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RESEARCH OF HARD CHEESE RIPENING REGIMES AS A FUNCTION OF THE COMPOSITION OF BACTERIAL STARTER CULTURES

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Abstract. The ripening of cheese, caused by the combined effect of the fermentative enzyme and microflora of the bacterial starter, occurs in certain intervals of temperature, humidity and acidity of the environment which depend on the type of cheese. The composition of bacterial starters for hard cheese is important for formation of the finished product properties and affects the choice of technological regimes. Even minor changes in ripening conditions can significantly affect the microbiological, physicochemical, biochemical parameters, organoleptic properties, and safety of the target product. The aim of the investigation was to study the influence of ripening temperature regimes on the physicochemical, biochemical and organoleptic characteristics of hard cheeses with a low temperature of the second heating aged 30 days, produced using different compositions of bacterial starters. It was shown that the inclusion of lactobacilli *Lactobacillus casei* in the composition of the bacterial starter "Active" makes it possible to intensify proteolysis, and the using of aroma-forming lactic acid bacteria *Leuconostoc mesenteroides* in the starter culture "Active-LN" has a positive effect on the formation of the taste-aromatic composition and texture of the cheese. Changes in the microbiological parameters of cheeses at different stages of production were monitored and the sanitary and hygienic properties of the finished products were evaluated. It was established that cheeses produced both with the use of starter cultures "Active" and "Active-LN" had been ripened best at a temperature of 12–13°C. The products had a good taste and aroma with distinctive features of each type of cheese microbiota, a plastic consistency and a pattern with correctly shaped eyes, larger in cheese with "Aktiv-LN". Thus, the temperature 12–13°C and humidity 75–85% are adequate for the ripening of these cheeses and ensure the formation of a specific taste and aromatic composition of the products and the required level of maturation during 30 days.

Key words: cheese, bacterial starter culture, *Lactobacillus casei*, *Leuconostoc mesenteroides*, ripening, regimes, quality.

Introduction. Formulation of the problem

Among dairy products, hard cheeses occupy a special place, as they are made by concentrating the most nutritionally valuable milk constituents and forming specific flavour and aroma compounds, as well as a wide range of biologically active substances. Cheese production is based on biochemical processes involving the breakdown of the main components of the cheese mass and the formation of many compounds specific to each type of cheese. The most important biological agents in cheese production are the milk coagulation enzyme, starter culture enzymes and foreign microflora. The use of starter cultures is crucial to obtaining a high-quality product, as the efficiency of

cheese production is determined by the level of biological activity of the starter cultures microflora. Prevention of cheese spoilage due to bacterial and technological defects can be achieved by using bacterial preparations containing specially selected strains of lactic acid bacteria with high biological activity [1].

The starter culture microflora of hard rennet cheeses with a low second heating temperature is represented by both mesophilic and thermophilic species. The strains *Lactococcus lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris* (belonging to the group of active acid-forming) and *L. lactis* ssp. *lactis* biovar. *diacetilactis*, *Leuconostoc mesenteroides* ssp. (representing the group of flavour-forming bacteria) are dominant in many cheeses of this type. The presence of

acid-forming bacteria in the starter culture composition ensures intensive lactose fermentation and hence the required level of active acidity in the cheese mass [2,3]. Flavouring lactic acid bacteria strains are important in shaping the taste and texture of cheese through the heteroenzymatic breakdown of lactose and citrates and the formation of diacetyl, volatile organic acids and CO₂ [4-6].

Mesophilic *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum* are often included in the composition of starter cultures in the production of cheeses with a low second heating temperature, characterised by high biological activity [7,8]. This ensures high stability of the technological process, despite the negative impact of seasonal variations in milk quality, resistance of the cheese to secondary bacterial contamination and the possibility of obtaining a quality product. These starter cultures in cheese production activate the lactic acid process, allow a harmonious combination of temperature regimes for processing cheese grains and the development of technologically important microflora, improve the taste and texture of cheese and reduce its ripening time [9,10]. Thus, the composition of the bacterial starter cultures for hard cheese is important in shaping the properties of the final product and influences the choice of technological regimes.

Analysis of recent research and publications

The technology of hard rennet cheeses can be divided into two stages: the process of milk and curd processing, which includes pressing, moulding and salting of the cheese, and cheese ripening. Each of these operations is important in the production of high-quality cheese, but the main focus is on ripening, with the starter culture microflora playing the most important role. During ripening, cheese acquires specific flavours, physical properties and appearance under the influence of microbial enzyme systems and rennet enzyme. The cheese mass undergoes a series of biochemical transformations: fermentation of lactose, hydrolysis of proteins and milk fat, which are the basis for the formation of the flavour and aroma composition of the product [11,12].

Cheese ripening conditions are an important technological factor determining the quality of a mature product. A number of authors have studied the dynamics of the microflora content and the changes in the biochemical parameters of cheese under different temperature conditions [13-17]. In particular, it was found that an increase in the ripening temperature of the typical French "Roquefort" cheese above 11°C at 95% relative humidity led to an accumulation of free organic acids in higher concentrations. The amount of acetic acid ranged from 3 mmol/kg at 11°C to 10 mmol/kg at 20°C and propionic acid from 0.5 to 3.0 mmol/kg. There was also an acceleration of proteolysis, which affected the intensity of the product's aroma. However, at a ripening temperature above 15–17°C, a significant

development of extraneous microflora was observed, with the formation of products that cause defects in the flavour and aroma composition [18]. Thus, the ripening regime of the product is an important regulator for obtaining the desired organoleptic characteristics of the product.

Some authors have researched the variation in the microflora content of cheeses with a low second heating temperature. It was found that in the initial stages of product production, up to 6 days of curd ripening, lactic acid bacteria dominated [19]. The content of other microorganisms, such as *L. paracasei*, *L. rhamnosus* and *L. fermentum*, pediococci, enterococci, increased from the formation of the rennet clot. The analysis of microbiological parameters of cheeses showed that the microflora content 24 hours after pressing was as follows, in log CFU/g: total number – 10.6; lactic acid bacteria of starter cultures – 10.34; flavour-forming bacteria – 9.35; *E. coli* bacteria – 3.09; yeasts – 2.44. The technically important microflora of cheese was represented by the following groups of microorganisms: enterococci – 39.8%, lactococci – 19%, lactobacilli – 12.3%, leucococci – 7.6% [20].

The nature of the transformations during cheese ripening depends not only on the composition of the bacterial starter cultures, but also on the level of microflora remaining after heat treatment, especially in the later stages of cheese ripening. The main group of extraneous microflora consists of heat-resistant bacteria, including spore-forming microorganisms. The number of lactic acid bacteria usually increases from insignificant amounts in the clot to a dominant content in ripened cheese. These cultures are resistant to unfavourable conditions during cheese ripening, such as moisture content of 32–37%, salt content of 4–6%, pH 4.9–5.3, temperature 5–13°C, lack of nutrients, etc [21]. The number of lactic acid bacteria in different types of cheese was calculated and ranged from 5.5 to 8.2 log CFU/g. The presence of lactic acid bacteria in cheese is explained both by post-pasteurization contamination and by the presence of heat-resistant microflora in raw milk remaining after heat treatment of milk [22].

Proteolysis is a complex process involving the combined action of rennet enzyme residues, natural milk proteases and extracellular proteolytic enzymes of starter culture and non-starter culture origin [23]. As a result, caseins are initially break down into large peptide fragments. Their further break down into low molecular weight peptides and amino acids occurs under the action of proteases and peptidases localised on the outer and inner surfaces of the lactic acid bacteria strains. Under the influence of non-proteolytic enzymes, amino acids are converted into volatile compounds that contribute to the aroma of the cheese. In addition, numerous low-molecular-weight substances formed as a result of enzymatic processes also contribute to the formation of the cheese "bouquet" [12,24].

In general, the amount of water-soluble nitrogen compounds, which increases in concentration during the ripening process, is an indicator of the degree of maturity of the cheese. The increase in soluble nitrogen compounds reflects the overall proteolytic hydrolysis of the protein substances in the cheese. The soluble nitrogen content is used to assess the age of the cheese and its type. The action of a complex of proteolytic enzymes in the cheese mass results in the formation of free amino acids. For example, the free amino acid content of sheep's milk cheese in Spain increased from 38.1 mg leucine/100 g cheese after 4 days to 136.1 mg after 28 days [25].

A number of authors have analysed the ability of different types of bacterial starter cultures used in cheese production to accumulate proteolysis products. Thermophilic strains have a higher proteolytic activity than mesophilic ones. A common feature of all types of lactic acid bacteria is the intensive accumulation of amine nitrogen during their active growth period of up to 3 days, as well as an increase in the content of soluble nitrogenous substances and a decrease in the content of total soluble and amine nitrogen throughout the research period [8,16,26,27].

The enzymatic break down of amino acids in cheese plays an important role in shaping the organoleptic characteristics of the product. The break down products of aromatic, branched chain and sulphur-containing amino acids contribute to the pleasant or unpleasant aroma of cheese. For example, branched-chain amino acids are precursors of cheese aroma formation, and aromatic amino acids are precursors of the fodder or phenolic odour of cheese [28]. The catabolism of amino acids by microorganisms in cheese leads to the formation of a wide range of volatile flavour compounds. The characteristic cheese flavour is formed under the influence of enzymes that promote the further conversion of amino acids and is added to the flavour base formed as a result of proteolysis during ripening. It has been found that volatile compounds – products of amino acid conversion – are the main components of the flavour composition of the product. In particular, diacetyl is one of the main components of cheese flavour and is a product of the fermentation of many microorganisms. This compound is a product of the conversion of α -acetolactate produced by lactic acid bacteria as a result of citric acid metabolism. The ability to utilize citric acid is mainly demonstrated by *Leuconostoc* ssp. and *Lactococcus lactis* biovar *diacetylactis*. However, *L. lactis* ssp. *lactis* can also synthesise a significant amount of diacetyl and other cheese compounds, depending on the cultivation conditions [29]. Diacetyl is formed during fermentation and then reduced to 2,3-butanediol, so its levels are higher in the clot and after salting, and decrease during ripening [30].

Free volatile acids play an important role in the formation of cheese flavour and aroma. The sources of

their accumulation are milk, lactose fermentation, deamination of some free amino acids, ketone oxidation, etc. The determination of the organic acid content is one of the indicators used to assess the ripening time of cheeses, which is related to glycolysis and the metabolism of the microflora during ripening [31,32].

Thus, the analysis of the literature has shown that biochemical and enzymatic processes during cheese ripening occur with the direct participation of starter culture bacteria and non-leavened microflora, and their intensity depends on the biological activity of these microorganisms. The main priority for improving the quality of hard rennet cheeses with a low second heating temperature is the inclusion of starter culture bacterial strains with a number of technologically important properties: high proteolytic activity, ability to accumulate flavour and aroma compounds and gas production, and antagonistic activity towards foreign microflora. At the same time, there is a lack of experimental data on the complex effect of temperature and humidity conditions on the ripening process of hard rennet cheeses with a low second heating temperature, depending on the microbiological composition of the starter cultures.

The aim of the study is to investigate the influence of ripening regimes on the quality indicators of hard rennet cheeses with a low second heating temperature as a function of the composition of the bacterial starter. To achieve this aim, the following **research objectives** were formulated:

- to determine the main physico-chemical and biochemical parameters of hard rennet cheeses aged 30 days under different ripening regimes, produced with bacterial starter cultures of different composition;
- to study the changes in the microbiological parameters of the cheese masses at different stages of production;
- to evaluate the organoleptic and sanitary properties of the finished products;
- to establish effective cheese ripening regimes depending on the composition of the bacterial starter culture.

Research materials and methods

Unpasteurized whole milk («EthnoProduct LLC, Ukraine»), liquid milk coagulation enzyme Marzyme XT 755 (Danisco, Denmark), granulated calcium chloride (Nedmag, The Netherlands), table salt («Artemsil» SE, Ukraine) and DelvoCoat cheese coating (DSM, France) were used for experimental cheese production.

The following reagents were used in the study: sodium hydroxide (standard titer (0.1H) 0.1 mol/dm³ (SPE «Alfarus» LLC, Ukraine), phenolphthalein (China), methylene blue (India).

All raw materials and reagents were in compliance with regulatory requirements and were stored under the required conditions as stated on the label.

For the research, we used strains of lactic acid bacteria from the collection of industrial crops of the Biotechnology Department of the Institute of Food Resources of the National Academy of Agricultural Sciences of Ukraine, on the basis of which we prepared bacterial starter cultures for hard rennet cheese with a low second heating temperature. The species and strain composition of the bacterial starter cultures for hard rennet cheese with a low second heating temperature are presented in Table 1.

Table 1 – Composition of bacterial starter cultures for cheeses

| Name | Microbiological composition: species, strain | Ratio between strains |
|---------------------------------------|--|-----------------------|
| Bacterial starter culture "Active" | <ul style="list-style-type: none"> • <i>Lactococcus lactis</i> ssp. <i>diacetylactis</i> IMB B-7061: • <i>Lactococcus lactis</i> ssp. <i>diacetylactis</i> IMB B-7062: • <i>Lactococcus lactis</i> ssp. <i>lactis</i> IMB B-7063: • <i>Lactococcus lactis</i> ssp. <i>cremoris</i> IMB B-7064: • <i>Lactobacillus casei</i> IMB B-7017. | 1:1:1:1:0: 5 |
| Bacterial starter culture "Active LN" | <ul style="list-style-type: none"> • <i>Lactococcus lactis</i> ssp. <i>diacetylactis</i> IMB B-7061: • <i>Lactococcus lactis</i> ssp. <i>diacetylactis</i> IMB B-7062: • <i>Lactococcus lactis</i> ssp. <i>lactis</i> IMB B-7063: • <i>Lactococcus lactis</i> ssp. <i>cremoris</i> IMB B-7064: • <i>Leuconostoc mesenteroides</i> IMB B-7065. | 0.5:0.5:1: 1:2 |

The experimental cheese production was carried out according to the technological scheme shown in Figure 1. The milk mixture was normalized in terms of fat mass, taking into account the protein content of the milk, up to a mass of 45% in the dry matter of the product. Pasteurization of the milk mixture was carried out at a temperature of $(74 \pm 2)^\circ\text{C}$ with a holding time of 20s. The following ingredients were constant throughout the production of the cheese: liquid milk coagulation enzyme, at a dose of 20–25 g per 100 kg of normalized mixture, and calcium chloride, added to the mixture in the form of a 40% solution at a rate of 40 g per 100 kg of milk. After the addition of a 1% bacterial starter culture and renneting at a temperature of $(30 \pm 1)^\circ\text{C}$, the finished cheese curd was cut within 15 minutes. Once the cheese curd had set to a size of 5–6 mm, 40–60% of the whey was removed and the grain was kneaded for 15–20 minutes. The second heating was carried out with hot $65\text{--}70^\circ\text{C}$ water to a temperature of $(40 \pm 1)^\circ\text{C}$. After whey extraction, the cheese grains were unloaded into cheese molds for self-pressing and pressing. Self-pressing was carried out for 30–40 minutes and pressing for 3–4 hours until the moisture content reached 44–46%. The cheeses were salted in brine with a NaCl concentration of 20–21% at a temperature of $8\text{--}10^\circ\text{C}$. The salting time was

determined according to the size of the cheese head and was 3–4 hours until the salt content in the cheese reached 2%. After drying, 3 layers of polyvinyl acetate coating were applied to the experimental cheeses and sent for ripening under different temperature and humidity regimes, which were measured using a thermohygrometer – ET175 data logger (Shenzhen FLUS Technology Co, China).

The following methods were used to study dairy raw materials and cheeses: assessment of active acidity (pH) – by potentiometric method; diacetyl content, mass fraction of fat, moisture, amount of volatile organic acids (distillation number) were determined according to [32]; the degree of proteolysis in cheeses was assessed by the content of free amino acids. The samples were prepared for analysis by grinding the cheese mass, dissolving the sample in a 1% sodium citric acid solution and removing the fat. The determination of these compounds was carried out by the Kjeldahl method according to DSTU 8063:2015.

The research of the microbiological parameters of the products was carried out by means of the following methods: the total content of LACTIC ACID BACTERIA, yeasts and moulds was determined by inoculating serially diluted cheeses on agarified media, respectively MRS, Sabouraud Dextrose with chloramphenicol (0.1 g/l), cultivated at 30°C for 48 hours. The content of butyric acid bacteria, flavour-forming lactic acid bacteria and the presence of *E. coli* bacteria were determined according to [Instruction on the organisation of production microbiological control at dairy enterprises / NAAS; Institute of Food Resources of NAAS]. K.: NSC "IAE", 2014. 372 p.] Organoleptic quality indicators of finished cheeses were determined according to SSU 6003:2008.

The experiments were performed in triplicate. The research data obtained were processed using Excel from the Microsoft Office 2007 service pack. The data are presented as mean \pm standard deviation.

Results of the research and their discussion

The research of the influence of ripening regimes on the characteristics of cheeses made with bacterial starter cultures of different composition.

The research was carried out to study the effect of different ripening temperatures on the quality indicators of hard rennet cheeses produced with the bacterial starter cultures "Active" and "Active-LN". Cheese ripening was researched at the following temperature regimes at a fixed relative humidity of 80%: regime 1 – $(9\text{--}10)^\circ\text{C}$, regime 2 – $(12\text{--}13)^\circ\text{C}$, regime 3 – $(14\text{--}15)^\circ\text{C}$. The ripened cheeses at the age of 30 days were analysed for the main physico-chemical and biochemical parameters, namely the level of accumulation of aroma compounds and low molecular weight proteolysis products. The results of the research are shown in Table 2.

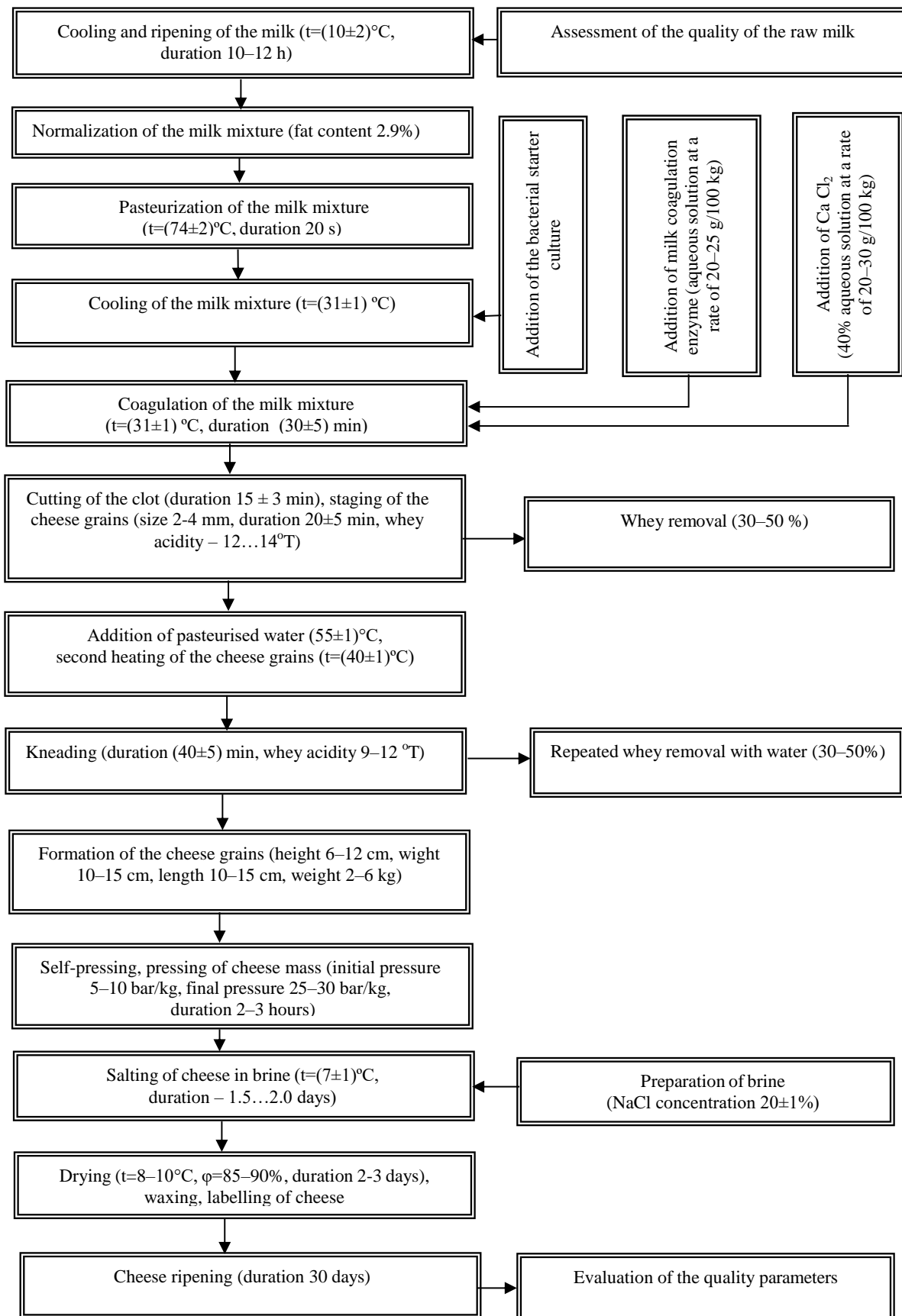


Fig. 1. Flow chart for producing hard rennet cheeses

Table 2 – Physical, chemical and biochemical parameters of cheeses aged 30 days under different ripening regimes (n=3, p≤0.05)

| Cheese, regime | Active acidity, pH | Moisture mass content, % | C | | |
|--|--------------------|--------------------------|---------------|------------------------------------|----------------------|
| | | | diacetyl, mg% | volatile organic acids, mcEq/100 g | free amino acids mg% |
| Cheese with the bacterial starter "Active": | | | | | |
| Regime №1 | 5.34±0.03 | 44.6±0.2 | 1.18±0.08 | 210±5 | 936±108 |
| Regime №2 | 5.30±0.03 | 43.3±0.1 | 1.36±0.10 | 425±6 | 1498±165 |
| Regime №3 | 5.15±0.04 | 41.9±0.1 | 1.28±0.07 | 540±8 | 1722±188 |
| Cheese with the bacterial starter "Active-LN": | | | | | |
| Regime №1 | 5.36±0.02 | 45.0±0.2 | 1.12±0.06 | 290±10 | 833±88 |
| Regime №2 | 5.33±0.03 | 44.1±0.1 | 1.40±0.12 | 495±6 | 1122±128 |
| Regime №3 | 5.20±0.03 | 42.4±0.2 | 1.26±0.08 | 585±5 | 1502±176 |

According to the data obtained, the influence of the ripening regime affected all the parameters studied. The general trend for both types of cheese was a decrease in the level of active acidity, cheese drying and an increase in the production of substances that determine the flavour and aroma composition of cheeses at high temperatures. Thus, the moisture content by mass during ripening at a temperature of 14–15°C was 41.9–42.4%, while at lower temperatures it was between 43.3–45.0%, which complies with current legislation.

When analyzing the accumulation of flavour-forming substances, it should be noted that the level of volatile organic acids was consistently higher in cheeses with "Active-LN", due to the presence of the flavour-forming strain *Leuconostoc mesenteroides* IMB B-7065 in this bacterial starter culture. Maximum diacetyl formation of 1.36±1.40 mg% was observed at a ripening temperature of 12–13°C, with a further increase in temperature of 2–3°C leading to the accumulation of lower amounts of this compound, apparently due to its reduction to the corresponding alcohol. The results obtained are in line with the literature. Thus, cheeses made using starter cultures with flavour-forming microflora had a more pronounced flavour, aroma and a characteristic pattern of regularly shaped gas eyes. These properties are mainly due to microorganisms of the genus *Leuconostoc* ssp. which are capable of active production of carbon dioxide, formation of acetic acid and other flavour and aroma components [6].

The higher temperature regimes of ripening contributed to a more intensive proteolysis, as the content of free amino acids was the highest in the samples of these cheeses. It is worth noting that cheeses made using the "Active" starter culture, which is characterized by the presence of lactobacilli with high proteolytic activity, *Lactobacillus casei* IMB B-7017, had a higher content of free amino acids in all the regimes studied, indicating an intensification of the ripening process. It has been reported that these

cultures have higher acid-formation energy and proteolytic properties compared to coccal forms, as well as antagonistic effects against sanitary indicator microflora [7,8].

Research of microbiological parameters of hard cheeses under different ripening regimes.

In addition to the determination of physico-chemical and biochemical parameters, the dynamics of the content of both the total number of lactic acid microflora and the number of flavour-forming lactic acid bacteria was analysed in the hard cheeses researched at different temperature regimes on a weekly basis. Comparing the respective indicators of the two types of cheeses, it is noteworthy that the total account of lactic acid bacteria remained constant at the end of ripening in the "Active" cheese (Fig. 2a), whereas their number decreased in the "Active-LN" cheese (Fig. 3a). This can be explained by the high content of *Lactobacillus casei* in the first variant, which is characterised by the ability to survive in a carbon-free environment and high resistance to harsh conditions: low moisture content of the cheese mass and the presence of common salt [8,21].

It should be noted that the results of the microbiological studies were consistent with the data obtained from the biochemical analysis of the cheeses. At low ripening temperatures, a slow development of lactic acid bacteria, especially flavour-forming bacteria, was observed (Fig. 2b; Fig. 3b). In particular, the maximum number of *L. diacetylactis* in "Active" cheeses under this regime was observed at 14 days of age, corresponding to 8.5 lg CFU/g, and its content gradually decreased over time. As a result, low production of volatile organic acids and diacetyl and inactive proteolysis were observed.

The results of the analysis of the content of extraneous microflora and the organoleptic evaluation of the cheeses of both types showed the unsuitability of the high-temperature ripening regime № 3 for this type of cheese (Table 3).

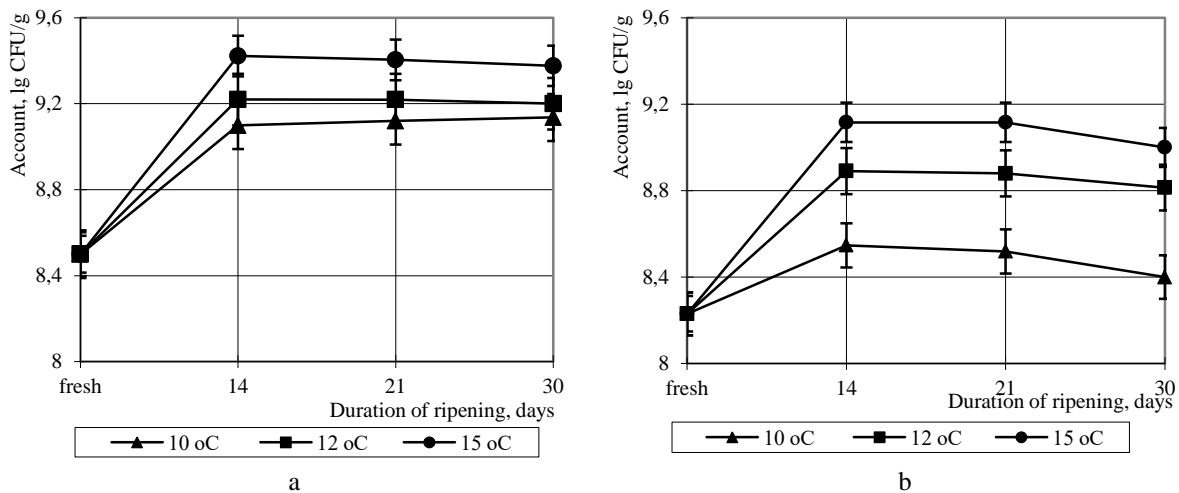


Figure 2. Dynamics of the lactic acid microflora content during the ripening of cheeses with the "Active" bacterial starter at different temperature conditions: a – the total account of lactic acid bacteria; b – the account of flavour-forming lactic acid bacteria

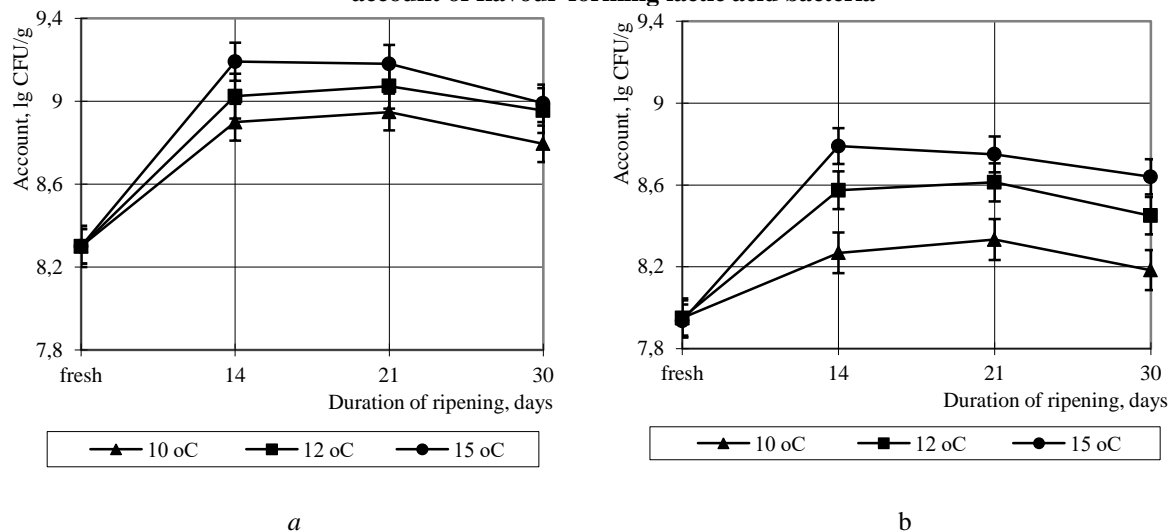


Figure 3. Dynamics of the lactic acid microflora content during the ripening of cheeses with the "Active-LN" bacterial starter at different temperature conditions: a – the total account of lactic acid bacteria; b – the account of flavour-forming lactic acid bacteria

Table 3 – Sanitary and hygiene indicators and characteristics of hard cheeses aged 30 days under different ripening regimes

| Cheese | Account, CFU/g | | | Organoleptic characteristic |
|--|-----------------|---------------------|---------------------|--|
| | Coliforms | Spore forming | Yeasts and | |
| Cheese with the bacterial starter "Active": | | | | |
| Regime №1 | 10 ¹ | 1.5·10 ² | 4.2·10 ² | Weakly expressed, slightly acid taste and smell, plastic consistency, without eyes |
| Regime №2 | 10 ¹ | 3.6·10 ² | 5.3·10 ² | Pronounced cheesy, moderately acid taste and smell, plastic consistency, small eyes of regular shape on the cut |
| Regime №3 | 10 ² | 3.8·10 ² | 9.8·10 ² | Sour taste with an off-flavour taste and smell, dense consistency, small eyes of different shape on the cut |
| Cheese with the bacterial starter "Active-LN": | | | | |
| Regime №1 | 10 ¹ | 1.2·10 ² | 3.8·10 ² | Weakly expressed, slightly acid taste and smell, plastic consistency, without eyes |
| Regime №2 | 10 ¹ | 3.3·10 ² | 6.5·10 ² | Pronounced cheesy, moderately acid and sharp taste and smell, plastic consistency, individual big eyes of regular shape on the cut |
| Regime №3 | 10 ² | 2.5·10 ² | 1.8·10 ³ | Sour taste with an off-flavour taste and smell, dense consistency, small eyes of different shape on the cut |

The intensive development of technically harmful microflora, in particular the surface microflora, led to the spoilage of the cheeses. In addition, an increase in volatile organic acids (apparently due to an increase in the proportion of butyric acid) resulted in unsatisfactory flavour and aroma, and increased acid formation resulted in a dense consistency. However, products ripened at low temperatures according regime № 1 also received unsatisfactory scores due to poor flavour and aroma and lack of pattern.

Positive results were obtained in the analysis of both types of cheese ripened at 12–13°C: the products had a good flavour and aroma, with characteristics specific to each type of cheese, a plastic consistency and a pattern with regular-shaped eyes, which were larger in the "Active-LN" cheese.

It was thus established that regime № 2 – temperature 12–13°C and relative humidity 75–85% – is effective for the ripening of these cheeses, ensuring the formation of a specific, pronounced flavour and aroma composition of the products and the required degree of ripening within 30 days.

Approbation of the research results. The results of the research can be used in production practice for modelling new and adjusting existing technological methods of ripening hard rennet cheeses with a low temperature of the second heating. Approbation of use

of bacterial starter cultures «Active» and «Active-LN» in industrial conditions was carried out at «Priyatyn Cheese Factory» LLC and other cheese-making enterprises of Ukraine.

Conclusion

The influence of ripening temperature regimes on the organoleptic, physico-chemical and biochemical parameters of hard cheeses with a low temperature of the second heating at 30 days of age, produced with bacterial starter cultures of different composition, was studied. It has been shown that the inclusion of *Lactobacillus casei* in the bacterial starter allows an intensification of proteolysis, and the use of the flavour-forming lactic acid bacteria *Leuconostoc mesenteroides* has a positive effect on the formation of the flavour and aroma composition and the cheese pattern. The changes in the microbiological parameters of the cheeses at different stages of production were followed and the sanitary and hygienic properties of the finished products were evaluated, on the basis of which an effective ripening regime at a temperature of 12–13°C and a relative humidity of 75–85% was established for cheeses produced using both types of starter cultures.

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ДОСЛІДЖЕННЯ РЕЖИМІВ ВИЗРІВАННЯ ТВЕРДИХ СИРІВ У ЗАЛЕЖНОСТІ ВІД СКЛАДУ БАКТЕРІАЛЬНОЇ ЗАКВАСКИ

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Анотація. Дозрівання сиру, спричинене комбінованим впливом молокозсідаального фермента і мікрофлори бактеріальної закваски, відбувається у певних інтервалах температури, вологості та кислотності середовища, які залежать від виду сиру. Склад бактеріальних заквасок для твердого сиру має важливе значення у формуванні властивостей готового продукту та впливає на вибір технологічних режимів. Навіть незначні зміни умов дозрівання можуть істотно позначитися на мікробіологічних, фізико-хімічних, біохімічних показниках, органолептиці та безпеці цільового продукту. Метою роботи було дослідження впливу температурних режимів визрівання на фізико-хімічні, біохімічні та органолептичні характеристики твердих сирів з низькою температурою другого нагрівання віком 30 дб, вироблених з використанням бактеріальних заквасок різного складу. Показано, що залучення лактобацил *Lactobacillus casei* до складу бактеріальної закваски „Актив” дає змогу інтенсифікувати протеоліз, а застосування ароматоутворюючих молочнокислих бактерій *Leuconostoc mesenteroides* у заквасці „Актив-ЛН” позитивно впливає на формування смако-ароматичної композиції та рисунку сиру. Простежено зміну мікробіологічних показників сирів на різних етапах виробництва та оцінено санітарно-гігієнічні властивості готових продуктів. Встановлено, що сир вироблений як зі застосуванням закваски „Актив”, так і „Актив-ЛН” найкраще визрівали за температури 12–13°C, продукти мали добрі смак та аромат із особливостями, притаманними кожному виду мікробіоти сиру, пластичну консистенцію та рисунок з вічками правильної форми, крупнішими у сирі з „Актив-ЛН”. Таким чином, температурний режим 12–13°C при відносній вологості повітря 75–85% є ефективним для визрівання даних сирів та забезпечує формування специфічної, вираженої смако-ароматичної композиції продуктів та необхідний рівень зрілості протягом 30 дб.

Ключові слова: сир, бактеріальна закваска, *Lactobacillus casei*, *Leuconostoc mesenteroides*, визрівання, режими, якість.