Microbiological indicators of cottage cheese using different rennet leavens

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Rennet cheeses occupy an important place in the diets of the population of Ukraine. The technology of cheese production depends both on the quality of raw materials and on the quality of the enzymes used to curdle milk. Therefore, the study of the effect of rennet enzymes obtained by improved biotechnology on the microbial state of the finished product is of scientific and practical importance. III groups of samples (n = 3) were formed to conduct the experiment. Cow's milk for research was collected from clinically healthy cows during the calving period. 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 was used, extracted from the rennet of dairy calves according to the method of Yu. Ya. Svyridenko In the II experimental group, an enzyme preparation obtained by the method of extraction according to the method of was used. S. V. Merzlova. Laboratory research was carried out in the conditions of the Research Institute of Food Technologies and Technologies of Processing Livestock Products and the Laboratory of Microbiological Research Methods of the Department of Microbiology of the Bilotserk National Agrarian University, which is certified according to (DSTU ISO 10012: 2005). To determine the qualitative and quantitative microbial composition, culture was carried out from cheeses obtained with the help of various enzyme preparations. Microbiological research was carried out using the methods defined by DSaNPiN 4.4.5-078-2004 “Microbiological standards and methods of control of food products”. To determine bacterial insenmination, two nutrient media such as Lees agar and Streptococcus thermophilus agar were used. The method of serial dilutions was used for seeding the suspension. Sowing was carried out by applying 1.0 cm³ of suspension (dilutions from 1 to 10-5) in melted and cooled agar in Petri dishes, followed by thermostating for 48 hours at 37 °.

Key words: milk processing, cheese production, rennet enzymes.

Introduction

Statement of the problem: the production of pickled cheeses occupies an important place in cheesemaking and belongs to the most dynamically developing segments. Salted cheeses are the most popular in Ukraine, and for many, including people with impaired carbohydrate metabolism, a daily product. Cheese is a source of complete proteins, calcium, magnesium and vitamins.

Rennet enzymes are used for the production of pickled cheeses, which are divided into two groups by origin. The raw material for the first group is natural rennet of kid goats, lambs and calves. Enzymes of the second group are obtained with the help of microorganisms. 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. The question of how different rennet enzymes affect the microbiological composition of cheese remains unexplored.

The purpose of the work is to establish the dependence of the microbiological state of cheese cheese on the action of various enzyme preparations during its production.

Analysis of research and publications. Rennet enzymes are used to produce rennet cheeses, which are highly nutritious protein products obtained from milk by curdling and processing. Cheese preserves all the main nutrients of milk except carbohydrates. Renal enzymes are obtained by extraction from the stomach of young ruminants that were fed only milk and dairy products. The rennet enzyme extract is purified by filtration, and then
passed through a bacterial filter and stored under sterile conditions. The two active protein components of rennet enzymes are chymosin and pepsin, the standard ratio of which is 80 % to 20 %, respectively. The activity of rennet enzymes depends on the factors of the technological conditions of their production (Silva et al., 2012; Tsisaryk, 2017). Soft cheeses occupy a special place among cheeses. As a result of the biochemical processes that occur during the ripening of cheeses, a large number of peptides and amino acids are formed in them in a shorter time compared to semi-hard and hard cheeses, which allows soft cheeses to be classified as dietary products. The wide taste range of soft cheeses fully satisfies the needs of consumers with any preferences (Bilyi et al., 2021; Bila & Merzlova, 2023). Interest in soft cheeses is growing especially rapidly in Ukraine. Today, this is one of the directions that is developing and occupies a special niche in the cheese industry. In particular, brynza cheese is popular in Ukraine (Kukhtyn et al., 2016; Johnson, 2017; Slyvka et al., 2018). The modern market makes high demands on the quality of food products, in particular dairy products. The quality of a food product is understood as the degree of perfection of the properties and characteristic features of the food product, which are able to satisfy the needs and wishes of those who consume or use this food product. The most acceptable way to determine the totality of product properties is to establish requirements for it in normative documents – technical conditions, standards, etc. (Milci et al., 2005; Yashkina & Makovetska, 2017). Dairy products must meet not only the taste preferences of consumers, but also be useful and safe for consumption. In order to popularize soft rennet cheeses of domestic production, it is first necessary to increase their quality, assortment and competitiveness both on the domestic and foreign markets (Ardo et al., 2002; Bilyi & Merzlova, 2022). During the ripening of cheeses, complex biochemical transformations take place, as a result of which, under certain conditions, there is an accumulation of taste and microbiological particles that determine the specific taste and aroma of cheese (Park et al., 2017).

### The purpose of the study

To investigate the effect of antibiotics in milk on the action of different leavens.

### Materials and methods

Laboratory research was carried out in the conditions of the Research Institute of Food Technologies and Technologies of Processing Livestock Products and the Laboratory of Microbiological Research Methods of the Department of Microbiology of the Bila Tserkva National Agrarian University, which is certified according to (DSTU ISO 10012:2005). Milk that met the requirements of DSTU 2661:2010 (Kholodenko et al., 2023) was collected from clinically healthy cows during the calving period for research. The filtered milk was cooled to a temperature of 4 °C and kept for 12 hours. Pasteurization was carried out at a temperature of 60–63 °C with a holding time of 30 minutes. Pasteurized milk was normalized by mass fraction of fat. Rennet enzyme and sourdough were added to normalized milk heated to a temperature of 33 °C while gently stirring it. The clot was cut into cubes of 15–20 mm in size and left alone for 10–15 minutes, then it was carefully 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. Five days after the ripening of the cheese, samples were taken from each sample to study the microbial composition.

Samples of cottage cheese obtained with the help of microbial enzymes and stabilized enzymes of animal origin were used for scientific research. The experiments were conducted according to the experimental scheme given in Table 1. Samples for research were taken for 20 days of storage at a temperature of 4–5 °C.

To determine the qualitative and quantitative microbial composition, culture was carried out from cheeses obtained with the help of various enzyme preparations.

### Table 1

<table>
<thead>
<tr>
<th>A group of samples</th>
<th>The studied factor</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>Cheese is obtained with the help of enzymes of microbial origin</td>
</tr>
<tr>
<td>I experimental</td>
<td>Brynza is obtained with the help of enzymes of animal origin extracted according to the method of Yu. Ya. Svyridenko</td>
</tr>
<tr>
<td>II experimental</td>
<td>Brynza is obtained with the help of enzymes of animal origin extracted according to the method of S. V. Merzlova</td>
</tr>
</tbody>
</table>

Microbiological research was carried out using the methods defined by DSaNPiN 4.4.5-078-2001 “Microbiological standards and methods of control of food products”.

Microscopy of bacterial cells was performed on fixed stained smears using the Vinogradsky-Shulgin-Brid method. The number of microorganisms in the test sample is calculated according to the formula:

\[ M = A \times S \times V \times X \times n \]

where M is the number of cells in 1 cm²; A is the average number of cells in a square field of view; S is the area of the square of the field of view and the test sample μm²; V is the volume of suspension applied to the glass in cm³; n – sample dilution.

To determine bacterial insemination, two nutrient media such as Lees agar and Streptococcus thermophilus agar were used.

The method of serial dilutions was used for seeding the suspension. Sowing was carried out by applying...
1.0 cm³ of suspension (dilutions from 1 to 10–5) in melted and cooled agar in Petri dishes, followed by thermostating for 48 hours at 37 °C.

The assessment of the quality of raw materials was carried out according to the methods of A. M. Polyvody, R. V. Strobykin, M. D. Lubetsky.

The reliability of the results is ensured by threefold repetition of determinations.

**Results and discussion**

In the (control) group, a rennet enzyme of microbial origin was used for milk curdling, in the 1st experimental group of samples, an enzyme preparation extracted from the rennet of dairy calves was used, the extraction was carried out according to the method described by Yu. Ya. Svyridenko (2011). The method of production of rennet enzyme, which includes crushing of rennet, extraction of the enzyme with a sodium chloride solution, introduction of the preservative sodium benzoate to prevent bacterial growth, separation of the liquid phase, filtering, which is characterized by the fact that the extraction of the enzyme is carried out once with 3.0–10.0 % with a solution of sodium chloride at a temperature of 35–40 °C for 3–3.5 h and with slow constant stirring, the extract is separated from the raw material, activation of the rennet in the extract is carried out by setting the pH to 4.6–4.7 and keeping it for 8–16 h at a temperature of 25–35 °C, the extract is treated with substances that facilitate and accelerate the filtration process, filtered under a pressure of 0.015–0.02 MPa at a temperature of 20–25 °C, the resulting enzyme is stabilized by adding dry sodium chloride to the content of the filtrate 17–18 % of it in the finished preparation and bringing the pH of the finished preparation to 5.3–7.0 units, pH, and the preservative sodium benzoate is added at the stabilization stage to a content of 0.1–0.5 % in the finished preparation.

The II experimental group was treated with an enzyme preparation, which was extracted from the rennet of dairy calves by the method of extracting rennet enzymes according to the method of S. V. Merzlova (2019). The essence of the technique is that a 40 % lactic acid solution is additionally introduced into the extractant, as a result of adding 2.3 % lactic acid to the hydrochloric acid extractant, it is possible to increase the extraction of rennet enzymes by 2.07 times.

With the use of various enzyme preparations, the level of lactic acid microflora becomes of particular importance, which affects the quality of the product and its shelf life. Therefore, we investigated the quantitative and qualitative composition of lactic acid bacteria in cheese. After microscopy of smears from cheese samples, groups of lactic acid bacteria and their number were identified (Table 2).

A high concentration of lactic bacteria was detected in the control group of samples where the level of titrated acidity was 210–220 °T, the total number of CFU was 28×10⁵. In the samples of the I research group, the number of microorganisms was 31×10⁵, which is 10.7 % more compared to the control.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>control</th>
<th>I experimental</th>
<th>II experimental</th>
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<tbody>
<tr>
<td>Total</td>
<td>28×10⁵</td>
<td>31×10⁵</td>
<td>33×10⁵</td>
</tr>
<tr>
<td><em>Streptococcus salivarius subsp. thermophiles</em></td>
<td>16×10⁵</td>
<td>22×10⁵</td>
<td>24×10⁵</td>
</tr>
<tr>
<td><em>Lactococcus lactis subsp. cremoris</em></td>
<td>11×10⁵</td>
<td>17×10⁵</td>
<td>19×10⁵</td>
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The largest number of microorganisms was observed in the samples of the II experimental group of samples with an acidity of 218–230 °T and was 33×10⁵ CFU, which is 17.8 % more than in the product with an acidity of 210–220 °T.

After seeding and thermostating the experimental samples on the nutrient medium, changes in the intensity of colony placement were detected (Fig. 1).

![Fig. 1. View of samples](image-url)
The presence of pathogenic microflora in cheese samples was assessed by the nature of growth on the appropriate media. The presence of *Staphylococcus aureus* bacteria was evidenced by the presence of yellow colonies and a change in pH of the medium (from purple to yellow) in the samples of the first experimental group. Samples of the II experimental group had a pronounced growth of colonies on the medium in separate groups. The medium on which the microorganisms were cultivated did not change color during the growth of the microorganisms, but had a slight turbidity. In the control, unlike the II experimental group of samples, the colonies did not have separate groups and merged with each other. The medium on which the microorganisms were cultivated had significant turbidity and contained traces of mold fungi.

Bacteria of the species *Streptococcus salivarius subsp. thermophilus* are of particular interest. Thermophilic lactacid streptococci. It is these types of lactic acid streptococci that are completely safe for human health, which is confirmed by many years of experience in their use in the production of various food products, including cottage cheese.

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The cells of lactic acid streptococci have a spherical or oval shape with a diameter of 0.5–1.2 μm, are located in pairs or in the form of chains of different lengths. The main type of biological activity of lactic acid streptococci, like other lactobacilli, is the fermentation of carbohydrates with the formation of lactic acid as the main metabolic product.

One of the most useful properties of lactic acid streptococci is the ability of certain strains to synthesize polysaccharides, the unique biological functions of which have been the subject of many studies in recent years. In particular, it was established that microbial polysaccharides have pronounced immunostimulating properties. This allows us to consider them as an important component of the complex therapy of patients with oncological pathology and infections, especially of viral etiology. The antioxidant properties of some strains of lactic acid streptococci are an additional factor preventing the formation of tumors. The anti-inflammatory properties of certain strains of lactic acid streptococci have been revealed. The enzymatic activity of some strains of *Streptococcus salivarius subsp. thermophilus* is the ability of certain strains to synthesize polymeric product.

During the fermentation of cheeses with enzyme preparations extracted from rennet, in comparison with microbial analogues, 1.3% more microorganisms remain in the curd and pass into the cheese grain (Borshch et al., 2019). The production of rennet cheeses is a complex multifunctional process, in which a change in 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 (Chuang et al., 2005).

**Conclusions**

The higher the nutritional and biological value of food depends on whether the product satisfies the body's needs for basic nutrients, i.e. corresponds to a balanced diet. One of the most important components of food products and their safety is the microbiological indicators of the finished product.

According to the results of this study, it can be seen that different enzyme preparations have different effects on the microbiological composition of cottage cheese. As a result, it was found that the high concentration of lactic bacteria in the control group of samples where the level of titrated acidity was 210–220 °Т, the total number of CFU was 28×10⁵. In the samples of the I research group, the number of microorganisms was 31×10⁵, which is 10.7% more compared to the control. The largest number of microorganisms was observed in the samples of the II experimental group of samples with an acidity of 218–230 °Т and was 33×10⁵ CFU, which is 17.8% more than in the product with an acidity of 210–220 °Т. The presence of pathogenic microflora in cheese samples was assessed by the nature of growth on the appropriate media. The presence of *Staphylococcus aureus* bacteria was evidenced by the presence of yellow colonies and a change in pH of the medium (from purple to yellow) in the samples of the first experimental group.

The obtained results are promising for further scientific research aimed at the study and improvement of enzyme extraction methods, the use of other organic extractants, and the study of finished products.

**Conflict of interest**

The authors declare that there is no conflict of interest.
References


