



Науковий вісник Львівського національного університету  
ветеринарної медицини та біотехнологій імені С.З. Гжицького.  
Серія: Сільськогосподарські науки

Scientific Messenger of Lviv National University  
of Veterinary Medicine and Biotechnologies.  
Series: Agricultural sciences

ISSN 2519–2698 print  
ISSN 2707-5834 online

doi: 10.32718/nvlvet-a9620  
<https://nvlvet.com.ua/index.php/agriculture>

UDC 637.334.2

## Effect of some current enzymes on milk coagulation indicators

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### Article info

Received 21.03.2022  
Received in revised form  
25.04.2022  
Accepted 26.04.2022

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**Bilyi, V. Y., & Merzlov, S. V. (2022). Effect of some current enzymes on milk coagulation indicators. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Agricultural sciences, 24(96), 144–147. doi: 10.32718/nvlvet-a9620**

Soft cheese technology involves using a significant number of enzymes involved in the circulation of milk. The market is filled with drugs of microbial origin; although they are cheaper than their counterparts made from ruminants, the demand for cheeses made with pure enzymes is relatively high. Brine cheeses are famous in Ukraine, and their production occupies an important place in cheesemaking and belongs to the dynamically developing food industries. Cheese is a source of complete protein, calcium, magnesium, and vitamins. Cheeses contain all the essential nutrients of milk except carbohydrates. The task of our work was to establish the effectiveness of the action on the circulation of milk and rennet enzymes obtained from rennet calves of different ages (from 2 to 20 weeks). The research was conducted in the conditions of the Research Institute of Food Technologies and Technologies of Processing of Livestock Products of Bila Tserkva National Agrarian University. It was found that enzymes derived from the abomasum of calves slaughtered at an earlier age coagulate milk faster, and enzymes obtained at an older age convert milk more slowly, but the quality of milk clots in organoleptic characteristics is almost the same. The effect of enzymes obtained from the abomasum of calves of different ages on serum acidity has not been established.

**Key words:** rennet, milk, clot formation, whey acidity, enzyme extraction.

### Introduction

Food quality is determined by its chemical composition, physical properties, and nutritional and biological value (Tsisaryk, 2013). The nutritional and biological value of the product is higher the more the product meets the body's needs for nutrients or meets the formula of a balanced diet, according to which the body's normal functioning is possible with a clear relationship between essential nutrients (Ardo et al., 2002; Guttyj et al., 2017; Merzlov et al., 2019).

In many countries of the world, there is an increase in the consumption of fermented milk products due to their nutritional value and health effects on the human body (Bos et al., 2003; Venher & Mishchenko, 2011). Milk coagulation enzymes are used for cheese production (Johnson, 2017; Bilyk et al., 2017), which can quickly break the bond between the hydrophilic and hydrophobic parts of  $\kappa$ -casein and not adversely affect the yield and organoleptic properties of cheeses by different technologies (Chuang et al., 2005). Milk coagulation enzymes differ in the ratio of chymosin and pepsin and milk coagu-

lation activity (Ozturk et al., 2018). Brine cheeses are famous in Ukraine, and their production occupies an important place in cheesemaking and belongs to the dynamically developing food industries. 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 producing rennet cheeses is the enzymatic coagulation of milk under the action of chymosin, resulting in the formation of a milk clot with most of the casein and whey. In animals, chymosin, similar to cheesemaking technology, coagulates milk at the beginning of its digestion. Coagulation of milk by rennet enzyme involves two irreversible processes. Several theories of rennet coagulation are known. The hydrolytic theory of rennet coagulation mechanisms explains that the rennet enzyme hydrolyzes the polypeptide chains of  $\kappa$ -casein of the casein calcium phosphate complex between phenylalanine and methionine as a result of the  $\kappa$ -casein molecule breaking down into hydrophobic captid paracodop. As a result, the micelles lose their negative charge, and the hydrate shell is partially destroyed – the system's stability

is lost, resulting in the appearance of protein flakes (stage I – induction). After the loss of protective colloid functions by k-casein, conditions are created for intensive coagulation with the participation of calcium ions (stage II). 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 (dissolved in water, milk sugar, proteins, and milk salts) (Borshch et al., 2019; Bilyi et al., 2021).

Rennet cheese production is a complex multifunctional process in which changing the influence of even one of the technological factors can change the dynamics of biochemical, microbiological, and physicochemical transformations of cheese mass, which affects not only the organoleptic properties but also the biological value of the final product (Kapreliants & Iorhachova, 2013; Melina et al., 2016).

Thus, the processing of milk in the production of cheese corresponds to natural physiological processes. Another function of enzymes in cheese production is to participate in the biotransformation of milk components into compounds that form the product's organoleptic characteristics.

**Table 1**  
General scheme

Indicator	Characteristic	Method of production
I sample	Enzymes are derived from abomasum from two-week-old calves	extraction
II sample	Enzymes are derived from abomasum from four-week-old calves	extraction
III sample	Enzymes are derived from abomasum from eight-week-old calves	extraction
IV sample	Enzymes are derived from abomasum from twelve-week-old calves	extraction
V sample	Enzymes are derived from abomasum from eighteen-week-old calves	extraction
VI sample	Enzymes are derived from abomasum from twenty-week-old calves	extraction

The first samples of enzyme extracts were obtained from the abomasum of calves slaughtered at two weeks of age II, III, IV, V, and VI; samples of extracts contained enzymes eliminated from the abomasum of calves aged 4; 8; 12; 18 and 20 weeks.

### Results and discussions

The effectiveness of rennet enzymes was determined by the rate of milk clot formation, organoleptic characteristics of this clot, and titrated acidity after 2 hours of thermostating.

It was experimentally established that under the action of rennet enzymes obtained from the first sample, the milk began to turn after 19 minutes (Table 2). The introduction into the milk of the enzyme obtained from the abomasum of four-week-old calves led to the formation of a clot 2 minutes later than in the version with the first test.

The use of enzymes extracted from the biomaterial of calves (8 weeks of age) leads to milk circulation, but this process began four minutes later compared to the first samples of enzymes. Thus, we prove that the beginning of the formation of a milk clot depends on the quality of rennet enzymes (Melina et al., 2016). The younger the

The study aimed to determine the effect of rennet enzymes extracted from rennet calves of different ages on the efficiency of milk clot formation using soft cheese technology.

### Material and methods

The research was conducted in the conditions of the Research Institute of Food Technologies and Technologies of Processing of Livestock Products of Bila Tserkva NAU. Milk was used for the experiment, which had the following physicochemical parameters: acidity of milk  $16.5 \pm 0.31$  °T, mass fraction of fat  $3.8 \pm 0.8$  %, mass fraction of protein  $2.95 \pm 0.31$  %, SZMZ  $11,7 \pm 0,35$ , number of somatic cells  $510 \pm$  five tns/cm<sup>3</sup>, degree of purity according to the standard I group. Pasteurization of milk, after maturation, was performed at a temperature of 75 °C. For the extremities, abomasums were selected from calves aged 2, 4, 8, 12, 18 and 20 weeks. Storage of abomasums, washing, and cleaning was carried out at a temperature of 4 °C. Obtaining enzymes from biological material was performed by extraction. The experiment was staged 5 times.

calves slaughtered (2–4 weeks) and the abomasum is selected, the faster the rennet enzymes extracted from such material lead to milk circulation.

An essential aspect of the study was establishing changes in titrated serum acidity. With the use of rennet enzymes extracted from rennet calves aged two weeks, the titrated serum acidity was 24 °T. No significant deviations of titrated serum acidity compared with the first sample were found during the introduction of enzymes from rennet calves at 4 and 8 weeks of age.

During fermentation of milk with enzymes in samples V and VI, whey acidity increased by 4.1 % and 4.6 % compared to the variant where enzymes from rennet calves were used at two weeks.

The use of enzymes from the abomasum of 12- and 18-week-old calves prolonged the onset of milk circulation by 7 and 9 minutes compared with enzymes extracted from the abomasum of calves slaughtered at two weeks of age.

Therefore, it can be assumed that the age of the calf from which the abomasum is selected affects not only the time of onset of clot formation but also the acidity of the serum (Kapreliants & Iorhachova, 2013) that was formed.

**Table 2**

Time of formation and quality of the milk clot, n = 5

Sample	The time of onset of clot formation min.	The quality of dairy clot	Serum acidity °T after 2 hours	Organoleptic indicators of the clot
I sample	19 ± 2.0	Dense, well cut	24.0 ± 0.10	Typical milk smell and taste
II sample	21 ± 0.5	Dense, well cut	24.2 ± 0.35	Typical milk smell and taste
III sample	23 ± 0.8	Dense, well cut	24.4 ± 0.38	Typical milk smell and taste
IV sample	26 ± 0.6	Dense, well cut	24.8 ± 0.20	Typical milk smell and taste
V sample	28 ± 0.4	Dense, well cut	25.0 ± 0.34	The sour milk smell, however, differs little in taste from previous samples
VI sample	30 ± 0.7	Dense, well cut	25.1 ± 0.10	The sour milk smell, however, differs little in taste from previous samples

Regarding organoleptic parameters, all samples, except for clots, were formed by the action of enzymes selected in 18 and 20 tons. age, they had a more sour smell had a typical milky smell, color, and taste.

### Conclusions

Therefore, the effect of enzymes derived from abomasum from calves aged two weeks to 2.5 months on milk coagulation was studied. The effect of rennet enzymes extracted from rennet calves of different ages on the efficiency of milk clot formation using soft cheese technology has been established.

Further research will focus on the study and improvement of enzyme extraction and immobilization methods.

### Conflict of interest.

The authors state that there is no conflict of interest.

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