

MILK-CLOTTING ACTIVITY OF RECOMBINANT BOVINE AND CAMEL CHYMOSIN FOR COW'S, GOAT'S AND EWES' MILK

Akischev Zhiger^{1,2}, Aktayeva Saniya^{1,2}, Shamsiyeva Yuliya^{1,2}, Tursunbekova Annelya³, Kalemshariv Begzhan³, Tultabayeva Tamara³, Khassenov Bekbolat^{1,4,*}

¹ National Center for Biotechnology, 13/5 Korgalzhyn Road, Astana, 010000;

² Faculty of natural sciences, L.N.Gumilyev Eurasian National University, 2 Kanysh Satpayev Street, Astana, 010008;

³ S.Seifullin Kazakh Agro Technical University, 62 Zhenis Avenue, Astana, 010001;

⁴ "GenLab" LLP, 19/1, 69, M. Gabdullin Street, Astana, 010000.

* khassenov@biocenter.kz

ABSTRACT

The Neolithic Age saw the domestication of goats and sheep. Dairy goats and lambs have been among the many new breeds developed since then. Milk from cows, goats, and sheep was used to examine the clotting ability of recombinant chymosins from bovine (*Bos taurus*) and camel (*Camelus bactrianus*). Recombinant bovine chymosin showed milk-clotting activities of 12.8540 ± 0.61 , 5.3850 ± 0.25 , and 14.8110 ± 0.72 U/mg when tested on milk from cows, goats, and ewes, respectively. Activity levels of recombinant camel chymosin were 29–46% higher, coming in at $16\,590 \pm 0.82$, 7850 ± 0.34 , and $20\,700 \pm 0.85$ U/mg, respectively. Both recombinant camel and bovine chymosins have proteolytic activities of 1679.97 ± 9.54 U/mg and $10,767 \pm 54.56$ U/mg, respectively. Milk from cows, goats, and sheep was used to make cheese with the use of recombinant camel chymosin. The output of cheese consisted of 18.0% cow's milk, 17.3% goat's milk, and 15.0% sheep's milk. In light of these findings, recombinant camel chymosin may be employed as a coagulation enzyme in cheeses produced from cow, goat, and sheep milk.

Key words: Cheese, dairy, enzyme, recombinant chymosin, Kazakh sheep breed

INTRODUCTION

During the Neolithic Revolution, goats (*Capra hircus*) and sheep (*Ovis aries*) were among the earliest animals domesticated by humans. About 10.2 thousand years ago, the domestication of the goat was first recorded in the Zagros Mountains in western Iran [1]. Wild bearded bezoar goats (*C. aegagrus*) and/or markhor goats are the most likely ancestors of current domestic goats (*C. falconeri*). Among the progenitors of domestic sheep are the wild mountain sheep species argali (*Ovis ammon*), urial (*Ovis vignei*), and moufflons, which range from the Mediterranean islands to Central Asia (*O. orientalis*, *O. musimon*, and *O. gmelini*). The domestication of sheep occurred in the Middle East, in the region of modern-day Turkey, around 8400 BCE [2]. Archaeological and paleogenetic studies indicated that sheep existed in Central Asia circa 6000 BCE. Analysis of collagen peptides and sequencing of ancient DNA verify the domestication of sheep in the Fergana Valley of Central Asia [3]. Despite the fact that sheep were domesticated in Central Asia later than in the Middle East, sheep breeding expanded throughout huge territories and became the primary source of income for nomadic peoples.

The following dairy goat breeds were obtained by selective breeding: Toggenbur, Russian, Megrelian, Cameroon, Gorky, Alpine, Zaanen, Nubian, and Lamancha. The seasonal production of dairy breeds ranges between 800 and 2500 liters [4]. Goat's milk may be used to make nearly the same products as cow's milk, including butter, sour cream, cottage cheese, and cheese. In addition to its organoleptic qualities, goat's milk cheese offers a number of physiologically relevant characteristics: goat's milk cheeses are regarded as lighter than cow's milk cheeses and are easier to digest [5]. The greatest advantage of goat cheese is that it is entirely hypoallergenic [6]. The majority of soft cheeses are created from goat's milk [5].

The fundamental distinction between sheep and dairy sheep is that the milk output ranges from 150 to 700 litres per lactation [7]. Dairy sheep, along with meat and wool sheep, are often bred for their wool and meat, and strong milk outputs are an additional source of income for the farmers. Sheep's milk is too fatty to be drunk in its natural state; instead, it is used to produce dairy products, including famous cheeses such as brynza, feta, and Roquefort [8]. Tsigai, Awassi, Lacaune, East Friesian, and Assaf are well-known milk-producing breeds.

There are 19 different breeds of sheep in Kazakhstan, including coarse-wooled (Gisar, Edilbay, Kazakh Kurdish coarse-wooled, Ordabasin, Saryarkin Kurdish), semi-coarse-wooled (Degereys meat-wooled, Kazakh Kurdish semi-coarse-wooled), semi-fine-wooled (Akzhaik meat-wooled, Kazakh meat-fresh semifine woolen, Kazakh meat-woolen, Kazakh semifine woolen with crossbred wool, Tsigay), fine-woolen (Meat Merino, Kazakh fine-woolen, Kazakh arharomerino, Kazakh merino, South Kazakh merino) and smushy (Kazakh karakul-kurnyaya, Karakulskaya) breeds. In general, the coarse-wooled and semi-coarse-wooled breeds are more productive in the meat category, whereas the rest of the sheep breeds, with the exception of the fur sheep breeds, combine fine-wooled and semi-fine-wooled directions with a meat category.

In conjunction with the lack of dairy sheep breeds cultivated in the country, the average milk production per ewe in Kazakhstan is much below the global average at less than 100 liters each season. For context, a single East Friesian dairy sheep may produce 400–500 liters of milk in a single season. Domestic breeds, on the other hand, have been developed specifically for year-round grazing in harsh climates like those found in Kazakhstan's deserts, semi-deserts, and arid steppes. The research and development of cheese-making techniques

using the milk of dairy goats and Kazakh sheep breeds is encouraging.

The primary components of milk from various farm animals have been compared in previous research [9-11]. Milk from cows, goats, and ewes ranges in protein (1.4–7.0), fat (0.3–9.0), lactose (3.2–7.2), and minerals (0.1–1.0) [12]. The clotting ability of milk is affected by a number of factors, including total casein and calcium content, milk acidity, lactation stage, season, and feeding frequency. Depending on the protein makeup (caseins, serum proteins), coagulating enzyme activity might be very different (or even absent).

Milk is coagulated by proteases from diverse sources [13]. Animal-derived pepsin-like enzymes, such as rennet enzymes pepsin and chymosin, are much sought after but are produced in low quantities [14]. The use of biotechnology to create recombinant enzymes with similar properties to those found in animal rennet shows promise [15]. The first recombinant enzyme authorized by the FDA was calf chymosin [16]. The majority of the rennet used is fermented, making it not only kosher and halal, but also suitable for usage by vegetarians and vegans [17]. Camel chymosin, like chymosin from calves, goats, and sheep, is promising and is active against cow [18-21], camel [21, 22], and mare [18, 21]. With improved thermostability and enhanced milk-clotting activity [20, 23], camel chymosin is a desirable alternative to bovine chymosin in the production of commercial cheese.

Earlier, we looked at yeast-produced recombinant bovine and camel chymosins [24-26]. The goal of this research was to examine the differences between bovine (*Bos taurus*) chymosin and camel (*Camelus bactrianus*) chymosin in their ability to coagulate milk from different mammals. According to the findings, camel chymosin has a higher level of milk-clotting activity compared to the more well-studied bovine chymosin. Cheeses were produced using recombinant camel chymosin and milk from cows, goats, and sheep.

MATERIALS AND METHODS

Preparation of recombinant bovine and camel chymosins

Previous descriptions of recombinant bovine and camel chymosin synthesis may be found in [24-26]. In brief, we synthesized de novo and cloned into pGAPZA on EcoRI and NotI restriction sites full-length bovine (1098 bp) (Genbank accession no. j00003.1) and camel prochymosin DNA (JARL00000000.1). Following the transformation of *P. pastoris* GS115 cells, 15 colonies were selected from YEPD-agar plates using zeocin at a concentration of 200 µg/mL and then grown in YEPD-broth. A 3 L flask was used to cultivate 500 mL of BMGY broth (1% yeast extract, 2% peptone, 100 mM potassium phosphate, pH 6.0, 1.34% YNB, 4×10⁻⁵% biotin, 1% glucose) with the clone that showed the highest clotting activity for 120 hours. Recombinant bovine chymosin was purified by centrifuging a yeast culture at 3500 × g for 15 minutes at +4 °C (BovChym). After being filtered (0.22 µm), 25 mM sodium acetate was used to lower the pH to 4.5, followed by 24 hours at rT °C, and finally 1 M HCl was used to bring the pH back down to 3. Sodium acetate buffer (pH 3.0), 25 mM NaCl, and 50 mM DEAE-Sepharose FF were used to load the mixture onto a column. A column of SP-Sepharose was pre-equilibrated in a 50 mM sodium acetate buffer (pH 3.0), 25 mM NaCl solution, and the flow through was placed

onto the column. The cells were cleaned with a 25 mM sodium acetate buffer (pH 5.5), 50 mM NaCl solution. The mixture was eluted using a 25 mM sodium acetate buffer (pH 5.5), 750 mM NaCl solution. The amount of NaCl that was present in the eluted fraction was adjusted such that it contained just 25 mM, and then the combination was loaded onto Q-Sepharose FF that had been pre-equilibrated with 25 mM sodium acetate buffer with a pH of 5.5 and 25 mM NaCl. Following column washings with 25 mM sodium acetate buffer (pH 5.5) and 25 mM NaCl, BovChym was eluted using a 50 mM - 2 M gradient of NaCl (pH 5.5). The most effective milk fractions were identified using coagulation tests and were combined for the study.

Goat's and ewes' milk

We utilized milk from cows of the Holstein breed, goats of the Saanen breed, and sheep of the Kazakh fat-tailed coarse-haired breed.

Bacterial strains

Strains of lactic acid bacteria were isolated from goat's, ewes' and mare's milk and identified with molecular genetic methods as *Lactobacillus brevis*, *Enterococcus faecium*, and *Lactobacillus casei*.

Milk-clotting assay

Rehydrated powdered cow's skim milk at 12% (w/v) in 0.025 mol/L sodium acetate buffer (pH 6.0) was used as the substrate in this test, which was performed according to ref. [27]. At least three repeats of the clone selection enzymatic processes were conducted at 37 °C in test tubes containing 1 mL of the substrate and 20 µL of an enzyme solution. The milk clots were exposed by inverting the tubes. As a control milk-clotting enzyme, chymosin from bovine rennet (BioRen, Langkamfen, Austria) was used. One unit of milk-clotting activity refers to the quantity of enzyme required to clot one milliliter of skimmed cow's milk in 40 minutes at a temperature of 35 °C. This equation (1) was used to determine the chymosin activity units (A).

$$A = \frac{V_{milk}}{V_{chymosin}} \times \frac{2400}{T_{mc}} \quad (1)$$

where V_{milk} is milk volume (mL), $V_{chymosin}$ is the volume of added chymosin (mL), and T_{mc} is milk-clotting time (s).

Proteolytic activity assay

Anson's [28] approach, with some modifications, was used to quantify the proteolytic activity of the sample. To be more specific, the reaction mixture was made up of 0.02 mL of enzyme and 0.5 mL of hemoglobin, both of which were suspended in 50 mM citrate buffer (pH 3.0). The mixture was stirred and incubated at 37 °C for 10 minutes. The reaction was terminated using 0.5 mL of trichloroacetic acid at 10%. The UV-1900i spectrophotometer was used to take the reading for the optical density at 280 nm (Shimadzu, Kyoto, Japan). The amount of enzyme necessary to release 1 µg of tyrosine per minute was used as the definition of one unit of activity.

Determination of protein concentration

The Bradford assay [29] was used to evaluate protein concentration using bovine serum albumin as the reference pro-

tein. Briefly, we mixed 100 µL of the Bradford reagent (protein assay dye; Bio-Rad, Munich, Germany) and 860 µL of 10% PBS with 1% glycerol and added 40 µL of a protein sample. After letting the solution remain at room temperature for 2 minutes, the spectrophotometer reading was taken at 595 nm to determine the optical density of the solution. Three biological replicates were measured, and the average of those results is shown below [29].

Production of cheese with recombinant camel chymosin

A cheesemaking experiment was conducted on a small scale in the lab, mostly following the protocol described in ref. [30]. We made two kinds of cheese: one with goat’s milk and one with cow’s. Only 5 liters of milk were used to make each kind of cheese. The Lactan 600 Ultra Milk Analyzer was used to analyze and standardize the milk components. During the 30-second pasteurization process, the milk was heated to 75 degrees Celsius. Lyophilized recombinant camel chymosin (35,700 U/g), 10 mL of 10% (m/v) CaCl₂, and the lactic acid strains were added to 5 L of pasteurized milk, and the mixture was then incubated at 38 °C for 60 minutes. At the conclusion of the incubation period, the whey was separated from the curd and its quantity was noted. The curd was squeezed at 8°C with 1 kilogram of weight. After 16 hours of pressing, the cheese weight (in grams) was measured to determine the production yield (in percent) for each production method. The cheese’s moisture content was tested and recorded using an Infrared Moisture Determination Balance MD 83 (VIBRA, Shinko Den-shi Co., LTD). The cheese yields (%) were determined using the cheese weight (g) and milk volume (mL). The solid yield (Y) was computed using the following formula (2):

$$Y = M \times \left(1 - \frac{H}{100\%}\right) \quad (2)$$

there, *M* is the cheese weight (g), and *H* is the cheese moisture (%).

Statistical analysis and software

All of the measurements were carried out three times. The program GraphPad Prism V.8.0.1 (San Diego, California, USA, www.graphpad.com) was used to compute the mean

values as well as the standard deviation (SD). The results of the milk-clotting activity are shown as the mean standard deviation (n = 3).

RESULTS

Table 1 displays the results of an experiment in which pure recombinant chymosins from *Bos taurus* and *Camel bactrianus* were tested for their ability to clot milk that had been previously reconstituted from milk from goats, cows, and ewes.

Table 1 - The milk-clotting activity of purified recombinant chymosins from *B. taurus* and *C. bactrianus* tested on reconstituted cow’s, goat’s and ewes’ milk

Chymosin	Milk Type		
	Cow’s	Goat’s	Ewe’s
Bovine rChymosin (U/mg)	12,854 ± 0,61	5385 ± 0,25	14,811 ± 0,72
Camel rChymosin (U/mg)	16,590 ± 0,82	7850 ± 0,34	20,700 ± 0,85

The proteolytic activity of camel rChymosin was 1679.97 ± 9.54 U/mg and for bovine rChymosin was 10,767.0 ± 54.56 U/mg.

The test of coagulation properties on fresh milk indicated that when 1000 for cow’s and ewes’ or 2000 U for goat’s per 1 L of milk, is added, a clot forms in 30–40 min. The figure shows clots obtained from cow’s (a), goat’s (b) and ewes’ (c) milk.

Since mammalian milk is difficult to standardize, cheese made from cow, goat, and ewe’s milk was manufactured in a lab setting, and the milk’s composition was examined using a milk analyzer. Our estimates are shown in Table 2.

Next, we determined how much cheese could be made from cow, goat, and ewe milk in a controlled laboratory setting. Following the completion of all cheesemaking steps, the final yields of cheese from 5 L of cow’s milk, 5 L of goat’s milk, and 1 L of ewe’s milk were nearly 900 g, 865 g, and 159 g, respectively. The information in Table 3 is converted to 1 L of milk.

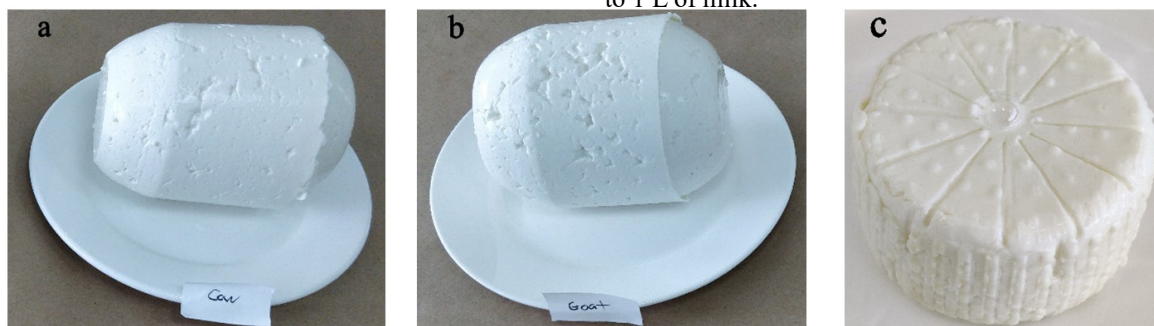


Figure 1 – Clot formation in cow’s (a), goat’s (b) and ewes’ (c) milk

Table 2 - Indicators of cow’s, goat’s, and ewes’ milk

Milk	Fat (%)	Proteins (%)	Total protein (%)	Lactose (%)	Salts (%)	Solid (%)	Density, g/cm ³
Cow’s	3.36±0.09	3.00±0.12	2.98±0.05	4.47±0.04	0.67±0.02	11.49±0.33	1.028±0.002
Goat’s	3.62±0.05	3.02±0.10	2.99±0.07	4.49±0.07	0.68±0.04	11.78±0.32	1.028±0.002
Ewes’	4.32±0.04	4.30±0.04	4.24±0.07	6.35±0.05	0.96±0.04	11.54±0.34	1.041±0.002

Table 3 - A comparison of parameters of cheese production with the camel rChymosin from cow's, goat's and ewes' milk

Milk	Volume (L)	Amount of Added Chymosin (U)	Whey Amount (L)	Postpress Cheese Yield (g)	Cheese Yield (%)	Moisture (%)	Yield of Solids (g)
Cow's	1	1000	0.73	180	18.0	32.0	122.4
Goat's	1	2000	0.69	173	17.3	44.4	96.18
Ewes'	1	1000	0.81	159	15.9	28.0	114.3

DISCUSSION

Recombinant chymosin from *C. bactrianus* has been shown to have stronger milk-clotting activity than recombinant chymosin from *B. taurus* in three different species: cows (29% higher), goats (46% higher), and ewes (40% higher). In contrast, camel rChymosin has proteolytic activity that is 6.4% lower than that of bovine rChymosin. As shown above, camel rChymosin is superior to bovine rChymosin as a cheese-making enzyme because of its higher specificity for milk proteins.

It's interesting to note that camel rChymosin's activity varies depending on the milk type. Goat milk has the lowest activity of this enzyme compared to that of cow milk and ewe milk. The protease chymosin hydrolyzes the cleavage site, which in turn destabilizes the casein complex and causes the casein mycelium to coagulate. Evidently, the sequence and context of the cleavage site influence the action of camel chymosin. The proportion of caseins in milk also varies from animal to animal, which affects the pace of syneresis and, in turn, milk coagulation. Due to a higher concentration of κ -casein, rennet has a greater effect on ewes' milk, and coagulation in ewes' milk occurs more quickly than in cow milk [31]. Ewes' milk has larger quantities of casein and colloidal calcium; therefore, although the pace of curd formation is faster than in cow milk, the rate of syneresis is slower. Cheeses made from cow's, goat's, camel's, and ewes' milk have all been successfully curdled using recombinant chymosin from *C. bactrianus*, demonstrating its versatility.

The production of cheese from cow's, goat's, and sheep's milk varied. The yield difference between cow's and goat's cheese was 7%, while goat's cheese was 12.4% moister than cow's cheese. When considered together, these factors resulted in a dry matter yield differential of 131.1 g in the cheese. A comparative total protein study of goat's milk and cow's milk similarly revealed just a slight difference (Table 2). Differences in protein content account for the dissimilar dry-matter yields of goat's and cow's cheeses. In comparison to cow's milk, the casein concentration in goat's milk is lower

(2.14 g per 100 g) [4]. This number is 2.55 g per 100 g of milk for the latter [4]. Casein concentration (w/v) varies between 2.20% and 2.62% between goat's and cow's milk, despite the densities of both being 1.028 g/cm³ (Table 2). Due to casein being the primary protein in charge of milk coagulation, the observed variation in cheese yields between goat's milk and cow's milk may be attributed mostly to the variation in casein content. Goat's milk is often used to manufacture soft cheeses due to its high moisture content.

Although ewes' cheese is 4% drier than cow's milk cheese, the difference in yield is just 2.1%. Cow's cheese and ewe's cheese may be compared to one another in terms of performance by recalculating the yield per 1 L. Cheeses made from cow's milk produce 122.4 grams of solids, whereas cheeses made from ewe's milk give 114.3 grams of solids, a difference of just 8.1 grams.

Based on the data shown in Table 3, cheeses made from goats and ewes have 21% and 6% less solids, respectively, than cow's cheese.

Caseins, lactoglobulin, and lactalbumin differentiation in the main proteins of whole milk from cows, ewes, camels, goats, and mares differed among the *Bovidae*, *Camelidae*, and *Equidae* families (Table 4). Bovidae milk has roughly 3.2–8.5 g/L of κ -casein, whereas camel and horse milk have less than 1 g/L. Hydrolysis of κ -casein causes the whole casein micelle to become unstable, which in turn causes casein precipitation and clot formation since κ -casein is essential for keeping the casein complex in a water-soluble condition.

* Sources: Adapted from [11, 12, 32, 33].

Table 4 demonstrates that camels and horses have a low κ -casein concentration and a high albumin percentage, which is not involved in clot formation. Cow, goat, and sheep's milk predominate in the cheese industry. A higher concentration of β -casein, as shown by Wedholm et al. [34], also increases the cheese's hardness. Table 5 shows that the average proportion of β -casein in milk from cows, goats, and ewes is 38%, 33%, and 56%, respectively. Cheeses manufactured from cow's milk and goat's milk have more moisture because they contain

Table 4 - Protein profile (g/L) of milk from different mammalian species

Protein Fraction	Cow	Goat	Ewe	Camel	Mare
Total casein	24.8–31.9	23.3–46.3	41.8–52.7	22.1–26.0	9.40–13.56
Caseins					
α_{s1} -Casein	8.0–10.8	0–13	15.4–22.1	4.9–5.7	2.4
α_{s2} -Casein	2.8–3.4	2.3–11.6	6–8	2.1–2.5	0.2
β -Casein	9.8–12.0	0–29.6	15.6–39.6	14.4–16.9	10.66
κ -Casein	4.2–6.7	3.5–13.4	3.2–12.2	0.8–0.9	0.24
Whey proteins					
β -Lactoglobulin	3.42–5.76	1.5–5.0	6.5–8.5	Absent	2.55
α -Lactalbumin	0.63–0.89	0.7–2.3	1–1.9	0.8–3.5	2.37

a lower percentage of β -casein (38% and 33%, respectively).

CONCLUSION

In comparison to bovine chymosin, camel chymosin demonstrates higher levels of milk-clotting activity on cow, goat, and ewe's milk, at 29.0%, 45.8%, and 39.8%, respectively. The strong selectivity of camel chymosin is demonstrated by the fact that it has a proteolytic activity that is 6.4 times lower than that of bovine chymosin. These two measures highlight camel chymosin's potential as a milk-clotting enzyme in the cheesemaking process. Cheese made from cow's, goat's, and ewe's milk demonstrates camel chymosin's coagulator characteristics. Cheese production yielded 18.0% from cow's milk, 17.3% from goat's milk, and 15.3% from ewe's milk. These findings support the idea that recombinant camel chymosin might be used in cheese made from cow's, goat's, or ewe's milk as a clotting enzyme.

FUNDING

The research is funded by the Ministry of Agriculture of the Republic of Kazakhstan (BR10764998).

LITERATURE

1. Daly K. G., Mattiangeli V., Hare A. J., Davoudi H., Fathi H., Doost S. B., Amiri S., Khazaeli R., Decruyenaere D., Nokandeh J., Richter T., Darabi H., Mortensen P., Pantos A., Yeomans L., Bangsgaard P., Mashkour M., Zeder M. A., Bradley D. G. Herded and hunted goat genomes from the dawn of domestication in the Zagros Mountains // *Proceedings of the National Academy of Sciences*. – 2021. – T. 118, № 25. – C. e2100901118.

2. Stiner M. C., Munro N. D., Buitenhuis H., Duru G., Özbaşaran M. An endemic pathway to sheep and goat domestication at Aşıklı Höyük (Central Anatolia, Turkey) // *Proceedings of the National Academy of Sciences*. – 2022. – T. 119, № 4. – C. e2110930119.

3. Taylor W. T. T., Pruvost M., Posth C., Rendu W., Krajcarz M. T., Abdykanova A., Brancaleoni G., Spengler R., Hermes T., Schiavinato S., Hodgins G., Stahl R., Min J., Alisher kyzy S., Fedorowicz S., Orlando L., Douka K., Krivoshapkin A., Jeong C., Warinner C., Shnaider S. Evidence for early dispersal of domestic sheep into Central Asia // *Nature Human Behaviour*. – 2021. – T. 5, № 9. – C. 1169-1179.

4. Stergiadis S., Nørskov N. P., Purup S., Givens I., Lee M. R. F. Comparative Nutrient Profiling of Retail Goat and Cow Milk // *Nutrients*. – 2019. – T. 11, № 10.

5. Pazzola M., Stocco G., Dettori M. L., Bittante G., Vacca G. M. Effect of goat milk composition on cheesemaking traits and daily cheese production // *J Dairy Sci*. – 2019. – T. 102, № 5. – C. 3947-3955.

6. Clark S., Mora García M. B. A 100-Year Review: Advances in goat milk research // *J Dairy Sci*. – 2017. – T. 100, № 12. – C. 10026-10044.

7. Nudda A., Atzori A. S., Correddu F., Battacone G., Lunesu M. F., Cannas A., Pulina G. Effects of nutrition on main components of sheep milk // *Small Ruminant Research*. – 2020. – T. 184. – C. 106015.

8. Bencini R., G P. The quality of sheep milk: A review //

Australian Journal of Experimental Agriculture. – 1997. – T. 37. – C. 485-504.

9. Verduci E., D'Elios S., Cerrato L., Comberiat P., Calvani M., Palazzo S., Martelli A., Landi M., Trikamjee T., Peroni D. G. Cow's Milk Substitutes for Children: Nutritional Aspects of Milk from Different Mammalian Species, Special Formula and Plant-Based Beverages // *Nutrients*. – 2019. – T. 11, № 8. – C. 1739.

10. Barłowska J., Sz wajkowska M., Litwińczuk Z., Król J. Nutritional Value and Technological Suitability of Milk from Various Animal Species Used for Dairy Production // *Comprehensive Reviews in Food Science and Food Safety*. – 2011. – T. 10, № 6. – C. 291-302.

11. Claeys W. L., Verraes C., Cardoen S., De Block J., Huyghebaert A., Raes K., Dewettinck K., Herman L. Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits // *Food Control*. – 2014. – T. 42. – C. 188-201.

12. Roy D., Ye A., Moughan P. J., Singh H. Composition, Structure, and Digestive Dynamics of Milk From Different Species-A Review // *Front Nutr*. – 2020. – T. 7. – C. 577759.

13. Aktayeva S. A. Z., Khassenov B. Proteolytic enzymes in cheese making // *Eurasian Journal of Applied Biotechnology*. – 2018. № 1. – C. 10-14.

14. Khan U., Selamoglu Z. Use of Enzymes in Dairy Industry: A Review of Current Progress // *Arch Razi Inst*. – 2020. – T. 75, № 1. – C. 131-136.

15. Noseda D. G., Recúpero M., Blasco M., Bozzo J., Galvagno M. Production in stirred-tank bioreactor of recombinant bovine chymosin B by a high-level expression transformant clone of *Pichia pastoris* // *Protein Expr Purif*. – 2016. – T. 123. – C. 112-21.

16. Flamm E. L. How FDA approved chymosin: a case history // *Biotechnology (N Y)*. – 1991. – T. 9, № 4. – C. 349-51.

17. Johnson M. E. A 100-Year Review: Cheese production and quality // *J Dairy Sci*. – 2017. – T. 100, № 12. – C. 9952-9965.

18. Bansal N., Drake M. A., Piraino P., Broe M. L., Harboe M., Fox P. F., McSweeney P. L. H. Suitability of recombinant camel (*Camelus dromedarius*) chymosin as a coagulant for Cheddar cheese // *International Dairy Journal*. – 2009. – T. 19, № 9. – C. 510-517.

19. Moynihan A. C., Govindasamy-Lucey S., Jaeggi J. J., Johnson M. E., Lucey J. A., McSweeney P. L. Effect of camel chymosin on the texture, functionality, and sensory properties of low-moisture, part-skim Mozzarella cheese // *J Dairy Sci*. – 2014. – T. 97, № 1. – C. 85-96.

20. Langholm Jensen J., Molgaard A., Navarro Poulsen J. C., Harboe M. K., Simonsen J. B., Lorentzen A. M., Hjerno K., van den Brink J. M., Qvist K. B., Larsen S. Camel and bovine chymosin: the relationship between their structures and cheese-making properties // *Acta Crystallogr D Biol Crystallogr*. – 2013. – T. 69, № Pt 5. – C. 901-13.

21. Gumus P., Hayaloglu A. A. Effects of blends of camel and calf chymosin on proteolysis, residual coagulant activity, microstructure, and sensory characteristics of Beyaz peynir // *J Dairy Sci*. – 2019. – T. 102, № 7. – C. 5945-5956.

22. Al-Zoreky N. S., Almathen F. S. Using recombinant camel chymosin to make white soft cheese from camel milk // *Food Chem.* – 2021. – T. 337. – C. 127994.

23. Kappeler S. R., van den Brink H. J., Rahbek-Nielsen H., Farah Z., Puhan Z., Hansen E. B., Johansen E. Characterization of recombinant camel chymosin reveals superior properties for the coagulation of bovine and camel milk // *Biochem Biophys Res Commun.* – 2006. – T. 342, № 2. – C. 647-54.

24. Akishev Zh.D. A. A. N., Abeldenov S.K., Khassenov B.B. Expression and characterization of bovine chymosin in *Pichia (Komagataella) pastoris* // *Eurasian Journal of Applied Biotechnology.* – 2017. № 2. – C. 64-67.

25. Akishev Z., Kiribayeva A., Mussakhmetov A., Baltin K., Ramankulov Y., Khassenov B. Constitutive expression of *Camelus bactrianus* prochymosin B in *Pichia pastoris* // *Helvion.* – 2021. – T. 7, № 5. – C. e07137.

26. Akishev Z., Aktayeva S., Kiribayeva A., Abdullaeva A., Baltin K., Mussakhmetov A., Tursunbekova A., Ramankulov Y., Khassenov B. Obtaining of Recombinant Camel Chymosin and Testing Its Milk-Clotting Activity on Cow’s, Goat’s, Ewes’s, Camel’s and Mare’s Milk // *Biology.* – 2022. – T. 11, № 11. – C. 1545.

27. Ageitos J. M., Vallejo J. A., Sestelo A. B. F., Poza M., Villa T. G. Purification and characterization of a milk-clotting protease from *Bacillus licheniformis* strain USC13 // *Journal of Applied Microbiology.* – 2007. – T. 103, № 6. – C. 2205-2213.

28. Anson M. L. THE ESTIMATION OF PEPSIN, TRYP-SIN, PAPAİN, AND CATHEPSIN WITH HEMOGLOBIN // *J Gen Physiol.* – 1938. – T. 22, № 1. – C. 79-89.

29. Bradford M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding // *Anal Biochem.* – 1976. – T. 72. – C. 248-54.

30. Hayaloglu A. A., Guven M., Fox P. Microbiological, biochemical and technological properties of Turkish White cheese ‘Beyaz Peynir’ // *International Dairy Journal.* – 2002. – T. 12. – C. 635-648.

31. da Silva V. B., da Costa M. P. Influence of Processing on Rheological and Textural Characteristics of Goat and Sheep Milk Beverages and Methods of Analysis // *Book Influence of Processing on Rheological and Textural Characteristics of Goat and Sheep Milk Beverages and Methods of Analysis / EditorElsevier, 2019.* – C. 373-412.

32. Amalfitano N., Stocco G., Maurmayr A., Pegolo S., Cecchinato A., Bittante G. Quantitative and qualitative detailed milk protein profiles of 6 cattle breeds: Sources of variation and contribution of protein genetic variants // *J Dairy Sci.* – 2020. – T. 103, № 12. – C. 11190-11208.

33. Amigo L., Fontecha J. Milk | Goat Milk // *Encyclopedia of Dairy Sciences (Second Edition) / Fuquay J. W.* – San Diego: Academic Press, 2011. – C. 484-493.

34. Wedholm A., Larsen L. B., Lindmark-Månsson H., Karlsson A. H., Andrén A. Effect of Protein Composition on the Cheese-Making Properties of Milk from Individual Dairy Cows // *J Dairy Sci.* – 2006. – T. 89, № 9. – C. 3296-3305.

REFERENCES

1. Daly K. G., Mattiangeli V., Hare A. J., Davoudi H., Fathi H., Doost S. B., Amiri S., Khazaeli R., Decruyenaere D., Nokandeh J., Richter T., Darabi H., Mortensen P., Pantos A., Yeomans L., Bangsgaard P., Mashkour M., Zeder M. A., Bradley D. G. Herded and hunted goat genomes from the dawn of domestication in the Zagros Mountains // *Proceedings of the National Academy of Sciences.* – 2021. – Vol. 118, No. 25. – P. e2100901118.

2. Stiner M. C., Munro N. D., Buitenhuis H., Duru G., Özbaşaran M. An endemic pathway to sheep and goat domestication at Aşıklı Höyük (Central Anatolia, Turkey) // *Proceedings of the National Academy of Sciences.* – 2022. – Vol. 119, No. 4. – P. e2110930119.

3. Taylor W. T. T., Pruvost M., Posth C., Rendu W., Krajcacz M. T., Abdykanova A., Brancaloni G., Spengler R., Hermes T., Schiavinato S., Hodgins G., Stahl R., Min J., Alisher kyzy S., Fedorowicz S., Orlando L., Douka K., Krivoshapkin A., Jeong C., Warinner C., Shnaider S. Evidence for early dispersal of domestic sheep into Central Asia // *Nature Human Behaviour.* – 2021. – Vol. 5, No. 9. – P. 1169-1179.

4. Stergiadis S., Nørskov N. P., Purup S., Givens I., Lee M. R. F. Comparative Nutrient Profiling of Retail Goat and Cow Milk // *Nutrients.* – 2019. – Vol. 11, No. 10.

5. Pazzola M., Stocco G., Dettori M. L., Bittante G., Vacca G. M. Effect of goat milk composition on cheesemaking traits and daily cheese production // *J Dairy Sci.* – 2019. – Vol. 102, No. 5. – P. 3947-3955.

6. Clark S., Mora García M. B. A 100-Year Review: Advances in goat milk research // *J Dairy Sci.* – 2017. – Vol. 100, No. 12. – P. 10026-10044.

7. Nudda A., Atzori A. S., Correddu F., Battacone G., Lunesu M. F., Cannas A., Pulina G. Effects of nutrition on main components of sheep milk // *Small Ruminant Research.* – 2020. – Vol. 184. – P. 106015.

8. Bencini R., G P. The quality of sheep milk: A review // *Australian Journal of Experimental Agriculture.* – 1997. – Vol. 37. – P. 485-504.

9. Verduci E., D’Elios S., Cerrato L., Comberiat P., Calvani M., Palazzo S., Martelli A., Landi M., Trikamjee T., Peroni D. G. Cow’s Milk Substitutes for Children: Nutritional Aspects of Milk from Different Mammalian Species, Special Formula and Plant-Based Beverages // *Nutrients.* – 2019. – Vol. 11, No. 8. – P. 1739.

10. Barłowska J., Szwajkowska M., Litwińczuk Z., Król J. Nutritional Value and Technological Suitability of Milk from Various Animal Species Used for Dairy Production // *Comprehensive Reviews in Food Science and Food Safety.* – 2011. – Vol. 10, No. 6. – P. 291-302.

11. Claeys W. L., Verraes C., Cardoen S., De Block J., Huyghebaert A., Raes K., Dewettinck K., Herman L. Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits // *Food Control.* – 2014. – Vol. 42. – P. 188-201.

12. Roy D., Ye A., Moughan P. J., Singh H. Composition, Structure, and Digestive Dynamics of Milk From Different Species-A Review // *Front Nutr.* – 2020. – Vol. 7. – P. 577759.

13. Aktayeva S. A. Z., Khassenov B. Proteolytic enzymes

in cheese making // Eurasian Journal of Applied Biotechnology. – 2018. No. 1. – P. 10-14.

14. Khan U., Selamoglu Z. Use of Enzymes in Dairy Industry: A Review of Current Progress // Arch Razi Inst. – 2020. – Vol. 75, No. 1. – P. 131-136.

15. Nosedá D. G., Recúpero M., Blasco M., Bozzo J., Galvagno M. Production in stirred-tank bioreactor of recombinant bovine chymosin B by a high-level expression transformant clone of *Pichia pastoris* // Protein Expr Purif. – 2016. – Vol. 123. – P. 112-21.

16. Flamm E. L. How FDA approved chymosin: a case history // Biotechnology (N Y). – 1991. – Vol. 9, No. 4. – P. 349-51.

17. Johnson M. E. A 100-Year Review: Cheese production and quality // J Dairy Sci. – 2017. – Vol. 100, No. 12. – P. 9952-9965.

18. Bansal N., Drake M. A., Piraino P., Broe M. L., Harboe M., Fox P. F., McSweeney P. L. H. Suitability of recombinant camel (*Camelus dromedarius*) chymosin as a coagulant for Cheddar cheese // International Dairy Journal. – 2009. – Vol. 19, No. 9. – P. 510-517.

19. Moynihan A. C., Govindasamy-Lucey S., Jaeggi J. J., Johnson M. E., Lucey J. A., McSweeney P. L. Effect of camel chymosin on the texture, functionality, and sensory properties of low-moisture, part-skim Mozzarella cheese // J Dairy Sci. – 2014. – Vol. 97, No. 1. – P. 85-96.

20. Langholm Jensen J., Molgaard A., Navarro Poulsen J. C., Harboe M. K., Simonsen J. B., Lorentzen A. M., Hjerno K., van den Brink J. M., Qvist K. B., Larsen S. Camel and bovine chymosin: the relationship between their structures and cheese-making properties // Acta Crystallogr D Biol Crystallogr. – 2013. – Vol. 69, No. Pt 5. – P. 901-13.

21. Gumus P., Hayaloglu A. A. Effects of blends of camel and calf chymosin on proteolysis, residual coagulant activity, microstructure, and sensory characteristics of Beyaz peynir // J Dairy Sci. – 2019. – Vol. 102, No. 7. – P. 5945-5956.

22. Al-Zoreky N. S., Almathen F. S. Using recombinant camel chymosin to make white soft cheese from camel milk // Food Chem. – 2021. – Vol. 337. – P. 127994.

23. Kappeler S. R., van den Brink H. J., Rahbek-Nielsen H., Farah Z., Puhán Z., Hansen E. B., Johansen E. Characterization of recombinant camel chymosin reveals superior properties for the coagulation of bovine and camel milk // Biochem Biophys Res Commun. – 2006. – Vol. 342, No. 2. – P. 647-54.

24. Akishev Zh.D. A. A. N., Abeldenov S.K., Khassenov B.B. Expression and characterization of bovine chymosin in *Pichia (Komagataella) pastoris* // Eurasian Journal of Applied Biotechnology. – 2017. No. 2. – P. 64-67.

25. Akishev Z., Kiribayeva A., Mussakhmetov A., Baltin K., Ramankulov Y., Khassenov B. Constitutive expression of *Camelus bactrianus* prochymosin B in *Pichia pastoris* // Heliyon. – 2021. – Vol. 7, No. 5. – P. e07137.

26. Akishev Z., Aktayeva S., Kiribayeva A., Abdullaeva A., Baltin K., Mussakhmetov A., Tursunbekova A., Ramankulov Y., Khassenov B. Obtaining of Recombinant Camel Chymosin and Testing Its Milk-Clotting Activity on Cow's, Goat's, Ewe's, Camel's and Mare's Milk // Biology. – 2022. – Vol. 11, No. 11. – P. 1545.

27. Ageitos J. M., Vallejo J. A., Sestelo A. B. F., Poza M., Villa T. G. Purification and characterization of a milk-clotting protease from *Bacillus licheniformis* strain USC13 // Journal of Applied Microbiology. – 2007. – Vol. 103, No. 6. – P. 2205-2213.

28. Anson M. L. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin // J Gen Physiol. – 1938. – Vol. 22, No. 1. – P. 79-89.

29. Bradford M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding // Anal Biochem. – 1976. – Vol. 72. – P. 248-54.

30. Hayaloglu A. A., Guven M., Fox P. Microbiological, biochemical and technological properties of Turkish White cheese 'Beyaz Peynir' // International Dairy Journal. – 2002. – Vol. 12. – P. 635-648.

31. da Silva V. B., da Costa M. P. Influence of Processing on Rheological and Textural Characteristics of Goat and Sheep Milk Beverages and Methods of Analysis // Book Influence of Processing on Rheological and Textural Characteristics of Goat and Sheep Milk Beverages and Methods of Analysis / EditorElsevier, 2019. – P. 373-412.

32. Amalfitano N., Stocco G., Maurmayr A., Pegolo S., Cecchinato A., Bittante G. Quantitative and qualitative detailed milk protein profiles of 6 cattle breeds: Sources of variation and contribution of protein genetic variants // J Dairy Sci. – 2020. – Vol. 103, No. 12. – P. 11190-11208.

33. Amigo L., Fontecha J. Milk | Goat Milk // Encyclopedia of Dairy Sciences (Second Edition) / Fuquay J. W. – San Diego: Academic Press, 2011. – P. 484-493.

34. Wedholm A., Larsen L. B., Lindmark-Månsson H., Karlsson A. H., Andrén A. Effect of Protein Composition on the Cheese-Making Properties of Milk from Individual Dairy Cows // J Dairy Sci. – 2006. – Vol. 89, No. 9. – P. 3296-3305.

МОЛОКОСВЕРТЫВАЮЩАЯ АКТИВНОСТЬ РЕКОМБИНАНТНЫХ БЫЧЬЕГО И ВЕРБЛЮЖЬЕГО ХИМОЗИНОВ В ОТНОШЕНИИ КОРОВЬЕГО, КОЗЬЕГО И ОВЕЧЬЕГО МОЛОКА

Акишев Жигер^{1,2}, Актаева Сания^{1,2}, Шамсиева Юлия^{1,2}, Турсунбекова Аннеля³, Калемшарив Бегжан³, Тултабаева Тамара³, Хасенов Бекболат^{1,4,*}

¹Национальный центр биотехнологии, Кургальжинское шоссе 13/5, Астана, 010000;

²Факультет естественных наук, Евразийский национальный университет им. Л.Н. Гумилева, ул. К.Сампаева, 2, Астана, 010008;

³Казахский агротехнический университет им. С.Сейфуллина, пр. Женис, 62, Астана, 010001;

⁴ТОО «GenLab», ул. М.Габдуллина, 19/1, кв.69, Астана, 010000

*khassenov@biocenter.kz

АННОТАЦИЯ

Одомашнивание коз и овец произошло во время неолитической революции. С тех пор были выведены различные породы коз и овец, в том числе молочные. В данной работе мы проверили молокосвертывающую активность рекомбинантных бычьего (*Bos taurus*) и верблюжьего (*Camelus bactrianus*) химозинов на коровьем, козьем и овечьем молоке. Молокосвертывающая активность рекомбинантного бычьего химозина на коровьем, козьем и овечьем молоке составила $12\ 854 \pm 610$, 5385 ± 250 и $14\ 811 \pm 720$ Ед/мг. Активность рекомбинантного верблюжьего химозина была выше на 29%-46% и составила $16\ 590 \pm 820$, 7850 ± 340 и $20\ 700 \pm 850$ Ед/мг. Протеолитическая активность составила $1679,97 \pm 9,54$ и $10\ 767,0 \pm 54,56$ Ед/мг для рекомбинантных бычьего и верблюжьего химозин, соответственно. С помощью рекомбинантного верблюжьего химозина были получены сыры из коровьего, козьего и овечьего молока. Выход сыра из коровьего, козьего и овечьего молока составил 18,0%, 17,3% и 15,3% соответственно. Полученные результаты свидетельствуют о перспективности использования рекомбинантного верблюжьего химозина в качестве фермента коагуляции при переработке коровьего, козьего и овечьего молока на сыры.

Ключевые слова: Сыр, молочные продукты, фермент, рекомбинантный химозин, казахская порода овец

СЫЫР, ЕШКІ ЖӘНЕ ҚОЙ СҮТІНЕ АРНАЛҒАН РЕКОМБИНАНТТЫ БҰҚА ЖӘНЕ ТҮЙЕ ХИМОЗИНІНІҢ КОАГУЛЯЦИЯЛЫҚ БЕЛСЕНДІЛІГІ

Акишев Жигер^{1,2}, Актаева Сания², Шамсиева Юлия², Турсунбекова Аннеля³, Калемшарив Бегжан³, Тултабаева Тамара³, Хасенов Бекболат^{1,4,*}

¹Ұлттық биотехнология орталығы, Қорғалжын тас жолы, 13/5, Нұр-Сұлтан қ., 010000;

²Жаратылыстану ғылымдары факультеті, Л. Н. Гумилев Еуразия ұлттық университеті, Қаныш Сәтбаев көшесі 2, Астана, 010008;

³С.Сейфуллин Қазақ агротехникалық университеті, Жеңіс даңғылы 62, Астана, 010001;

⁴ЖШС “GenLab”, М. Фабдуллин көшесі 69, 19/1, Астана, 010000;

*khassenov@biocenter.kz

ТҮЙІН

Неолит төңкерісі кезінде ешкілер мен қойлар қолға үйретілді. Содан бері ешкі мен қойдың әртүрлі тұқымдары, соның ішінде сүт тұқымдары өсірілді. Бұл жұмыста біз сыыр, ешкі және қой сүтіндегі рекомбинантты бұқа (*Bos taurus*) және түйе (*Camelus bactrianus*) химозиндерінің коагуляциялық белсенділігін тексердік. Сыыр, ешкі және қой сүтіне арналған рекомбинантты бұқа химозинінің коагуляциялық белсенділігі $12\ 854 \pm 610$, 5385 ± 250 және $14\ 811 \pm 720$ бірлік/мг құрады. Рекомбинантты түйе химозинінің белсенділігі 29%-46% жоғары болды және $16\ 590 \pm 820$, 7850 ± 340 және $20\ 700 \pm 850$ бірлік/мг құрады. Протеолитикалық белсенділік сәйкесінше рекомбинантты бұқа мен түйе химозиндері үшін $1679,97 \pm 9,54$ және $10\ 767,0 \pm 54,56$ бірлік/мг құрады. Рекомбинантты түйе химозинінің көмегімен сыыр, ешкі және қой сүтінен ірімшіктер алынды. Сыыр, ешкі және қой сүтінен алынған ірімшік сәйкесінше 18,0%, 17,3% және 15,3% құрады. Алынған нәтижелер сыыр, ешкі және қой сүтін ірімшіктерге өндеуде рекомбинантты түйе химозинін ұю ферменті ретінде пайдалану перспективаларын көрсетеді.

Негізгі сөздер: ірімшік, сүт өнімдері, фермент, рекомбинантты химозин, қазақ қой тұқымы