 2003; Law et al., 2010). In Figure 2 it is shown the PAG plate after preparative fractionation of total casein, as well as the areas from which casein substrates were isolated. The obtained electrophoretic fractions of α_{S1}-, β- and ϰ-caseins were used to establish the specificity of the CLM coagulant proteolytic action.

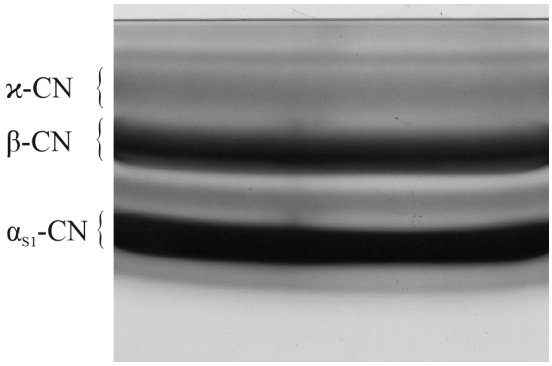

Figure 2. Preparative electrophoresis of cow's milk total casein

The results of proteolysis studies are shown in Figures 3–8. It can be seen that CLM coagulant, on the background of the identical milk-clotting activity, showed at the same time a lower proteolytic activity against α_{S1}- and β-caseins compared to pepsin and close to, but also lower than the standard rennet enzyme.

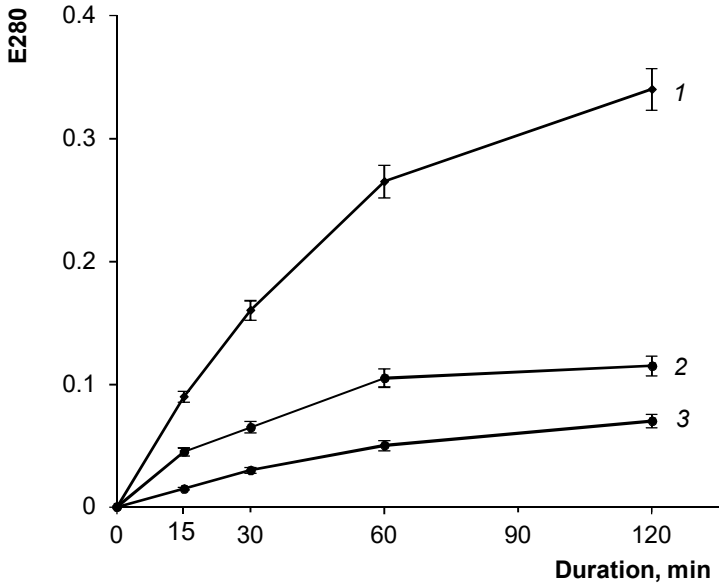

Figure 3. The course of the α_{S1}-casein substrate proteolysis:
1, pepsin; 2, standard rennet enzyme; 3, CLM coagulant

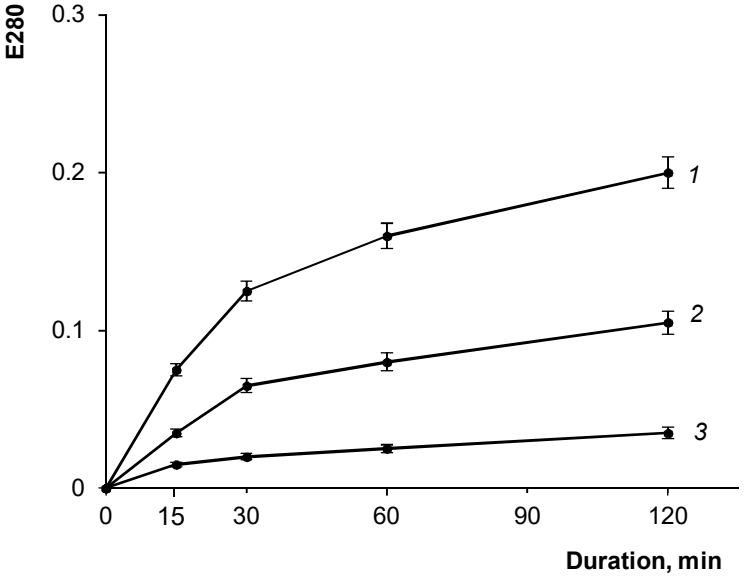

Figure 4. The course of proteolysis of the β-casein substrate:
1, pepsin; 2, standard rennet enzyme; 3, CLM coagulant

Figure 3 shows that the optical density (E_{280}) of cleavage products of the α_{S1}-casein substrate by CLM coagulant (0.05) at the end of proteolysis is 6 times lower compared to pepsin (0.32). It indicates a lower proteolysis products's concentration, caused by the low activity of CLM coagulant proteases in relation to α_{S1}-casein. During the β-casein substrate proteolysis (Figure 4), optical densities (E_{280}) are 0.02 and 0.2, respectively for CLM coagulant and pepsin. Such specificity of proteolytic action in relation to α_{S1}- and β-casein fractions is typical for the natural milk-coagulant enzyme – chymosin (Barbano et al., 1992; Iukalo, 2015). This may be due to the high content of chymosin (EC 3.4.23.4) in the CLM coagulant. The slightly higher proteolytic activity of standard rennet (Figures 3 and 4), compared to CLM coagulant, is caused by the presence in it of two minor proteases: pepsin A (EC 3.4.23.1) and gastricsin (EC 3.4.23.3) in small amounts, in addition to chymosin (Corredig et al., 2016).

These results are confirmed by electrophoretic analysis (Figures 5 and 6).

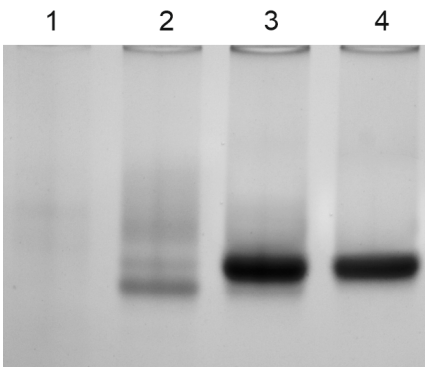

Figure 5. Analytical electrophoresis:
1, α_{S1}-casein substrate after proteolysis by pepsin (60 min);
2, α_{S1}-casein substrate after proteolysis by standard rennet enzyme (60 min);
3, α_{S1}-casein substrate after proteolysis by CLM coagulant;
4, α_{S1}-casein substrate

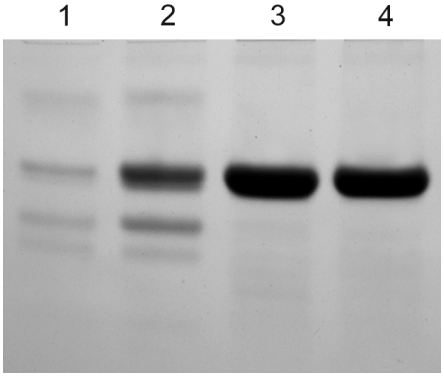

Figure 6. Analytical electrophoresis:
1, β-casein substrate after proteolysis by pepsin (60 min);
2, β-casein substrate after proteolysis by standard rennet enzyme (60 min);
3, β-casein substrate after proteolysis by CLM coagulant;
4, β-casein substrate

Figure 5 shows that the α_{S1}-casein substrate remained almost completely unchanged during proteolysis by CLM coagulant enzymes (sample 3), while it was completely cleaved when pepsin was used – absent in sample 1. Similar results are visible in Figure 6: the β-casein substrate in sample 3 (after CLM coagulant proteolysis) is the same as in sample 4, which was not subjected to proteolysis. It apparently indicates a weak enzymatic activity of CLM coagulant in relation to this casein fraction. Similar results are noticeable for standart rennet enzyme (sample 2, Figure 6). Only after proteolysis with pepsin (sample 1, Figure 6) the β-casein substrate was cleaved almost completely.

When analyzing the results of κ-casein proteolysis, a certain divergence can be noted between the results of determining the concentration of peptides and polypeptides soluble in TCA (Figure 7) and the data of analytical electrophoresis (Figure 8). This is due to the separation's peculiarities of the main products of κ-caseins proteolysis by lactic coagulation enzymes (glycomacropeptide and para-κ-casein) in the anode electrophoresis system (Fox et al., 2015).

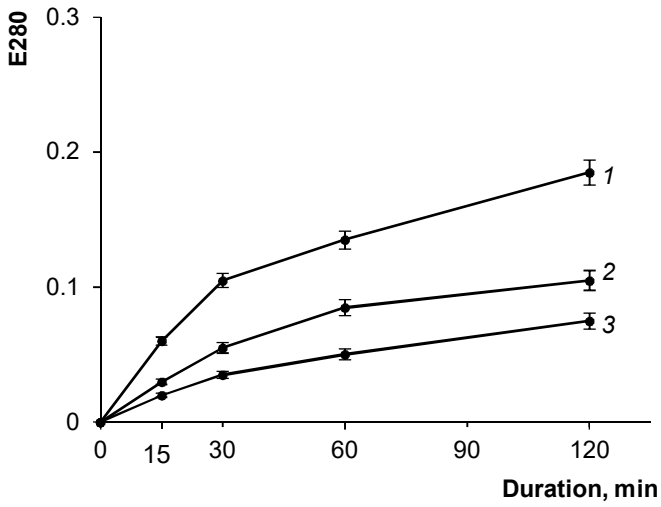

Figure 7. The course of proteolysis of the κ-casein substrate:
1, pepsin; 2, standard rennet enzyme; 3, CLM coagulant

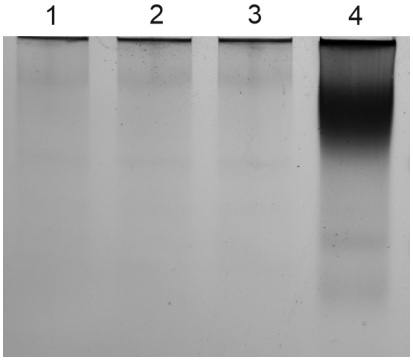

Figure 8. Analytical electrophoresis:
1, κ-casein substrate after proteolysis by pepsin (60 min);
2, κ-casein substrate after proteolysis by standard rennet enzyme (60 min);
3, κ-casein substrate after proteolysis by the CLM coagulant;
4, κ-casein substrate

According to electrophoresis, κ-casein is completely splited under the action of all three milk coagulants. This is fully consistent with literature data (Hyslop, 2003). However, the concentration of κ-casein proteolysis products is much lower than in the case of other casein substrates. Obviously, this is related to the features of the primary structure of κ-casein and the specificity of its proteolysis by milk coagulants. In all three cases, para-κ-casein (f 1-105) and glycomacropeptide (f 106-169) are formed. Both of these fragments are absent in the PAG plate. Para-κ-casein has a positive charge and is not included in the PAG of the anode electrophoretic system (Sharma et al., 2021). Glycomacropeptide (GMP) is not fixed in the gel plate. Thus, the cleavage of only one peptide bond (105-106) produces two proteolysis products that are not fixed in the gel plate (Figure 8). On the other hand, during precipitation

with a 12% TCA solution, GMP and low-molecular-weight products of ϰ-casein proteolysis remain in the solution. At the same time, para-ϰ-casein is precipitated. It should be noted here that GMP does not contain aromatic amino acid residues in its composition and does not absorb at 280 nm (Fox et al., 2015). Only the breakdown products of para-ϰ-casein can be absorbed. Such products can be formed to a large extent by the action of pepsin and to a lesser extent by the action of standard rennet, which contains impurities of pepsin. This is also evidenced by the formation of casein bands on electropherograms of α_{S1}- and β-casein proteolysis under the action of pepsin and standard rennet (Figures 3 and 4).

A low level of cleavage of ϰ-casein (mainly one peptide bond 105-106) is characteristic under the action of purified chymosin, the main component of the standard milk coagulation enzyme (Hyslop, 2003). A similar low level of ϰ-casein proteolysis is shown by the CLM coagulant.

Conclusion

1. Carpathian liquid milk (CLM) coagulant is characterized by relatively high coagulant activity, which increases during its storage. CLM coagulant exhibits maximum proteolytic activity 8100 SU at 18-th month of its storage. During further storage, CLM coagulant reduces its activity, therefore, in order to ensure the required quality characteristics of cheeses, it is necessary to use the coagulant in a larger amount.
2. To form a strong clot, CLMCP is recommended to be added to milk heated to a temperature of 32-35°C, in an amount that ensures the beginning of coagulation within 20-25 minutes.
3. Analysis of the concentration and composition of proteolysis products of homogeneous α_{S1}-, β- and ϰ-casein fractions indicates that the main enzyme in the composition of CLM coagulant is chymosin. It is confirmed by the high specificity for the ϰ-casein cleavage and the lowest activity among the studied milk-clotting preparations in relation to α_{S1}- and β-casein substrates. In further studies, it is important to study in more detail the structure and biological activity of proteolysis products, as well as their influence on the product's taste properties.

Reference

Barbano D.M., Rasmussen R.R. (1992), Cheese yield performance of fermentation-produced chymosin and other coagulants, *Journal of Dairy Science*, 75(1), pp. 1-12, https://doi.org/10.3168/jds.S0022-0302(92)77731-5

Blazic M., Pavic K., Zavadlav S., Marcac N. (2017), The impact of traditional cheeses and whey on health, *Croatian Journal of Food Science and Technology*, 9(2), pp.198-203, https://doi.org/10.17508/CJFST.2017.9.2.11

Braichenko O., Hrymych M., Lylo I., Reznichenko V. (2020), *Ukraine: Food and History,* Kyiv.

Cais-Sokolińska D., Kaczyński Ł. K., Bierzuńska P., Skotarczak E., Dobek A. (2020), Consumer acceptance in context: Texture, melting, and sensory properties of fried ripened curd cheese, *International Journal of Dairy Technology*, 74(1), pp. 225-234, https://doi.org/10.1111/1471-0307.12747

Cetinkaya A., Kaban G.(2021), Some quality properties and volatile compound profile of Ardahan Tel cheese, a traditional cheese in Turkey, *Ukrainian Journal of Food Science*, 9(1), pp. 18-29, https://doi.org/10.24263/2310-1008-2021-9-1-4

Commission regulation (EC) No 642/2007, (2007), *Official Journal of the European Union*, L 150/4.

Commission Regulation (EC) No 676/2008, (2008), *Official Journal of the European Union*, L 189, pp. 19–20.

Corredig M., Salvatore E. (2016), Enzymatic coagulation of milk: In: P.L.H. McSweeney and J.A. O'Mahony (Eds.), *Advanced Dairy Chemistry: Proteins: Applied Aspects,* (Fourth edition), Springer, New York, NY, pp 287–307.

Donnelly C., Kehler M. (2016), *The Oxford Companion to Cheese*, Oxford Companions. Oxford University Press, p. 81.

Dimitrova, K., Vuchkov, A. (2018), Tulum cheese - cheese making technology and main characteristics, *Journal of Mountain Agriculture on the Balkans,* 21(3), pp.1-26.

El-Sheikh M., Farrag A., Zaghloul A. (2010), Ricotta cheese from whey protein concentrate, *Journal of American Science*, 6(8), pp. 321–325.

Farrell H.M., Jimenez-Flores R., Bleck G.T., Brown E.M., Butler J.E., Creamer L.K., Hicks L.C., Hollar C.M., Ng-Kwai-Hang K.F., Swaisgood H.E. (2004), Nomenclature of the proteins of cows' milk sixth revision, *Journal of Dairy Science*, 87(6), pp. 1641–1674, https://doi.org/10.3168/jds.S0022-0302(04)73319-6

Fox P.F., Uniacke-Lowe T., McSweeney P.L.H., O'Mahony J.A. (2015), *Dairy Chemistry and Biochemistry* (Second edition), Springer, New York.

Iukalo A.V. (2014), New approach for isolation of individual caseins from cow milk by the preparative electrophoresis, *Advances in Biololical Chemistry*, 4(6), pp. 382–387, https://doi.org/10.4236/abc.2014.46043

Iukalo A.V. (2015), Milk-coagulation and proteolytic activity of «Glek» carpathian enzyme preparation, *Biotechnologia Acta*, 8(2), pp. 91–95, https://doi.org/10.15407/biotech8.02.091

Guinee T.P. (2003), Role of protein in cheese and cheese products: In *Advanced dairy chemistry Volume 1: Proteins (Third edition). Part B*, edited by P.F. Fox and P.L.H. McSweeney, Kluwer academic / Plenum publishers, New York, pp. 1083–1174.

Hernik J., Walczycka M., Sankowski E., Harris B.J. (2022), *Cultural Heritage—Possibilities for Land-Centered Societal Development*, Springer Nature, Berlin/Heidelberg, , https://doi.org/10.1007/978-3-030-58092-6

Hyslop D.B. (2003), Enzymatic coagulation of milk: In: P.F. Fox and P.L.H. McSweeney (Eds.), *Advanced Dairy Chemistry: Proteins* (Third edition), Part B, Kluwer academic / Plenum publishers, New York, pp. 839–878.

Kačániová M., Nagyová L., Štefániková J., Felsöciová S., Godočí-ková L., Haščík P., Horská E., Kunová S. (2020), The characteristic of sheep cheese "Bryndza" from different regions of Slovakia based on microbiological quality. *Potravinarstvo Slovak Journal of Food Sciences*, 14, pp. 69–75, https://doi.org/10.5219/1239

Kamel B., Boubaker K., Attia H. (2013), Implementation of ricotta cheese production process in Tunisia, *International Food Research Journal*, 20(5), pp. 2343–2348.

Karagozlu C., Kilic S., Akbulut N. (2009), Some characteristics of cimi tulum cheese from producing goat milk, *Bulgarian Journal of Agricultural Science*, 15(4), pp. 292–297.

Law B. A., Tamime A.Y. (2010), *Technology of cheesemaking (Second Editon)*, Blackwell Publishing Ltd, Oxford, https://doi.org/10.1002/9781444323740

Najgebauer-Lejko D., Domagała J., Walczycka M. (2022), Traditional Cheeses from the Malopolska Region. In: *Cultural Heritage—Possibilities for Land-Centered Societal Development*, Springer Nature, Berlin/Heidelberg, Germany, pp. 171–190.

Nalepa B., Markiewicz L.H. (2023), Microbiological biodiversity of regional cow, goat and ewe milk cheeses produced in Poland and antibiotic resistance of lactic acid bacteria isolated from them, *Animals,* 13(1), pp.168, https://doi.org/10.3390/ani13010168

Pappa E.C., Samelis J., Kondyli E., Pappas A.C. (2016), Characterisation of Urda whey cheese: Evolution of main biochemical and microbiological parameters during ripening and vacuum packaged cold storage, *International Dairy Journal*, 58, pp. 54-57, https://doi.org/10.1016/j.idairyj.2015.12.016

Rasovic M., Nikolic N., Rasovic R. (2017), Quality of "urda" obtained after production of montenegrin semi-hard cheese, *Food Research,* 1(5), pp. 166-170, https://doi.org/ 10.26656/fr.2017.5.107

Rybak O. (2016), Milk fat in structure formation of dairy products: a review, *Ukrainian Food Journal*, 5(3), pp. 499–514, https://doi.org/10.24263/2304-974X-2016-5-3-9

Ryzhkova T., Odarchenko A., Silchenko K., Danylenko S., Verbytskyi S., Heida I., Kalashnikova L., Dmytrenko A. (2023), Effect of Herbal Extracts Upon Enhancing the Quality of Low-Fat Cottage Cheese, *Innovative Biosystems and Bioengineering*, 7(2), pp. 22–31, https://doi.org/10.20535/ibb.2023.7.2.268976

Semjon B., Reitznerová A., Poláková Z., Výrostková J., Maľová J., Koréneková B., Lovayová V. (2018), The effect of traditional production methods on microbial, physico-chemical and sensory properties of 'Slovenská bryndza'Protected Geographical Indication cheese, *International Journal of Dairy Technology*, 71(3), pp. 709–716, https://doi.org/10.1111/1471-0307.12522

Sharma N., Sharma R., Rajput Y. S., Mann B., Sight R., Gandhi K. (2021), Separation methods for milk proteins on polyacrylamide gel electrophoresis: Critical analysis and options for better resolution, *International Dairy Journal*, 114, 104920, https://doi.org/10.1016/j.idairyj.2020.104920

Slyvka I., Tsisaryk O., Dronyk O., Musiy L. (2018), Strains of lactic acid bacteria isolated from traditional Carpatian cheeses, *Regulatory Mechanisms in Biosystems*, 9(1), pp. 62–68, https://doi.org/10.15421/021808

Teplý M., Mašek J., Havlová J. (1976), *Syřidla živočišná a mikrobiální (Annimal and microbial rennets),* SNTL – Nakladatelství technické literatury, Praha.

Tremblay L., Laporte M.F., Onil J. U, Dupont D., Paquin P. (2003), Quantitation of proteins in milk and milk products: In: P.F. Fox and P.L.H. McSweeney (Eds.), *Advanced Dairy Chemistry: Proteins* (Third edition), Part A, Kluwer academic / Plenum publishers, New York, pp. 49–138.

Trichopoulou A., Soukara S., Vasilopoulou E. (2007), Traditional foods: A science and society perspective. *Trends in Food Science & Technology,* 18, pp. 420–427, https://doi.org/10.1016/j.tifs.2007.03.007

Walstra P., Wouters Jan T.M., Geurts T.J. (2006), *Dairy science and technology* (Second edition), CRC Press, NY.

Weichselbaum E., Benelam B., Soares Costa H. (2009), *Traditional foods in Europe*. Norwich: EuroFIR Project, Available at: https://www.eurofir.org/wp-admin/wp-content/uploads/EuroFIR%20synthesis%20reports/Synthesis%20Report%206_Traditional%20Foods%20in%20Europe.pdf

Yukalo V.G., Storozh L.A., Datsyshyn K.Y., Krupa O.M. (2018), Electrophoretic systems for the preparative fractionation of proteins-precursors of bioactive peptides from cow milk, *Food Science and Technology*, 12(2), pp. 26–32, https://doi.org/10.15673/fst.v12i2.932

Yukalo V., Krupa O., Storozh L. (2019), Characteristics of proteolytic processes during the isolation of natural casein phosphopeptides, *Ukrainian Food Journal,* 8(1), pp. 61–69, https://doi.org/10.24263/2304-974X-2019-8-1-7