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THE USE OF BACCONCENTRATE HEROBACTERIN IN BRINE CHEESE TECHNOLOGY

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Abstract. In the article a comparative analysis of the use of the bacterial preparation Herobacterin and the starter RSF-742 (*Chr. Hansen*, Denmark) in the technology of brine cheese was conducted. Herobacterin is a bacterial preparation created using bacteria *Lactococcus lactis*, *Lactobacillus plantarum*, *Enterococcus faecium*, *Leuconostoc mesenteroides* and *Lactococcus garvieae*, isolated from traditional Carpathian brine cheese brynza and identified using classical microbiological and modern molecular genetic methods (RAPD-PCR, RFLP-PCR, sequencing of the 16S rRNA gene). The results of investigations of organoleptic, physico-chemical, syneretical and microbiological parameters of cheese brynza with use of preparation Herobacterin are presented in comparison with the starter RSF-742, which includes cultures: *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. lactis, Streptococcus thermophilus, Lactobacillus helveticus.* The use of Herobacterin has a positive effect on organoleptic, physico-chemical and microbiological parameters, all parameters complied with the requirements of DSTU 7065:2009. The level of survival of lactic acid bacteria in brynza during maturation and storage is high, which confirms the correctness of the selection of strains to preparation Herobakterin, which demonstrated good adaptability to the composition and properties of ewe's milk.

Key words: brynza, technology, starter Herobakterin, starter RSF-742, organoleptic parameters, physical and chemical parameters, number of viable cells.

ВИКОРИСТАННЯ БАККОНЦЕНТРАТУ ГЕРОБАКТЕРИН У ТЕХНОЛОГІЇ БРИНЗИ

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Анотація. Показано результати порівняльного аналізу використання створеного бакконцентрату Геробактерин із бактеріальним препаратом RSF-742 (Chr. Hansen, Данія) у технології розсільного сиру бринза. Геробактерин − бактеріальний препарат, створений на міжвидовій, багатоштамовій основі з використанням бактерій *Lactococcus lactis, Lactobacillus plantarum, Enterococcus faecium, Leuconostoc mesenteroides і Lactococcus garvieae,* виділених із традиційної карпатської бринзи та ідентифікованих із використанням класичних мікробіологічних і сучасних молекулярно-генетичних методів (RAPD–PCR, RFLP–PCR, секвенування гену 16S рРНК). Наведено результати досліджень органолептичних, фізико-хімічних, синеретичних та мікробіологічних показників сиру бринза із використанням бакконцентрату Геробактерин у порівнянні із препаратом прямого внесення RSF-742, до складу якого входять такі культури: *Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Streptococcus thermophilus, Lactobacillus helveticus.* Використання препарату Геробактерин здійснює позитивний вплив на органолептичні, фізико-хімічні та мікробіологічні показники бринзи, усі параметри відповідали вимогам ДСТУ 7065:2009. Рівень виживання молочнокислих бактерій у бринзі протягом визрівання і зберігання є високим, що підтверджує правильність відбору штамів до бакконцентрату Геробактерин, які продемонстрували добру пристосованість до складу і властивостей овечого молока.

Ключові слова: бринза, технологія, бакконцентрат Геробактерин, препарат мікробіальний RSF-742, органолептичні показники, фізико-хімічні показники, кількість життєздатних клітин.

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Introduction. Formulation of the problem

"Brynza" from the Romanian language means "cheese", it is from the territory of modern Romania that its production begins. The first written mention of brynza dates from the end of the 15th century (Slovakia), and in the territory of modern Ukraine – 16 centuries [1]. This brine cheese is a traditional product of the Carpathians and the Southern region of Ukraine, Poland, Slovakia and Romania. They make it from ewe's milk, which graze on the Carpathian mountain valleys from April to October. For centuries, microbiota was formed, which participates in the process of

producing this cheese. Today, the attention of scientists focuses on the study of the microflora of traditional dairy products, because it is the source of new strains that can be endowed with valuable technological and probiotic properties. Unfortunately, the microflora of the traditional Carpathian brynza has not been investigated for today. For the production of brynza in industrial conditions, milk-raw must be pasteurized, and for protein coagulation are used various starters, created for cheese other groups. There are no special microbial Ukrainian starters for brine cheeses. We believe that the reproduction of a natural microbial composition, which has been formed in natural habitats for centuries, and which manifests itself in symbiotic interactions, can serve as the development of brynza production on an industrial scale. In addition, it is possible that among the bacteria of this composition are those that are endowed with special biological properties. Taking into account that the inhabitants of the Carpathians have good health and longevity, we assume that the microflora of brynza, which is a daily product for them, plays a role in it.

Literature review

Attention of many researchers today focuses on the study of natural microbiocenoses of traditional dairy products in many geographic areas [2]. Strains of bacteria with valuable properties are used to construct starters [3]. Abroad, bacterial preparations for traditional sour-milk products are developed on the basis of natural populations of lactic acid bacteria characteristic of these products [4]. In Ukraine, for the production of brynza import starters are used, which are characterized by different composition of microbial cultures. Instead, there is no bacterial preparation, the composition of which would be most closely related to the natural composition of lactic acid bacteria (LAB) dominant in raw ewe's milk. The use of isolated strains of LAB from Carpathian brine cheese in the composition of a starter will preserve the classical nature of traditional products [5–7]. We believe that the creation of starters with the involvement of typical natural strains of the LAB and the development of modern technologies for the industrial production of national dairy products and cheeses with their use is an actual and timely task. In addition, the production of national products in industrial conditions can give a significant consequence to the development of traditional centuries-old technology. Such product the Carpathian brine cheese brynza is in Ukraine, which has its own characteristics, which are determined by the conditions of the climatic zone of the region and the peculiarity of its production. Traditionally, brynza is made from raw ewe's milk in private farms. In the process of producing cheese from raw milk bacterial cultures are not added, so during maturation of cheese natural lactic microflora prevails over other groups of microorganisms. For the production of brynza in industrial conditions, the composition of the bacterial preparation is of great importance, since raw milk must be pasteurized, during which not only undesirable microflora is destroyed, but also lactic acid flora of raw milk [8].

The existing technology for the production of brynza in industrial conditions involves the addition of starter in milk after pasteurization, which, together with the coagulation enzyme, provides the formation of a casein clot and biochemical transformations during the maturing of cheese. The microflora of fermentation crops determines specific physico-chemical, organoleptic properties, ensures product safety and preserves qualitative characteristics during storage. Thanks to the microflora, you can control the biochemical processes during maturation and direct them, as well as provide the product with functional properties [9].

Therefore, for the production of a bacterial preparation for brynza in industrial conditions, it is important to select the cultures of LAB that are typical of a particular geographical region. The lactic microflora involved in the production of traditional cheese and exhibits unique properties can be taken as the basis for the bacterial composition of microorganisms.

Herbacterin is a bacterial preparation created on an interspecific, multi-strain basis using bacteria of genera *Lactococcus, Lactobacillus*, *Leuconostoc* and *Enterococcus – Lactococcus lactis ssp. lactis, Lactobacillus plantarum, Enterococcus faecium, Leuconostoc mesenteroides ssp. mesenteroides* and *Lactococcus garvieae*. It is a factor in the preservation of the natural bacterial system of traditional ewe's cheese – brynza, which is produced in non-industrial conditions of the Carpathian region of Ukraine, whose role is directly related to the natural fermentation of milk. Cultures of bacteria (95) identified using classical microbiological and modern molecular genetics methods (RAPD-PCR, RFLP-PCR, sequencing of 16S rRNA gene) [10,11]. To construct the preparation Herobacterin strains that are endowed with valuable technological and probiotic properties were selected: *Lactococcus lactis ssp. lactis* TW54-2, *Lactobacillus plantarum* WCFS1, *Enterococcus faecium* L3-23, *Leuconostoc mesenteroides ssp. mesenteroides* A7 and *Lactococcus garvieae* JB282647 2.

Strain *Lactococcus lactis ssp. lactis* TW54-2 has the highest resistance to high concentrations of NaCl (6.5%) and high acid-forming energy. Brynza cheese is a brine cheese; therefore resistance to high salt concentrations is an important technological indicator of the bacteria used for its production. *Lactobacillus plantarum* WCFS1 is selected as a functional strain [12], it exhibited resistance to high concentrations of NaCl. Inclusion of *Enterococcus faecium* is based on the fact that its strains are found in all samples of cheese, selected from different regions of the Carpathians and made from milk of sheep various breeds. In quantitative terms, Enterococci were a significant part of the microbiota. Today enterococci are included in the starting cultures for many fermented dairy products [13].

Their presence positively affects the formation of the taste, aroma, color and structure of cheese [14], as well as the storage of cheese [15]. They exhibit high survival ability for extreme values of temperature, pH and salt concentration [16], conditions of the digestive tract [17]. Enterococci are known for their probiotic properties, primarily antagonistic due to the synthesis of bacteriocins [13,18]. Recently, their positive role in the metabolism of lipids has been experimentally confirmed, which is especially relevant for the prevention of cardiovascular diseases and metabolic syndrome [19]. Strain *Enterococcus faecium* L3-23 has acid-forming energy of 87 °T for 24 hours of cultivation and a resistance to a salt concentration of 6.5%. Strain *Leuconostoc mesenteroides ssp. mesenteroides* A7 is involved in the bacterial preparation as an aroma-forming agent. The *Lactococcus garvieae* strain JB282647 2 was isolated from all samples of brynza, thus it is a typical representative of the microbiota of dairy products with natural fermentation [20] and was characterized by high salt tolerance.

Today, using molecular genetics methods, it became possible to identify LAB at the level of strains [21], since it is known that there are strains of the features of many properties of bacteria. In previous studies, we have identified the LAB of the Carpathian brynza, made in the highlands and foothills zone from milk of sheep different breeds. Selected strains characteristic of all samples of brine, their technological features and antibiotic resistance are investigated. From the strains, which showed the best properties, a bacterial preparation Herobacterin was created.

In order to find the optimal correlation of cultures in the composition of a bacterial preparation for the production of brynza in industrial conditions, three samples of brynza were made and evaluated. The cheeses were evaluated on a 100-point scale, according to the score, the following ratio was chosen: 50% *Lactococcus lactis*, 15% *Lactobacillus plantarum*, 20% *Enterococcus faecium*, 10% *Leuconostoc mesenteroides* and 5% *Lactococcus garvieae* [22].

The purpose of the work is the production of cheese from ewe's milk with the preparation Herobakterin, a study of its properties compared with the cheese made with the starter RSF-742 (*Chr. Hansen*, Denmark).

To accomplish this purpose you need to solve the following **tasks**:

- to study the composition and properties of ewe's raw milk;
- to made brine cheese with preparation Herobakterin and a Direct Vat Set RSF-742;
- to analyze the effect of Herobakterin and RSF-742 on the increase of acidity during the protein coagulation and the syneretic properties of clots;
- to study the composition and properties of cheese made using Herobakterin and the preparation of RSF-742;
- to investigate the change of physicochemical and microbiological properties of brynza, made using Herobakterin and RSF-742 during storage;
- to improve the technology of brynza making with application of starter Herobakterin.

Research Materials and Methods

The raw material for the production of brynza was the ewe's milk of the Ukrainian mountain-Carpathian breed of sheep. Organoleptic, physico-chemical and microbiological parameters were determined in milk in accordance with TU 10.16 USSR 71-89 "Sheep's milk. Requirements for purchases". Two samples of brynza (three times repeated) were made – control (C) using the starter DVS RSF-742 (Chr. Hansen, Denmark) at a dose of 35.7 g per 1 ton of cheese milk and experimental (E) using preparation bacteria Herobacterin (lyophilic drying), which was administered at a dose of 25 g per 1 ton of cheese milk after pre-activation in skimmed milk (the dose selection was carried out in the calculation of the final yield of the LAB number in an amount not less than 1×10^7 CFU/g of the product).

The starter RSF-742 contains the following LAB: *Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Streptococcus thermophilus, Lactobacillus helveticus.* Since RSF-742 is a Direct Vat Set, there was no need for prior activation. In addition to fermentation preparations for protein coagulation, an enzyme preparation of microbial origin CHY-MAX (*Chr. Hansen*, Denmark) in a dose of 10 g and calcium chloride in a dose of 100 g per 1 ton of cheese milk were used.

After fermentation agents and milk-enzyme introduction at a temperature (33±1)°C during sowing, the growth of acidity (active) and syneretic properties of clots were monitored. The pH-meter ARN-9 was used to determine the active acidity. To determine syneretic properties, 100 cm^3 clots with serum isolated on the surface were cut and placed on a paper filter by measuring the serum volume every 10 minutes during one hour [23].

Properties of the finished product have been researched in accordance with DSTU 7065:2009 (Brynza: General Specifications). Organoleptic (taste, odor, color and consistency according to GOST 7616-85), physico-chemical (fat mass by the butyrometric method, mass fraction of moisture by drying method according to GOST 3626-73, active acidity by means of pH meter ARN-9) and microbiological parameters (the number of lacobacilli and lactococci by the method of serial dilutions on nutrient media MRS and M17 according to GOST 10444.11-89) were identified in fresh cheese and during storage.

Results of the research and their discussion

Special requirements apply to the quality of raw milk in the dairy production. Analyzing the data in ta-

ble 1, we can conclude that ewe's milk meets the requirements of DSTU 7412-2013 and is suitable for the production of brynza. The raw ewe's milk did not have sediment and flakes, had a sweet taste, without ineligible flavors and odors. For bacterial contamination milk belonged to the First class.

Table 1 – Physico-chemical parameters of ewe's milk (n=3)

Raw	Protein, %	Fat, %	Lactose, %	Dry matter, %	Density, kg/m^3	Active acidity, pH	Titrated acidity, °T
Ewe's milk	5.20±0.12	6.60±0.11	4.65±0.11	17.20±0.12	1034±0.11	6.25±0.12	25±0.12

Ewe's milk was matured at a temperature of (10±2)°C during 10–12 hours. After maturation, milk was normalized by skimmed milk. Cheese milk pasteurized at a temperature of (73±2)°C during 20 s, then cooled down to a temperature (33±1)°C. In a pasteurized and cooled cheese milk, a bacterial preparation Herobacterin or RSF-742, milk enzymes and a 40% calcium chloride solution added and stirred (15 min^{-1}) during 10 minutes. The duration of protein coagulation, along with other factors, depends on the type of milk. This, first of all, is related to the physical and chemical composition of raw milk, namely, the content of proteins. It is known, in ewe's milk the protein content is 1.5 times higher than in the cow's milk. Figure 1 shows the change in active acidity during coagulation of proteins This process is accompanied by a decrease in the active acidity index, which was shown to be more intense for the control sample with the use of RSF-742, which includes more active acid formers.

One of the indicators of quality of the finished cheese is the mass fraction of moisture, which depends on the syneretic properties of the clots. Syneresis is the process of serum separating from the clot, which involves the imperceptible sealing of the structure by regrouping the particles and increasing the number of contacts between them, compressing the gel and extortion out the dispersion medium from it. Figure 2 shows the results of studying the syneretic properties of clots, obtained by using RSF-742 and Herobacterin. Data from the studies indicate that there are no significant differences between the samples.

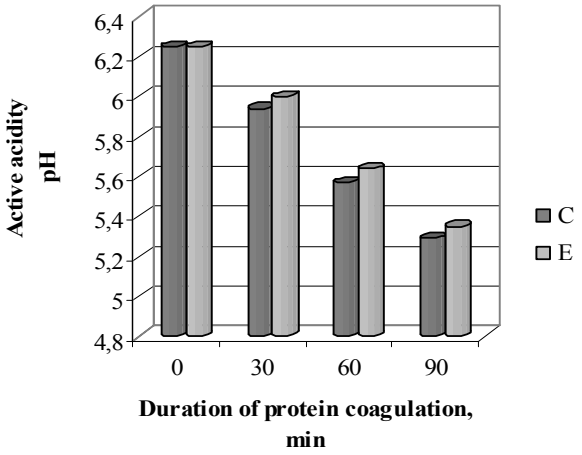

Fig. 1. Change of active acidity of cheese

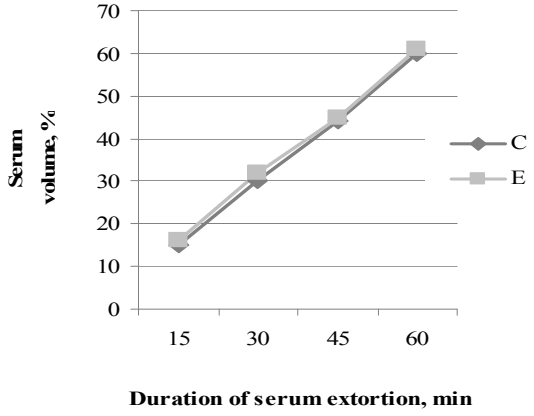

Fig. 2. Syneretic properties of clots milk during coagulation

C –using RSF-742; E –using Herobacterin

The clots were cut in (5±2) min with received a cheese grains in size 1 cm^3. After serum extortion, cheese grains were formed and self-pressed during 12 hours and pressed for 2–3 hours at a temperature of +18°C. Cheesewas salted for 4 hours under concentration of salt in brine 18% and temperature +10–12°C). The maturation of brynza was carried out in 18% brine at a temperature (6±2)°C for 20 days as well as storage for 40 days.

During maturation of brynza it is important to control the number of microflora, which is an important factor in the course of biochemical processes and the formation of organoleptic characteristics of the finished product. During the maturation of brine (0, 5, 15 and 20 days) the dynamic of changes in the number of lactococci and lactobacilli was studied. The number of lactococci was 5.2–5.6 lg CFU/g at the beginning of maturation. During the 20 days of maturation, the number of lactococci increased by 75–77% and reached 6.9–7.3 lg CFU/g (Fig. 3). Sample of cheese with using Herobacterin was characterized a larger number of lactococci. A similar tendency was observed also in relation to the quantity of lactobacilli. Number of viable lactobacilli cells increased from 4.8–5.1 to 6.7–7.1 lg CFU/g of cheese during the maturation (Fig. 4), experimental sample had a larger number of lactobacillus. In our opinion, is due to the better adap-

tation of these cultures to the composition and properties of ewe's milk, which is their natural environment, as well as their high salt tolerance.

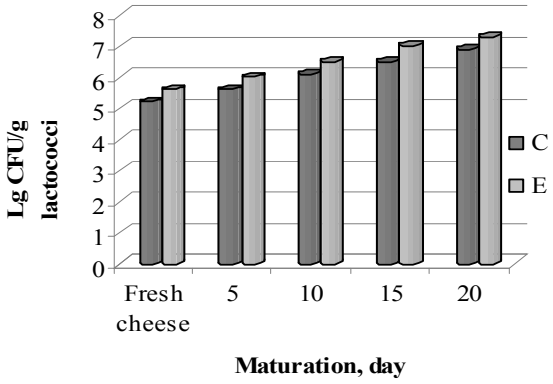

Fig. 3. Change in the number of lactococci during maturation of cheese

Fig. 4. Change the number of lactobacilli during maturation of cheese

The choice of bacterial preparation was reflected on the organoleptic parameters of cheese. Samples were characterized by organoleptic parameters (Table 2), suitable for brynza. it is important that the use of the created preparation Herobacterin did not lead to deterioration of organoleptic parameters of cheese.

Table2 – Organoleptic parameters of brynza made with different bacterial preparations

Indicator/Samples	C	E
Outward	The surface is clean, with nipple impressions, the crust is absent. Insignificant deformation	The surface is clean, with nipple impressions, the crust is absent
Taste and flavor	Pure, sour milk, salty, characteristic of ewe's milk	Pure, sour milk, salty, characteristic of ewe's milk
Consistency	Slightly crap, moderately dense and plastic	Slightly crap, moderately dense and plastic
Drawing	Unique eyes of irregular shape	Small eyes of irregular shape
Color	Light yellow, connatural	Light yellow, connatural

The use of Herobacterin did not significantly affect on the physical and chemical properties of brynza (Table 3). The active acidity of the experimental samples of brine was by 0.2 units higher than control, due to the presence in the preparation RSF-742 more active acid-forming agents –*Streptococcus thermophilus* and *Lactobacillus helveticus*.

Table 3 – Physico-chemical parameters of brine made with different bacterial preparations (n=3)

Indicator/Samples	C	E
Fat in dry matter, %	45.0±0.01	44.2±0.03
Moisture, %	68.9±0.02	69.2±0.01
Salt, %	4.9±0.12	5.0±0.11
Active acidity, pH	4.0±0.12	4.2±0.12

The next stage of experimental studies was to determine the change of the active acidity and the amount of viable cells of lactococci and lactobacilli in cheese during storage at a temperature of 2–6ºC, relative humidity of not more than 85% for 60 days, taking into account 20 days of maturation. Analyzing the acidity of the brynza during storage (Table 4), it was observed that the acidity increased in all samples of brynza, especially in the samples using the bacterial preparation RSF-742. The results are indicated the possibility of cheese consumption up to 40 days of storage, since the acidity is within limits that do not affect the organoleptic and microbiological parameters.

Table 4 – Acidity of the cheese during storage, pH (n = 3)

Samples	Duration of storage (age of cheese), days				
	0 (20)	10 (30)	20 (40)	30 (50)	40 (60)
C	4.23±0.11	4.19±0.12	4.11±0.10	4.05±0.12	3.98±0.10
E	4.26±0.13	4.21±0.11	4.16±0.12	4.13±0.11	4.07±0.11

During the maturation and storage of cheese for 60 days the number of lactococci cells (Fig. 5) and lactobacillus (Fig. 6) was determined. The maximum amount of lactococci was observed on the end of maturation (on the 20th day), – 6.9 and 7.3 lg CFU/g for C and E samples respectively. At low temperatures, enzymes are inactivated, which eventually leads to the death of cells. On the 60th day (40th day of storage) the number of viable lactococci cells was 5.8–5.9 lg CFU/g. Similarly, the change in the number of viable lactococci cells in the samples of cheese brynza the number of lactobacilli varied (Fig. 6).

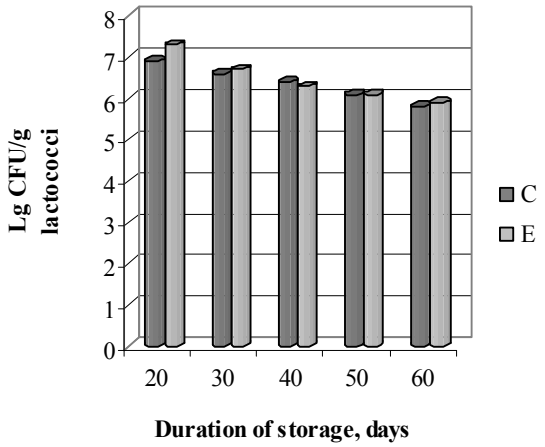

Fig. 5. Change in the number of lactococci during storage of cheese

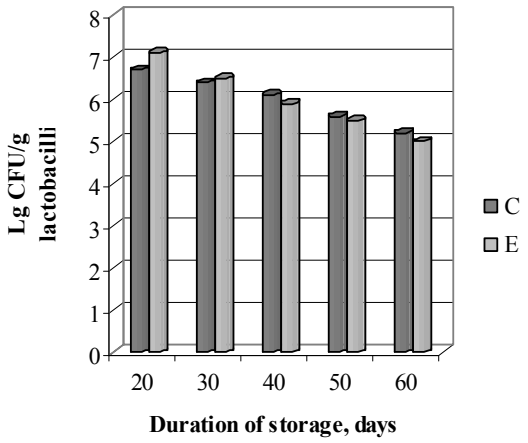

Fig. 6. Change the number of lactobacilli during storage of cheese

At the beginning of the storage of cheese, the amount of lactobacilli was in the range of 6.7–7.1 lg CFU/g. Subsequently, there was a death of cells and up to 40 days of storage their amount decreased to 5.2 and 5.0 lg CFU/g in C and E samples respectively.

The use of preparation Herobakterin in the production of cheeses allows providing organoleptic, physico-chemical and microbiological parameters of cheese, which according to the complied with the requirements of DSTU 7065:2009 (Brynza. General technical conditions). Thus, the use of Herobacterin has a positive impact on both the quality of the manufactured product and the number of viable lactic acid bacteria in the finished product and during storage.

Based on the functional properties of strains *Lactobacillus plantarum* WCFS1 and *Enterococcus faecium* L3-23, we can position brynza made with Herobacterin as a product as a product with useful properties. Herobacterin is a bacterial preparation that was first created in Ukraine for the production of brynza, based on strains of LAB identified by their genetic features. It is a factor in the preservation of the natural bacterial system of traditional ewe's cheese – brynza, which is produced in non-industrial conditions of the Carpathian region of Ukraine, whose role is directly related to the natural fermentation of milk. From the point of view of nutrition it is incorrect to compare bacterial preparations that are different in composition and biological action. In the composition of Herobacterin enter strains of LAB, which are typical for brynza, which is made in non-industrial conditions of the Carpathian region of Ukraine. Starter RSF-742, witch used for comparison, includes representatives of the genus *Lactococcus* and *Lactobacteriaceae*, which are selected according to technological indicators. There are no analogues of Herobacterin present on the Ukrainian market, which indicates the promise of its application in the technology of brine cheese.

Conclusions

The complex of organoleptic and physico-chemical indicators of ewe's milk is suitable for use in brine technology.

The influence of preparation Herobacterin and starter RSF-742 on the change of milk cheese acidity during protein coagulation and syneretic properties of clots was established. The decrease of the active acidity index was more pronounced for

the sample with the use of RSF-742, which includes active acid formers. Regarding the syneretic properties of the clots, no significant differences between the samples were observed.

The use of bacterial preparation Herobacterin did not lead to deterioration of the organoleptic and physico-chemical parameters of cheese brynza. The active acidity of the experimental samples of brine is 0.2 pH higher compared to the control, due to the presence in the RSF-742 more active acid-forming agents – *Streptococcus thermophilus* and *Lactobacillus helveticus*.

Brynza made using bacterial Herobacterin according to the organoleptic, physico-chemical and microbiological parameters complied with the requirements of DSTU 7065:2009 and was characterized by a high concentration of viable cells of lactic acid bacteria – more than 1×10^7 CFU/g.

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ИСПОЛЬЗОВАНИЕ БАККОНЦЕНТРАТА ГЕРОБАКТЕРИН В ТЕХНОЛОГИИ БРЫНЗЫ

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Аннотация. В статье показаны результаты сравнительного анализа использования созданного бакконцентрата Геробактерин с бактериальным препаратом RSF-742 (Chr. Hansen, Дания) в технологии рассольного сыра брынза. Геробактерин – бактериальный препарат, созданный на межвидовой, многоштаммовой основе с использованием бактерий *Lactococcus lactis, Lactobacillus plantarum, Enterococcus faecium, Leuconostoc mesenteroides* и *Lactococcus garvieae*, изолированных из традиционной карпатской брынзы и идентифицированных с использованием классических микробиологических и современных молекулярно-генетических методов (RAPD-PCR, RFLP-PCR, секвенирование гена 16s рРНК). Приведены результаты исследований органолептических, физико-химических, синеретичних и микробиологических показателей сыра брынза с использованием бакконцентрата Геробактерин по сравнению с препаратом прямого внесения RSF-742, в состав которого входят такие культуры: *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. lactis*, *Streptococcus thermophilus*, *Lactobacillus helveticus*. Использование препарата Геробактерин осуществляет положительное влияние на органолептические, физико-химические и микробиологические показатели брынзы, все параметры соответствовали требованиям ДСТУ 7065: 2009. Уровень выживания молочнокислых бактерий в брынзе в течение созревания и хранения является высоким, что подтверждает правильность отбора штаммов для конструирования бакконцентрата Геробактерин, которые продемонстрировали хорошую приспособленность к составу и свойствам овечьего молока.

Ключевые слова: брынза, технология, бакконцентрат Геробактерин, препарат микробиальный RSF-742, органолептические показатели, физико-химические показатели, количество жизнеспособных клеток.

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