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Comparison of the effect of different rennet enzymes on the amino acid composition and sensory parameters of Lyubitelskyi cheese

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Rennet cheeses occupy an important place in the diets of the population of Ukraine. Cheese technology depends on both the quality of raw materials and the quality of enzymes used to curdle milk. Therefore, the study of the influence of rennet enzymes, obtained by improved biotechnology, on the transformation of milk proteins into a finished product has scientific and practical significance. Based on the above, the aim of the work was to study the amino acid composition of Lyubitelskyi cheese using various rennet preparations. For setting up the experiment, II groups of milk samples (n = 5) were formed. Cow's milk for research was collected from clinically healthy cows during the calving period. In the first group of samples, rennet enzyme of microbial origin was used for milk curdling. In the II experimental group, an enzyme preparation was used, obtained by the method of extracting rennet enzymes according to the method of S. V. Merzlova The content of amino acids in cheeses was determined by capillary electrophoresis. As a result of the experiment, it was found that the content of amino acids in Lover's cheese (lysine, methionine + cystine, tryptophan, valine, leucine, isoleucine, phenylalanine + tyrosine, proline, serine, alanine, glycine, histidine, arginine, aspartic acid and glutamine) probably did not differ according to the indicators of cheese obtained in central Ukraine. It was established that the use of rennet enzymes extracted according to the method of S. V. Merzlova is accompanied by an increase in the content of amino acids in the curd mass by an average of 5.6 %.

Key words: *milk coagulation, casein, whey proteins, milk processing, smell, consistency, amino acids.*

Introduction

An important task of modern biotechnology is the development of scientific bases and solutions for obtaining products with wide prospects for practical use. Such products include enzymes of animal origin, which are used in various branches of the national economy (Gurung et al., 2013). Rennet enzymes are used in the technology of rennet cheeses, which are highly nutritious protein products obtained from milk by curdling and processing. Renal enzymes are obtained by extraction from the stomach of dairy calves. The extract of rennet enzymes is purified by filtration, and then passed through a bacterial filter and stored under sterile conditions. The two active protein components of calf enzymes are chymosin and pepsin, the standard ratio of which is 4:1, respectively. The activity of rennet enzymes depends on the factors of the technological conditions of their production (Tsisaryk, 2013).

Salted cheeses are popular in Ukraine, and their production occupies an important place in cheesemaking and belongs to the dynamically developing branches of the food industry. Cheese is a source of complete proteins, calcium, magnesium and vitamins. Cheeses contain all the main nutrients of milk with the exception of carbohydrates.

Lover's cheese is a high-calorie protein product, the nutritional value of which is determined by the presence of proteins, fats, peptides, essential amino acids, calcium salts, phosphorus, which are easily absorbed by the body. The cheese belongs to the group of soft rennet cheeses without ripening. Lover's cheese has a gentle, homogeneous consistency and a mild sour-milk taste. Eating cheese has a beneficial effect on the functioning of the body, in particular on the digestive system.

The main technological process in the production of rennet cheeses is the enzymatic coagulation of milk under the action of chymosin, resulting in the formation of a milk clot with a large proportion of casein and whey. In the body of animals, chymosin, similar to cheese-making technology, coagulates milk at the beginning of its digestion. Thus, the processing of milk in the process of cheese production corresponds to natural physiological processes.

Another function of enzymes in cheese production is participation in the biotransformation of milk components into compounds that form organoleptic indicators of the product.

Today, in connection with the shortage of rennet enzyme and its high cost, other enzymes similar in action to rennet are widely used: pepsin and microbial enzymes (Ardo et al., 2012). However, the use of enzymes of microbial origin can negatively affect the sensory parameters of cheeses. In addition, the demand for cheeses made with the use of natural rennet enzymes has been growing recently.

Coagulation of milk by rennet enzyme involves two irreversible processes. There are several theories of rennet coagulation. From the standpoint of the hydrolytic theory, the mechanism of rennet coagulation is explained as follows: under the action of introduced rennet enzyme, the polypeptide chains of k-casein of the casein calcium phosphate complex between phenylalanine and methionine are hydrolyzed. As a result, k-casein molecules disintegrate into hydrophobic para-do-casein and hydrophilic glycomacropeptide. As a result, the micelles lose their negative charge, and the hydration shell is partially destroyed – the system loses its stability, resulting in the appearance of protein flakes (I stage - induction). The loss of protective colloid functions by k-casein creates conditions for intensive coagulation with the participation of calcium ions in the structuring of paracasein (II stage). At this stage, a spatial network of the clot is formed, which later, after appropriate processing, is divided into two phases: solid (casein + fat) and liquid (milk sugar, proteins and milk salts dissolved in water) (Johnson, 2017).

Acid composition is an important indicator of dairy products and characterizes their biological value (Bilyi et al., 2021; Bily & Merzlov, 2022). The physiological value of such amino acids is diverse, for example, lysine helps to increase the level of hemoglobin in the blood and the body's assimilation of phosphorus, calcium, and iron (Merzlov, 2019). Also, lysine plays a vital role in muscle formation, maintaining bone strength, promotes recovery after injuries, regulates the synthesis of hormones, antibodies and enzymes, and can have an antiviral effect. Histidine participates in hematopoiesis and tissue regeneration, is part of myelin sheaths nerve cells. The body metabolizes histidine into histamine, which is critical for immunity, reproductive health, and digestion. Methionine and cysteine in the body of animals and humans are necessary for the formation of immune bodies, elasticity of skin and hair. Methionine promotes the assimilation of Selenium and Zinc, the removal of heavy metals (in particular, lead and mercury). Tryptophan is necessary for the proper growth of babies and is a precursor to serotonin and melatonin. Valine is necessary for mental activity, muscle coordination and emotional calm. Phenylalanine helps the body use other amino acids, as well as proteins and enzymes. The body converts phenylalanine into tyrosine, which is necessary for specific brain functions. Isoleucine helps with wound healing, blood sugar regulation, and hormone synthesis. It is mainly present in muscle tissue and regulates energy levels. Older people are more prone to isoleucine deficiency than young people. Leucine affects the level of sugar in the blood, participates in the growth and restoration of muscles and bones (Bila & Merzlova, 2023). 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 (Nelson & Cox, 2008). The quality of food products is determined by their chemical composition, physical properties, as well as nutritional and biological value. At the same time, the biological value is a leading indicator of quality, as it determines the degree of compliance of food products with optimal human needs according to physiological norms. The biological value of cheese lies not only in its protein content, but also in its quality. The most important indicator of protein quality is its amino acid composition (Tsisaryk, 2013). Whey can be considered as a biologically active liquid that contains valuable components for the human body: lactose, milk fats, proteins, minerals (calcium, magnesium, phosphorus, sodium), as well as immunoglobulins, lactoferrin and lactoperoxidase. The main milk protein – casein – consists of several fractions, of which only one – χ-casein – is hydrolyzed by chymosin. Numerous peptide bonds are subjected to hydrolysis at the same time, and χ-casein is split into both soluble and insoluble fractions. A glycoprotein with a carbohydrate content of up to 28% was identified as a hydrolysis product. The insoluble fraction, which is formed as a result of the hydrolysis of χ-casein by chymosin, is called para-χ-casein. Shallow hydrolysis of χ-casein by chymosin leads to the loss of its protective colloidal effect in milk, resulting in the separation of serum and the formation of a milk clot (Park, 2001; Kammerlehner, 2009; Ardo et al., 2012; Benamouzig & Tomé, 2013; Ozturk et al., 2015; Johnson, 2017; Vlasenko et al., 2018; Merzlov et al., 2019).

The purpose of the study

The purpose of the work is to establish the amino acid composition of cheese and its sensory analysis under the action of various enzyme preparations.

Materials and methods

Cow's milk for research met the requirements of DSTU 2661:2010. The amino acid composition of milk, whey, and cheese proteins was determined at the State Research Control Institute of Veterinary Medicines and Feed Additives (Lviv) by capillary electrophoresis using the Kapel-105/105M system (Ukraine). The system is equipped with a positive polarity high voltage source (internal diameter of the capillary 75 μm, total length of the capillary 60 cm, effective length 50 cm), equipped with special software based on a personal computer.

The method of determining amino acids in milk is based on the decomposition of samples by acid hydrolysis

with their transition into free forms of phenylthiocarbamyl derivatives (FTK derivatives), their subsequent separation and quantitative determination. Detection was carried out in the UV region of the spectrum at a wavelength of 254 nm. For setting up the experiment, II groups of milk samples (n = 5) were formed. In the control group of samples, rennet enzyme of microbial origin was used for curdling milk. In the 1st experimental group of samples, an enzyme preparation from the rennet of dairy calves, extracted according to the method of Yu. Ya. Svyridenko, was used. In the II experimental group, an enzyme preparation was used, which was extracted from the rennet of dairy calves according to the method of S. V. Merzlova (Merzlov, 2019).

In the experimental groups, each sample was 2.0 dm³. The filtered milk was cooled to a temperature of 3.8 °C and kept for 12 hours. Pasteurization was carried out at a temperature of 62–64 °C with a holding time of 33 minutes. Pasteurized milk was normalized by mass fraction of fat. The rennet enzyme was added to the normalized milk heated to a temperature of 34°C while gently stirring it. The clot was cut into 10–15 mm cubes and left alone for 10–15 minutes, then gently mixed for 20–30 minutes to compact and dehydrate. Mixing was carried out using stops for 2–3 minutes.

The second heating of the curd mass was not used. After sufficient compaction, the cheese mass was moved to a forming table covered with gauze in two layers for self-pressing, which was carried out for 2 hours.

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

Results and discussion

Along with the organoleptic indicators of the obtained Lyubitelskyi cheese, its output was studied using various enzymes. It was established that in experimental groups I and II, 252.2 ± 1.16 g and 257.7 ± 1.04 g of cheese were obtained from 2 dm³ of milk, respectively.

So, studies show that the use of enzymes obtained according to the method of S. V. Merzlov (II group of samples) contributes to the fact that the yield of the finished product increases by 3.7 %.

Examining the organoleptic indicators of Lyubitelskyi cheese in both groups of samples, it was established that the taste was clean, sour-milk without extraneous odors. The consistency is uniform, brittle, not brittle. The pattern, color of the cheese grain, and appearance also corresponded to the current regulatory documents for Lyubitelskyi cheese (Table 1).

Table 1
Sensory analysis of Lyubitelskyi cheese

Indicator	Characteristics of the indicator
I group of samples	
Taste and smell	Clean sour milk tastes and smells, without extraneous
Consistence	Uniform, fragile, but not fragile
The color of the cheese dough	Weak yellow, homogeneous throughout the mass
Appearance	The surface is clean, with traces of a serration. There is no pickaxe. Slight deformation of the head
II group of samples	
Taste and smell	Pure sour milk, without extraneous tastes and smells
Consistence	Uniform, fragile, but not fragile
The color of the cheese dough	Weak yellow, homogeneous throughout the mass
Appearance	The surface is clean, with traces of a serration. There is no pickaxe. Slight deformation of the head

Table 2
Amino acid composition of Lyubitelsky cheese (% of the total amount) (n = 5, P ≥ 0.96)

Amino acids	I group of samples	II group of samples
Lysine	4.98	5.07
Methionine + Cystine	3.21	3.44
Tryptophan	3.96	4.12
Valin	3.26	4.28
Leucine	8.94	9.12
Isoleucine	4.58	5.13
Phenylalanine + Tyrosine	9.42	10.12
Proline	8.98	9.16
Serin	4.79	5.03
Alanine	2.03	2.38
Glycine	2.36	2.40
Histidine	2.41	2.42
Arginine	2.95	3.0
Aspartic acid	5.75	5.78
Glutanin	15.91	16.0
∑	83.53	87.35

For the use of rennet enzymes obtained by the method of S. V. Merzlov (II research group) established a more effective transfer of proteins, and accordingly, amino acids of milk into the curd mass, in comparison with options where an enzyme preparation of microbial origin was used. This can be explained by the fact that in the II research group, the enzymes are stabilized with increased activity and are more resistant to the effects of environmental factors. As a result, the hydrolysis of k-casein polypeptide chains and the breakdown of its molecules into hydrophobic and hydrophilic fractions is more effective. K-casein completely loses the function of a protective colloid and at the same time coagulation takes place to the maximum and cheese dust is formed to a lesser extent.

With the use of rennet enzymes in the II research group, the strength of the clot was the greatest, which also led to an increase in the content of amino acids in the cheese.

Conclusions

Rennet cheeses are concentrated protein dairy products, which are obtained by enzymatic coagulation of milk proteins with further processing of the curd and ripening of the cheese mass. Their quality is affected not only by the quality of the milk and compliance with the technological process, but also by the quality and origin of the rennet.

It has been experimentally proven that the use of different enzyme preparations does not have the same effect on the transformation of amino acids of milk into cheese. The use of rennet enzymes extracted according to the method of S. V. Merzlov is accompanied by an increase in the content of amino acids in cheese by an average of 5.6 %, and the yield of the finished product by 3.7 %.

Conflict of interest

The authors declare that there is no conflict of interest.

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