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Amino Acid Composition of Whey and Cottage Cheese Under Various Rennet Enzymes

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Abstract. Rennet cheeses occupy an important place in the diets of the population of Ukraine. The technology of making cheeses depends on both the quality of raw materials and the quality of enzymes that are used to curdle milk. Therefore, the study of the effect of rennet enzymes obtained using advanced biotechnology on the transformation of milk protein and amino acids into a finished product is of scientific and practical importance. Considering the above, the purpose of this study was to investigate the amino acid composition of whey and cottage cheese for the use of various enzyme preparations. Three groups of samples (n=5) were formed to set up the experiment. Cow's milk for research was selected from clinically healthy cows during the milking period. In the control group of samples, rennet of microbial origin was used for milk clotting. In the first experimental group of samples, an enzyme preparation extracted from rennet of dairy calves was used according to the method of Yu.Ya. Svyrydenko. In the second experimental group, an enzyme preparation obtained by extraction of rennet enzymes according to the method of S.V. Merzlov was used. The content of amino acids in milk, whey, and cheeses was determined by capillary electrophoresis. Studying the milk used for the experiment, it was found that the content of amino acids (lysine, methionine + cystine, tryptophane, valine, leucine, isoleucine, phenylalanine + tyrosine, proline, serine, alanine, glycine, histidine, arginine, aspartic acid and glutamine) did not significantly differ from the typical indicators of milk obtained in central Ukraine. It was found that the use of rennet enzymes extracted according to the method of S.V. Merzlov is accompanied by a decrease in the content of amino acids in serum by an average of 15.9%

Keywords: milk clotting, casein, whey proteins, enzyme preparations

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INTRODUCTION

An essential task of modern biotechnology is to develop scientific foundations and solutions for obtaining products with broad prospects for practical use. Such products include enzymes of animal origin, which are used in various branches of the national economy [1]. Rennet enzymes are used in the technology of making rennet cheeses, which are highly nutritious protein products obtained from milk by its coagulation and cutting. Rennet enzymes are obtained by extraction from the stomach of dairy calves. Rennet enzyme extract is purified by filtration, then passed through a bacterial filter and stored in sterile conditions. The two active protein components of calf enzymes are chymosine and pepsine, the standard ratio of which is 4:1, respectively. The activity of rennet enzymes depends on the factors of technological conditions of their production [2].

Brine cheeses are popular in Ukraine, and their production occupies an essential place in cheese making and belongs to the dynamically developing food industries. Cottage cheese is a source of complete protein, calcium, magnesium, and vitamins. Cheeses contain all the essential nutrients of milk, except carbohydrates.

The main operation in the production of rennet cheeses is the enzymatic coagulation of milk under the action of chymosine, resulting in a milk clot with most of the casein and whey. In animals, chymosine, similar to cheese-making technology, curdles milk at the beginning of its digestion. Thus, milk processing in the cheese production corresponds to natural physiological processes. Another function of enzymes in cheese production is to take part in the biotransformation of milk components into compounds that form the organoleptic parameters of the product.

Today, rennet enzymes are expensive, so microbial enzymes that are similar in action are widely used [3]. However, the use of enzymes of microbial origin can adversely affect the sensory parameters of cheeses. Furthermore, the demand for cheeses made using natural rennet enzymes has recently been growing.

Coagulation of milk with rennet enzyme involves two irreversible processes. Several theories of rennet coagulation are known. The hydrolytic theory of rennet coagulation mechanisms explains: under the action of the rennet enzyme, the polypeptide chains of the K-casein casein calcium phosphate complex between phenylalanine and methionine are hydrolysed, as a result of which the K-casein molecules break down into hydrophobic para-K-casein and hydrophilic glycomacropeptide. As a result, micelles lose their negative charge, and the hydrate shell is partially destroyed – the stability of the system is lost, resulting in protein flakes (stage I – induction). After K-casein loses its protective colloid functions, conditions are created for intensive coagulation involving calcium ions (stage II). At this stage, a spatial network of the clot is developed, which then, after appropriate treatment, is divided into two phases: solid (casein + fat) and liquid (milk sugar, milk proteins and salts dissolved in water) [4].

In whey, fat is present in small amounts (0.05… 0.4%), its value is in its dispersibility to balls with a diameter of less than 2 microns [5]. The main importance of proteins lies in their indispensability by other food substances [6]. Whey milk proteins (albumines and globulines) are the most biologically valuable concerning the content of essential amino acids (methionine, lysine, threonine, tryptophane). From the standpoint of nutritional physiology, they approach the amino acid scale of the "ideal" protein.

Amino acid composition is an essential indicator of dairy products and describes their biological value [7; 8]. The physiological significance of amino acids is diverse: for example, lysine promotes the body's absorption of phosphorus, calcium, and ferrum [9]. Lysine is also actively involved in muscle development, promotes recovery from injuries, maintains bone strength, regulates the synthesis of hormones, antibodies and enzymes, and can have an antiviral effect. Histidine is involved in hematopoiesis and tissue repair, and is part of the myelin sheaths of nerve cells. The body metabolises histidine to histamine, which is crucial for immunity, reproductive health, and digestion. Methionine and cysteine in animals and humans are essential for the development of immune bodies, skin and hair elasticity. Methionine promotes the absorption of selenium and zinc, the elimination of heavy metals (namely plumbum and mercury). Tryptophane is essential for the proper growth of newborns and is involved in the production of serotonin and melatonin. Valine promotes mental activity and muscle coordination. Phenylalanine is involved in the use of other amino acids. Phenylalanine is transformed by the body into tyrosine, which provides important brain functions. Isoleucine is involved in blood sugar regulation and hormone synthesis. It is mainly present in muscle tissue and regulates energy levels. Older people are more likely to be deficient in isoleucine than young people. Leucine affects blood sugar levels, is involved in the growth and repair of muscles and bones [10]. A violation of the balance of essential amino acids leads to the use of protein at the minimum level determined by the limited essential amino acid [11].

The quality of food products is affected by their chemical composition, physical properties, as well as nutritional and biological value, which depends on its amino acid composition [2]. Whey contains valuable components for the human body: lactose, milk fats, proteins, mineral salts (calcium, magnesium, phosphorus, sodium), as well as immunoglobulins, lactoferrin, and lactoperoxidase. Whey is a source of whey proteins, the amino acid composition of which is closest to the amino acid composition of human muscle tissue [12]. It is important that during the development of a milk clot, amino acids pass into cheese as much as possible, and not into

whey. When cheese is fermented with enzyme preparations extracted from rennet, compared to microbial analogues, 1.3% more amino acids remain in the cheese clot and pass into the cheese grain [13]. Milk protein in terms of the composition of essential amino acids is very close to the "ideal or reference protein" proposed by FAO/WHO [14].

The production of rennet cheeses is a complex multifunctional process wherein changing the influence of even one of the technological factors can change the dynamics of biochemical, microbiological, and physicochemical transformations of the cheese mass, which affects not only the organoleptic properties, but also the biological value of the final product [15]. Whey proteins are globular proteins and hydrophilic colloids. The presence of essential amino acids (lysine, tryptophane, methionine, threonine) and cysteine is the most valuable part of milk proteins, so their use in the cheese production is of great practical importance [16].

According to the technology of cheeses, whey is not further used in most cases, a considerable part of it is poured into the sewer. Therefore, it is important that the amino acids and minerals of milk are transferred to cheese, and not to whey as much as possible. The question of how various rennet enzymes affect the amino acid composition of the product remains unexplored.

The purpose of this study is a determination of the amino acid composition of cheese and the resulting whey due to the action of various enzyme preparations.

MATERIALS AND METHODS

In clinically healthy cows during the milking period, milk that met the requirements of DSTU 2661:2010 was selected for research [17].

Three groups of milk samples (n=5) were formed to set up the experiment. In the control group (CG), rennet of microbial origin was used for milk clotting. The experimental group I (EG-I) was tested with an enzyme preparation from rennet of dairy calves, extracted according to the Yu.Ya. Sviridenko's method (using sodium chloride solution). The experimental group II (EG-II) was tested with an enzyme preparation extracted from rennet of dairy calves according to the S.V. Merzlov method (using a mixture of lactic and hydrochloric acids).

Each sample, both in the control and experimental groups, was 1.0 dm^3. The filtered milk was cooled to a temperature of 4°C and kept for 12 hours. Pasteurisation was carried out at a temperature of 60-63°C with an exposure time of 30 minutes. Pasteurised milk was normalised by mass fraction of fat. Rennet enzyme was added to normalised milk heated to a temperature of 33°C, stirring it smoothly. The clot was cut into 15-20 mm cubes and left for 10-15 minutes, then carefully kneaded for 20-30 minutes to seal and dehydrate. Kneading was carried out with 2-3-minute stops.

The second heating of the cheese mass was not used. After sufficient compaction, the cheese mass was moved to a moulding table covered with gauze in two layers for self-pressing, which lasted 2 hours. Two days after the cheese matured, samples were taken from each sample to investigate the amino acid composition.

The amino acid composition of milk, whey, and cheese proteins was determined at the State Research Control Institute of Veterinary Drugs and Feed Additives (city of Lviv) by capillary electrophoresis using the capillary electrophoresis system "Kapel-105/105M" (Ukraine).

The method for determining amino acids in milk is based on the decomposition of samples by acid hydrolysis with their transition to free forms of phenylthiocarbamyl derivatives (FTC derivatives), subsequent separation and quantitative determination. Detection was performed in the UV region of the spectrum at wavelengths of 254 nm.

The under-study materials were processed using the method of variational statistics based on the calculation of the arithmetic mean, root-mean-square error and the reliability of the difference between the compared indicators. The probability of the results obtained and the difference between the indicators were calculated according to the Student's t-test.

RESULTS AND DISCUSSION

In the experiment, milk had the highest content of glutamine. As the concentration decreased, the amino acids were in the following sequence: leucine, phenylalanine + tyrosine, proline, lysine, serine, asparagine, isoleucine, valine, tryptophan, histidine, and glycine (Table 1). Notably, the content of amino acids in raw materials did not significantly differ from the standard indicators of milk [11].

Table 1. *Amino acid composition of experimental milk g/100 g*

Amino acids	Milk
Lysine	0.198
Methionine+Cystine	0.102
Tryptophane	0.138
Valine	0.145
Leucine	0.296
Isoleucine	0.150

Table 1, Continued

Amino acids	Milk
Phenylalanine+Tyrosine	0.288
Proline	0.247
Serine	0.173
Alanine	0.119
Glycine	0.066
Histidine	0.083
Arginine	0.100
Asparagine	0.172
Glutamine	0.561

Studying the effect of various starter cultures on the content of amino acids in milk serum after clot coagulation, it was found that under the action of enzymes of microbial origin, the lysine content was 0.044 g per 100 g.

Using an enzyme preparation from calf rennet obtained using a solution of sodium chloride (EG-I), the lysine content in milk serum was 9% lower compared to the CG. In milk whey from the EG-II (use of enzymes from calf rennet obtained by using a mixture of acids) the lysine content was significantly lower compared to the CG. The difference was 13.6%. There was a decrease in the error of methionine and histidine in milk serum from Experimental Groups I and II relative to the CG. In the experimental groups of samples, this indicator was the same (Table 2).

***Table 2**. Amino acid composition of serum, g/100 g*

Amino acid	CG	EG-I	EG-II
Lysine	0.044±0.0060	0.040±0.0043	0.038±0.0030*
Methionine + Cystine	0.009±0.0022	0.008±0.0012	0.008±0.0017
Tryptophane	0.011±0.0021	0.010±0.0032	0.009±0.0007
Valine	0.031±0.0026	0.028±0.0014	0.026±0.0013
Leucine	0.085±0.0042	0.082±0.0033	0.079±0.0028
Isoleucine	0.050±0.0047	0.048±0.0053	0.048±0.0019
Phenylalanine + Tyrosine	0.026±0.0017	0.024±0.0035	0.023±0.0026
Proline	0.027±0.006	0.024±0.0021	0.022±0.0018
Serine	0.018±0.0035	0.016±0.0027	0.016±0.003*
Alanine	0.025±0.0021	0.023±0.0019	0.020±0.0017
Glycine	0.005±0.0004	0.004±0.0002	0.003±0.0003*
Histidine	0.006±0.0004	0.006±0.0003	0.005±0.002
Arginine	0.029±0.0012	0.025±0.0014	0.023±0.0009*
Aspartic acid	0.014±0.0009	0.016±0.0011	0.016±0.0008
Glutanine	0.120±0.0012	0.108±0.0007**	0.102±0.0014**

***Note**: possible differences with the CG: p<0.05 – *; p<0.01 – ***

Studying the content of tryptophane, a decrease in this amino acid in milk serum from the second experimental group was revealed by 18.2%. The difference was a trend. The content of leucine and isoleucine was found to be within the trend in milk whey for the use of natural enzyme preparations extracted from calf rennet. It was proved that in the serum from the second experimental group of samples, the content of phenylalanine and tyrosine was 11.5% lower than in the CG. Under the action of enzyme preparations from calf rennet (Experimental Groups I and II), the residue of proline, alanine, histidine, and aspartic acid in milk serum decreases relative

to the serum obtained under the action of enzymes of microbial origin. Comparing the experimental groups with each other, it was found that in the second experimental group of samples, the content of proline, alanine, and histidine was lower by 8.3%, 13.0%, and 16.7% relative to the Experimental Group, respectively. A significant decrease in serine, glycine, arginine, and glutanine was established in milk serum of the EG-II. The difference with the control was 11.1%, 40.0%, 20.7%, and 15.0%, respectively. Thus, it was established that the use of enzymes from calf rennet extracted with a mixture of acids (EG-II) leads to the lowest content of essential and non-essential amino acids in milk whey.

Upon processing milk clots developed from each group of samples, samples of the finished product – cheese – were obtained. In cheese obtained using calf rennet enzymes (Experimental Groups I and II), the lysine content was higher by 0.08% and 0.51% compared to the CG. An increase in methionine + cystine, tryptophane, and valine was detected in cheese from the EG-II. The indicators were higher than in the CG, respectively, by 0.07%, 0.2%, and 0.07%. The content of leucine and isoleucine in cheese from the EG-II was higher than in the CG and EG-I. Indicators of the content of phenylalanine + tyrosine, proline, serine, glycine, histidine, arginine, aspartic acid, and glutanine in cheese from the EG-II were higher than in the CG by 0.6%, 0.074%, 0.18%, 0.46%, 0.38%, 0.13%, 0.15%, and 0.6%, respectively (Table 3). Thus, it is proved that upon using enzymes extracted from calf rennet (EG-II), the transformation of milk amino acids into cheese is the highest. Similarly, the yield of the finished product increases by 12%.

***Table 3**. Amino acid composition of cheese (% of the total amount) (n=5, P≥0.96)*

Amino acids	Control	EG-I	EG-II
Lysine	5.46	5.54	5.97
Methionine + Cystine	3.47	3.51	3.54
Tryptophane	3.92	4.03	4.12
Valine	4.21	4.26	4.28
Leucine	9.14	9.48	10.10
Isoleucine	4.30	4.58	4.59
Phenylalanine + Tyrosine	10.3	10.5	10.90
Proline	9.46	9.79	10.20
Serine	4.82	5.0	5.0
Alanine	2.18	2.46	2.64
Glycine	1.96	2.14	2.34
Histidine	2.02	2.08	2.40
Arginine	3.12	3.21	3.25
Aspartic acid	5.02	5.18	5.17
Glutanine	15.82	16.31	16.42

For the use of rennet enzymes obtained according to the method of S.V. Merzlov (EG-II), an effective transition of milk proteins and, accordingly, amino acids to the cheese mass was established in comparison with the variants where an enzyme preparation of microbial origin was used (CG) and rennet enzymes obtained according to the method of Yu.Ya. Sviridenko (EG-I).

It is known that casein comprises fractions, of which only one – χ-casein – is hydrolysed by chymosin. Numerous peptide bonds are also hydrolysed, and χ-casein is broken down in both soluble and insoluble fractions. Para-χ-casein is an insoluble fraction developed as a result of hydrolysis of χ-casein by chymosin. Hydrolysis of χ-casein by chymosin weakens its protective colloidal action in milk, which leads to the separation of whey and the development of a milk clot [18; 19].

Studies by A.V. Gudkov note that the nutrients of milk do not fully pass into cheese, since such components as whey proteins, lactose, and water-soluble vitamins pass into whey. However, the loss of proteins can be compensated to a certain extent by working out the technology and using efficient coagulants [12]. Scientific studies of W.L. Claeys described that the value of milk proteins depends on their digestibility and the amount of essential amino acids. Approximately 80% of milk proteins comprise casein (AS1, AS2-, B-, and KSHIN), and an important aspect in cheese production is the maximum transition of milk proteins to cheese, – similar statements are found in experiments conducted by W.Y. Park [20; 21].

Achieving enzyme stability and activity is often a complex effort. As noted by T. Hwang, their biological

activity depends on the three-dimensional native structure, hence the catalytic activity, and any considerable conformational changes can lead to their inactivation, which leads to the loss of most of the milk's nutrients [22].

Manifestations of enzyme instability arise from aggregation, loss of biological functionality, and exposure to extreme conditions or even minor changes in temperature or pH, can cause sudden changes and subsequent loss of their biological activity, accompanied by the inevitable loss of milk proteins and their transformation into whey, – states J.T. Kim [23].

CONCLUSIONS

According to these processes, in the EG-II, hydrolysis of polypeptide chains of *K*-casein and the breakdown of its molecules into hydrophobic and hydrophilic fractions occurred more efficiently, since the stabilised enzymes used had increased activity and were more resistant to environmental factors. As a result, *K*-casein completely lost the function of a protective colloid and coagulation occurred as efficiently as possible, cheese dust is formed to a lesser extent. The strength of the clot was the highest, which caused a decrease in the content of amino acids in the serum.

It has been experimentally proven that the use of enzyme preparations made using different methods does not equally affect the transformation of milk amino acids into cheese. The use of rennet enzymes extracted according to the S.V. Merzlov's method is accompanied by a decrease in the content of amino acids in whey by an average of 15.9%.

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Амінокислотний склад молочної сироватки та сиру під впливом різних сичужних ензимів

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Анотація. Сичужні сири займають важливе місце у раціонах населення України. Технологія виготовлення сирів залежить як від якості сировини, так і від якості ензимів, які застосовують для зсідання молока. Тому дослідження впливу сичужних ензимів, отриманих за удосконаленої біотехнології на трансформацію білка та амінокислот молока у готовий продукт, має наукове та практичне значення. З огляду на вищезазначене, метою цієї статті було дослідження амінокислотного складу сироватки молока та сиру за використання різних ензимних препаратів. Для постановки досліду було сформовано III групи проб (n=5). Молоко коров'яче для досліджень відбирали в клінічно здорових корів у період роздою. У контрольній групі проб для зсідання молока використовували сичужний фермент мікробіального походження. У I дослідній групі проб застосовувався ферментний препарат, екстрагований із сичугів молочних телят за методикою Ю.Я. Свириденко. У II дослідній групі застосовували ензимний препарат, отриманий способом екстракції сичужних ензимів за методикою С.В. Мерзлова. Вміст амінокислот у молоці, сироватці та сирах визначали методом капілярного електрофорезу. Досліджуючи молоко, яке використовували для експерименту було виявлено, що вміст амінокислот (лізин, метіонін + цистин, триптофан, валін, лейцин, ізолейцин, фенілаланін + тирозин, пролін, серин, аланін, гліцин, гістидин, аргінін, аспарагінова кислота та глутамін) вірогідно не відрізнявся від типових показників молока, яке отримують в центральній Україні. Встановлено, що застосування сичужних ензимів, екстрагованих за методикою С.В. Мерзлова, супроводжується зменшенням вмісту амінокислот у сироватці в середньому на 15,9 %

Ключові слова: згортання молока, казеїн, сироваткові білки, ферментні препарати